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ORIGINAL ARTICLE

A study of leptin and its gene 2548 G/A Rs7799039 single-nucleotide polymorphisms in Egyptian children: A single-center experience

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Abstract
Background/objectives: The pathophysiology of obesity is multifactorial, including genetic and environmental factors. Previous studies had highlighted the association of the leptin gene/receptor with obesity. We aimed to study the leptin gene rs7799039 single nucleotide polymorphism (SNP) in children, and its association with the children's characteristics.
Methods: A cross-sectional analytic study that included 143 children with obesity (cases) and a comparable group of 86 lean children as controls. The anthropometric measures, blood pressure, and biochemical testing were done for all participants. The real-time polymerase chain reaction was used to detect rs7799039 SNP variant alleles and ELISA for leptin level assessment.
Results: The distribution of rs7799039 SNPs genotypes GG/GA/AA was comparable between both groups. Testing children regardless of their body mass index showed that the abnormalities in blood pressure, lipids values, insulin resistance, and hepatic insulin sensitivity were significantly associated with increased leptin levels. Among cases, the abnormal metabolic status was associated with higher leptin levels.

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Conclusions: The genotype' distribution of leptin gene rs7799039 SNP was similar in both children with obesity and those with normal-weight. The high blood pressure, abnormal lipid profile, and metabolic disturbances, were significantly associated with higher leptin levels and not with leptin gene rs7799039 SNP.

Introduction

The burden of obesity has augmented in the last decades owing to its escalated rates in adults and children. According to the World Health Organization definition, obesity is an extra-deposition of fat in the human body, which can adversely affect health. Accordingly, the use of the body mass index (BMI) defines obesity in children. Obesity-related comorbidities are enormous, with excessive rates of lifelong morbidities, mortalities, and national financial burden [1]. The prevalence of obesity in the last decade reached 18% worldwide [1] and 15% in Egyptian school-age children [2].

Obesity is currently considered a metabolic and neuroendocrine disorder, in which pathogenesis is multifactorial. Its occurrence required obesogenic environmental factors, lifestyle, social, and ecological factors, and predisposed genetic background. The effects and their interactions of these factors resulted in energy intake excessively exceeding consumed energy, with subsequent excess adiposity [3,4].

The genetic influences over the bodyweight control are conducted via different pathways, including many hormones [5,6]. Among them, leptin is a polypeptide hormone secreted by the adipose tissue, with some structural similarity to the cytokines. It acts as a mediator for bodyweight control via suppressing appetite and augmentation of the expenditure of energy [7]. Serum leptin can represent the energy stores in the adipose tissues, reflecting the total body fat [8]. Leptin act through binding to its specific receptor producing a neuroendocrine influence via interacting with different central neuronal pathways [9,10]. Multiple human studies had confirmed the role of both the gene and its receptor and obesity development [11,12], while other reports did not find an association [13].

The G2548A (rs7799039) is a single nucleotide polymorphism (SNP) in the leptin gene promoter, with G to A substitution at nucleotide -2548 upstream. This SNP had proven associations with type 2 diabetes mellitus [14], coronary artery disease [15], and the development of cancer [16], with controversy in its relation to high leptin levels [17] in adult studies.

Aim of the study

To study the leptin gene 2548 G/A rs7799039 single-nucleotide polymorphism (SNP) in healthy and obese Egyptian children. Also, to explore its association with clinical phenotype, leptin levels, lipid profile, and insulin resistance.

Methods

Our work is a cross-sectional analytic study that included children with obesity and a non-obese control group. All participants were screened for the leptin gene 2548 G/A rs7799039 SNPs polymorphism. Children were recruited from those coming for routine assessment at the outpatient clinics of the National research center, Giza, Cairo, Egypt, and the National Institute for diabetes and endocrinology, Cairo, Egypt, for six months duration. We obtained approval from the National research center-Research Ethics Committee of the consent from one of the parents.

We recruited all children aged between 2 and 16 years, of both sexes, and at any pubertal stage. For the group with obesity, we included those with BMI above 85th percentiles with nutritional obesity. We excluded children with eating disorders, on regular corticosteroids, or with a secondary cause for obesity as pathological and syndromic obesity, i.e., Prader Willi and Cushing syndrome. Children on lipid-lowering drugs or known comorbid conditions, such as renal failure, thyroid disease, and hepatic pathology, were excluded as well.

All recruited children were subjected to:

Clinical assessment included:

Anthropometric measurements: *we measure the* weight and height, then we calculated body mass index (BMI) as body weight (kg)/height^2 (m^2), and calculated BMI Z scores online [18]. Children were categorized as overweight if their BMI plotted between 85th but below 95th percentiles. As obese if at or above 95th percentile on the corresponding age and sex-matched growth references, as per the Center for Disease Control and Prevention (CDC) definition [19].

Blood pressure measurement: using a sphygmomanometer with an appropriate-sized cuff. Readings were plotted to appropriate age & sex percentiles and categorized to hypertension stages as per the American Academy of Pediatrics - Clinical Practice Guidelines for Management of Hypertension in Children and Adolescents definition [18].

Blood sampling for biochemical analysis and genotyping testing:

Participants were asked to attend in the morning after a fasting period of 10—12 h. Samples were drawn at a similar timing for all participants to avoid the diurnal rhythm of leptin, which is the norm in individuals regardless of their BMI [20]. A 10 mL venous sample was drawn. About 3 mL were taken in coagulant-free sterile tubes to be used in the analysis of biochemical markers. About 7 mL was taken in EDTA tubes to be used for complete blood count (CBC) and DNA extraction and gene polymorphism analysis. Samples were centrifuged for coagulation, serum was obtained immediately and stored at −80° to determine serum leptin by Eliza.

Table 1 Characteristics of children with overweight/obesity and normal weight children.

	Non-obese children N = 86	Children with overweight/obesity N = 143	P value
Age in years (range)	12.3 ± 2.2 (5–15)	11.8 ± 1.9 (7–16)	0.1
Sex; N (%)			
[•]*Male*	43 (50)	61 (42.7)	0.3
[•]*Female*	43 (50)	82 (57.3)	
Residency; N (%)			
[•]*Urban*	66 (76.7)	101 (70)	0.3
[•]*Rural*	20 (23.3)	43 (30)	
Meal frequency; N (%)			
[•]*Up to two*	0	1 (0.7)	0.4
[•]*Three*	30 (34.9)	60 (42)	
[•]*Four or more*	56 (65.1)	82 (57.3)	
Overeating habit; N (%)	52 (60.5)	87 (60.8)	0.9
No diet regimen; N (%)	44 (51.2)	88 (61.5)	0.1
No regular physical activity (not active); N (%)	57 (66.3)	83 (58)	0.2
Two or more children in the family; N (%)	82 (95.3)	140 (97.9)	0.5
Mother education; N (%)			
[•]*Up to secondary school*	49 (57)	77 (53.8)	0.6
[•]*College degree*	37 (43)	66 (46.2)	
Father education; N (%)			
[•]*Up to secondary school*	34 (39.5)	82 (57.3)	0.02*
[•]*College degree*	52 (60.5)	61 (43.4)	
Systolic blood pressure	107.5 ± 6.5	111.8 ± 7.4	0.000*
Systolic blood Percentile	64.1 ± 20.2	76.95 ± 20.7	0.000*
Diastolic blood pressure	67.7 ± 5	69.4 ± 7.7	0.06
Diastolic blood Percentile	69.8 ± 15.9	72.5 ± 19.2	0.2
Blood pressure stage; N (%)			
[•]*Normal*	71 (82.6)	85 (59.4)	0.000*
[•]*Elevated*	15 (17.4)	21 (14.7)	
[•]*Stage 1*	0	33 (23.1)	
[•]*Stage 2*	0	4 (2.8)	
Weight in kg	38.6 ± 9.9	64 ± 14.6	0.000*
Percentile; median (IQR)	29.5 (5.1–51.9)	97.4 (90.5–99.3)	0.000*
Z score; median (IQR)	−0.5 (−1.6–0.05)	1.9 (1.3–2.4)	0.000*
Height in cm	146 ± 11.4	144.7 ± 9.5	0.4
Percentile; median (IQR)	17.9 (6.8–44)	30.9 (10.7–55.6)	0.01*
Z score; median (IQR)	−0.9 (−1.5 to −0.15)	−0.5 (−1.2 to 0.1)	0.01*
BMI	18 ± 2.8	30.8 ± 5.2	0.000*
Percentile; median (IQR)	46.6 (21.6–73.6)	98.9 (96.9–99.4)	0.000*
Z score; median (IQR)	−0.1 (−0.8 to 0.6)	2.3 (1.9–2.5)	0.000*
Hemoglobin (g/dL)	11.9 ± 1.4	11.4 ± 1.4	0.01*
Total leukocytic count ($\times 10^3/mm^3$)	6.8 ± 2.3	7.6 ± 3.5	0.05*
ALT (up to 37 IU/mL)	29.6 ± 6.2	30.6 ± 5.8	0.2
AST (up to 37 IU/mL)	30.7 ± 7.7	33.7 ± 8	0.007*
Total bilirubin (up to 1 mg/dL)	0.7 ± 0.2	0.8 ± 0.2	0.2
Direct bilirubin (up to 0.25 mg/dL)	0.1 ± 0.06	0.2 ± 0.06	0.6
ALP (up to 150 IU/mL)	194.3 ± 47.8	228.6 ± 96.2	0.002*
GGT (up to 30 IU/mL)	35.7 ± 9.8	36.7 ± 9.7	0.5
Albumin (3.5–5.3 gm/L)	3.9 ± 0.2	3.8 ± 0.2	0.007*
FBG (up to 100 mg/dL)	91.7 ± 6	101.2 ± 15.3	0.000*
Cholesterol (mg/dL)	149.9 ± 19.9	176.1 ± 34.4	0.000*
Triglycerides (mg/dL)	144.6 ± 18.6	159.9 ± 31.7	0.000*
HDL-c (mg/dL)	45.2 ± 8	41.1 ± 9.2	0.000*
LDL-c (mg/dL)	107.2 ± 10.9	118.3 ± 19.4	0.000*
AFP (up to 10 ng/mL); median (IQR)	5.5 (3.2–6.8)	6 (5–7.2)	0.006*

Table 1 (*Continued*)

	Non-obese children N = 86	Children with overweight/obesity N = 143	P value
Fasting insulin (μIU/mL)	20.6 ± 7.1	23.9 ± 6.7	0.001*
HOMA-IR	4.7 ± 1.7	6 ± 1.9	0.000*
HOMA IR > 3.5; N (%)	59 (68.6)	130 (90.9)	0.000*
Hepatic insulin sensitivity	0.25 ± 0.1	0.1 ± 0.07	0.000*
Unhealthy Metabolic status; N (%)	4 (4.7)	129 (90.2)	0.000*
Leptin (up to 15 ng/mL); median (IQR)	3.7 (2.6—4)	23 (3.8—32)	0.000*
Genetic alleles; N (%)			
[•]*GG*	50 (58.1)	78 (54.5)	0.7
[•]*GA*	30 (34.9)	51 (35.7)	
[•]*AA*	6 (7)	14 (9.8)	

Data are shown as mean ± SD unless otherwise mentioned. AFP: Alfa-fetoprotein, ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index, FBG: Fasting blood Glucose; GGT: gamma-glutamyl transpeptidase; HDL-c: High density lipoprotein-cholesterol, HOMA-IR: homeostasis model assessment-insulin resistance, IQR: interquartile range; LDL-c: low-density lipoprotein —cholesterol.

* $P < 0.05$ was considered significant. Statistical tests used were using Student's t-test, Mann Whitney U, and chi-square test.

The biochemical analysis included:

A liver panel included aspartate transaminase (AST), and alanine aminotransferase (ALT) enzymes were assessed by wet chemistry Bachman machine; total, direct bilirubin, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), albumin. Lipid profile included cholesterol (TC), triglycerides (TG), High-density lipoprotein-cholesterol (HDL-c), low-density lipoprotein-cholesterol (LDL-c). Besides, fasting blood glucose (FBG), alfa-fetoprotein (AFP), fasting insulin, and CBC were done. Leptin was assessed by use DRG® Leptin (Sandwich) ELISA (EIA-2395), Inc., USA. The normal range is 1—15 ng/mL.

Genotyping testing included:

DNA extraction and genotyping: it was carried out using QIA amp® DNA Blood Mini Kit (QIAGEN GmbH, Hilden, Germany) [21] according to recommended instructions. We measured the concentration of the extracted DNA using the Nano Drop® (ND-1000) Spectrophotometer (Nano Drop Technologies Inc., Washington, USA). The ratio of DNA extracted absorbance was 1.7—1.9 at 260/280 nm.

Functional testing of the leptin gene 2548 A/G rs7799039 SNPs polymorphism: on chromosome 7: 128238730 on GRCh38, catalog number: 4351379. This was carried out using real-time polymerase chain reaction with TaqMan® allelic discrimination assay software (Applied Biosystems Step One TM Real-Time PCR system Thermal Cycling Block, Singapore) the manufacture's instructions.

Calculation of Insulin resistance:

We calculated the homeostasis model assessment (HOMA) using the equation = (fasting insulin (mIU/mL) × fasting glucose (mmol/L)/22.5). HOMA values of more than or equal to 3.5 were representative for insulin resistance [22].

Calculation of Hepatic insulin sensitivity:

Using the equation = K/FPG × FPI. [K = 22.5 × 18, FPG: fasting glucose (mg/dL), FPI: fasting plasma insulin mU/mL] [23].

Assessment of the metabolic status in Children with obesity:

We used the criteria of Damanhoury et al. [24] to classify participants into metabolically healthy or unhealthy. Children with obesity, having FBS less than 100 mg/dL, TG level less than 150 mg/dL, HDL-c more than 40 mg/dL, and blood pressure readings less than 90th percentile were termed metabolically healthy.

Statistical analysis

Data analysis was done using Statistical Package for Social Science (SPSS, Chicago, IL 60606-6412, USA) program version 17.0. Quantitative data were presented as mean ± standard deviation (SD), and differences were tested using Student's t-test or One-Way ANOVA, or median and interquartile range (IQR), and the difference was tested Mann Whitney U or Kruskal—Wallis Tests. Qualitative data were presented as frequency and percentage, and by chi-square test was used to test differences between groups. Person correlations were used to test linear associations of leptin and scale clinical and laboratory variables. The difference was statistically significant when a two-sided P-value is at or below 0.05. We applied Hard-Weinberg equilibrium using an online calculator [25].

Results

Among recruited 251 children, we used data of 143 children with overweight/obesity (25 overweight and 118 obese) and 86 controls who were age and gender comparable. We used the Equilibrium Hardy-Weinberg to calculate expected and observed genotypes in our population, Fig. 1. The p allele frequency was 0.736, while the q allele frequency: 0.264.

Cases had significantly higher anthropometric indices, systolic pressure percentiles, leucocyte count, AST, ALP, FBG, AFP, lipid profile values, fasting insulin, HOMA-IR, and leptin level than controls. They have significantly lower albumin, hemoglobin levels, and hepatic insulin sensitivity. Also, they had significantly more frequent hypertension and unhealthy metabolic status than controls. The distribution of rs7799039 SNPs genetic variants GG/GA/AA were compa-

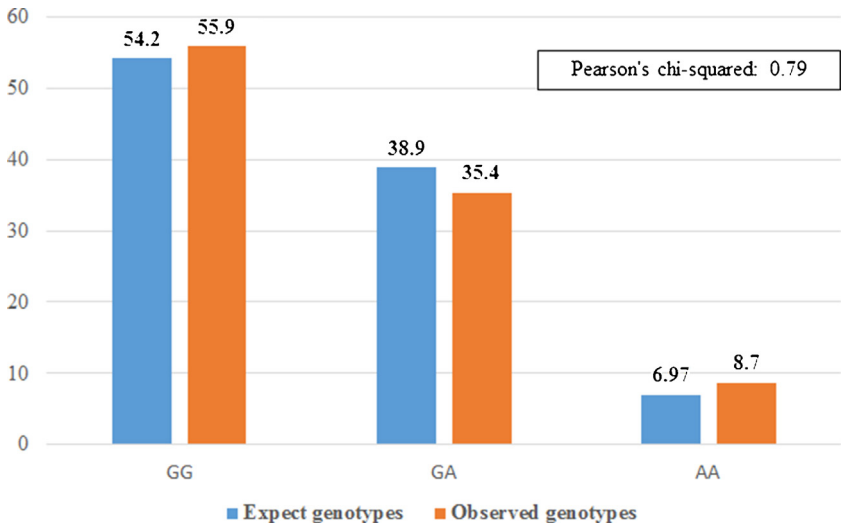

Figure 1 Frequency of expected and observed genotypes in the studied population using Hard-Weinberg equilibrium.

rable between both groups, Table 1. None of the enrolled children was receiving chronic medications.

Subject characteristics were compared between cases and controls again with the age of 10 years as demarcation level. Children older than ten years showed the same positive relations as the whole group, but cases were significantly younger than controls. Children aged ten years or less also showed significantly higher diastolic pressure percentiles, direct bilirubin, and GGT in cases than in controls (Tables 2).

Then, we compared characteristics between cases and controls regarding individual variants GG/GA/AA. Non-obese children with AA alleles had significantly higher ALP, fasting insulin, and HOMA-IR, while those with GG had higher hepatic insulin sensitivity. Overweight/obese children showed no difference regarding genetic variants except for leptin level, which was higher in cases with AA alleles. When pure AA was compared to that of GG/GA alleles, the control group showed the same previous positive relations. At the same time, overweight/obese children lost a significant relation to leptin level (Tables 3 and 4).

We used the median leptin level for each group was used to compare children's characteristics. In the control group, children with high leptin levels had significantly higher ALP, FBG, insulin levels, and HOMA-IR. Among cases, children with high leptin levels had significantly higher anthropometric measures, ALP, GGT, lipid profile apart from HDL, insulin levels, and HOMA-IR, and lower hepatic insulin sensitivity, Table 5.

We tested the linear associations of leptin and patient's clinical and laboratory values in different genotypes and with BMI categories. In all studied children, leptin positively correlated with weight, BMI indices, TC, and HOMA; and negatively with hepatic insulin sensitivity with no difference related to genotypes. Children with GG/GA genotypes showed positive correlations of leptin with blood pressure percentiles, ALP, GGT, TG, LDL-c, FBG, insulin, and a negative correlation HDL-c. Leptin showed significant correlations with most tested variables only in children with overweight/obese and not in lean children, Table 6.

Discussion

Obesity is the most widely-spread malnutrition disease affecting children currently. Leptin gene mutations, especially rs7799039 G > A SNP, were incriminated in the development of Obesity [26]. To our knowledge, our study is among few testing rs7799039 G > A SNP in Egyptian children.

The distributions of genotypes GG, GA, and AA were comparable between children with obesity/overweight and children with average weight in our study group. Similarly, Eldosouky et al. [27] studied the rs7799039 SNPs in Saudi children and reported no difference in genotype distribution among studied children with obesity and lean children. On the contrary, Aboelros et al. [28] studied G2548A among a group of adult obese and non-obese Egyptians, observed significantly more prevalent AG and AA among the obese group & more homozygous GG among the non-obese group. They suggested that leptin gene polymorphism (G2548A) may be a marker of obesity in the Egyptian population. The controversy in results regarding G2548A SNIP at the promoter region of the leptin gene may arise from its interactions with other polymorphisms, other socio-environmental factors, or the suggested inhibitory effects from transcription in adipocytes [28].

Our results showed that the AA allele of rs7799039 SNPs was significantly associated with high leptin levels among cases. Similarly, a previous study in children found that the presence of AA alleles was associated with higher leptin levels [27]. The rs7799039 has been considered a promotor SNP in the leptin gene that could alter the gene expression and serum leptin level [29]. The presence of the A allele of leptin geneG2548A polymorphism is positively correlated with leptin and insulin resistance. This polymorphism was claimed for inadequate metabolism of insulin and leptin hormones, which can induce resistance of their action [28].

Our group of children with obesity/overweight had significantly higher leptin levels than children with average weight. Among cases, higher leptin values were highly associated with children's weight and BMI indices and well correlated with them. To stabilize other confounders affect-

Table 2 Characteristics of cases and controls with age stratification.

	Children ≤ 10 years N = 41			> 10 years N = 188		
	Non-obese children N = 12	Children with overweight/obesity N = 29	P value	Non-obese children N = 74	Children with overweight/obesity N = 114	P value
Age in years; mean ± SD	8.3 ± 1.6	8.9 ± 0.4	0.08	13.2 ± 1.5	12.5 ± 1.4	0.002*
Sex; N (%)						
[•]*Male*	2 (16.7)	14 (48.3)	0.06	41 (55.4)	47 (41.2)	0.06
[•]*Female*	10 (83.3)	15 (51.7)		33 (44.6)	67 (58.8)	
Systolic blood pressure	101.7 ± 3.9	114.8 ± 7.5	0.000*	108.4 ± 6.4	11 ± 7.2	0.01*
Systolic blood Percentile	72.2 ± 16.2	86.6 ± 14	0.01*	61 ± 23.3	68.1 ± 21.3	0.04*
Diastolic blood pressure	66.7 ± 7.2	72.4 ± 10.1	0.01*	67.8 ± 4.6	68.7 ± 6.8	0.3
Diastolic blood Percentile	63.5 ± 27.3	79.2 ± 18.6	0.04*	68 ± 13.4	69 ± 18.5	0.7
Blood pressure stage; N (%)						
[•]*Normal*	10 (83.3)	5 (17.2)		61 (82.4)	80 (70.2)	
[•]*Elevated*	2 (16.7)	8(27.6)	0.001*	13 (17.6)	13 (11.4)	0.000*
[•]*Stage 1*	0	12 (41.4)		0	21 (18.4)	
[•]*Stage 2*	0	4 (13.8)		0	0	
Weight in kg; mean ± SD	28.4 ± 5.9	54.8 ± 12.1	0.000*	40.3 ± 9.4	66.4 ± 14.3	0.000*
Percentile; median (IQR)	55.7 (28.2)	99.6 (1.3)	0.000*	25.5 (42.9)	96.6 (11.3)	0.000*
Z score; median (IQR)	0.1 (0.7)	2.7 (0.8)	0.000*	−0.7 (1.7)	1.8 (1.1)	0.000*
Height in cm; mean ± SD	129.7 ± 10.6	134.5 ± 7.7	0.2	148.6 ± 9.1	147.2 ± 8.1	0.4
Percentile; median (IQR)	48.4 (36.4)	56.7 (45.1)	0.6	14.2 (28.97)	26.3 (37.2)	0.01*
Z score; median (IQR)	−0.04 (0.96)	0.2 (1.2)	0.6	−1.1 (1.2)	−0.6 (1.2)	0.01*
BMI; mean ± SD	16.8 ± 1.9	30.8 ± 5.6	0.000*	18.2 ± 2.9	30.8 ± 5.2	0.000*
Percentile; median (IQR)	58.1 (57.4)	99.4 (0.7)	0.000*	46.6 (52.6)	98.7 (3.1)	0.000*
Z score; median (IQR)	0.2 (1.6)	2.5 (0.4)	0.000*	−0.09 (1.5)	2.2 (0.6)	0.000*
Hemoglobin (g/dL)	11.2 ± 1.4	11.5 ± 1.7	0.5	12.1 ± 1.4	11.4 ± 1.4	0.003*
Total leukocytic count ($\times 10^3/mm^3$)	6.8 ± 1.9	7.7 ± 2.1	0.2	6.8 ± 2.4	7.5 ± 3.8	0.002*

Table 2 (*Continued*)

	Children ≤ 10 years N = 41			> 10 years N = 188		
	Non-obese children N = 12	Children with overweight/obesity N = 29	P value	Non-obese children N = 74	Children with overweight/obesity N = 114	P value
ALT (up to 37 IU/mL)	33.3 ± 5.6	32.1 ± 7.2	0.3	28.9 ± 5.6	30.5 ± 5.6	0.06
AST (up to 37 IU/mL)	33.3 ± 7.7	32.1 ± 7.2	0.6	30.3 ± 7.9	34.1 ± 8.2	0.002*
Total bilirubin (up to 1 mg/dL)	0.9 ± 0.2	0.8 ± 0.2	0.2	0.7 ± 0.2	0.1 ± 0.05	0.05
Direct bilirubin (up to 0.25 mg/dL)	0.2 ± 0.07	0.2 ± 0.07	0.03*	0.1 ± 0.05	0.2 ± 0.05	0.07
ALP (up to 150 IU/mL)	188.8 ± 29.7	195 ± 21.7	0.8	195.2 ± 50.2	237.1 ± 105.6	0.002*
GGT (up to 30 IU/mL)	31.7 ± 6.3	39.5 ± 13.6	0.02*	36.4 ± 10.1	35.99 ± 8.4	0.8
Albumin (3.5—5.3 gm/L)	3.8 ± 0.3	3.7 ± 0.3	0.6	3.9 ± 0.2	3.8 ± 0.2	0.01*
FBG (up to 100 mg/dL)	91.2 ± 5.7	100.5 ± 14.8	0.04*	91.7 ± 6	101.4 ± 15.5	0.000*
Cholesterol (mg/dL)	144.3 ± 20.7	178.1 ± 38.9	0.001*	150.8 ± 19.7	175.6 ± 33.3	0.000*
Triglycerides (mg/dL)	136.8 ± 8.2	161.3 ± 36.3	0.03*	137.5 ± 8.7	159.6 ± 30.6	0.000*
HDL-c (mg/dL)	46.2 ± 6.7	38.8 ± 8.9	0.01*	45.7 ± 7.6	41.6 ± 9.3	0.001*
LDL-c (mg/dL)	107 ± 11.7	119.1 ± 22.9	0.03*	107.2 ± 10.9	118 ± 18.5	0.000*
AFP (up to 10 ng/mL);	6.2 (2.8)	5 (3)	0.9	5 (3.6)	6.2 (2.4)	0.003*
Fasting insulin (μIU/mL)	18.8 ± 7.2	24.3 ± 7.3	0.04*	20.9 ± 7.1	23.8 ± 6.6	0.006*
HOMA-IR	4.3 ± 1.8	6.1 ± 2.3	0.01*	4.7 ± 1.6	5.9 ± 1.9	0.000*
HOMA IR > 3.5; N (%)	6 (50)	26 (89.7)	0.005*	53 (71.6)	104 (91.2)	0.000*
Hepatic insulin sensitivity	0.3 ± 0.1	0.2 ± 0.07	0.005*	0.2 ± 0.1	0.2 ± 0.07	0.000*
Leptin (up to 15 ng/mL); median (IQR)	3.7 (2.2)	23 (29.7)	0.000*	3.7 (1.4)	23 (28.2)	0.000*
Genetic alleles; N (%)						
[•]*GG*	8 (66.7)	15 (51.7)	0.6	42 (56.8)	63 (55.3)	0.8
[•]*GA*	4 (33.3)	13 (44.8)		26 (35.1)	38 (33.3)	
[•]*AA*	0	1 (3.4)		6 (8.1)	13 (11.4)	

Data are shown as mean±SD unless otherwise mentioned. AFP: Alfa-fetoprotein, ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index, FBG: Fasting blood Glucose; GGT: gamma-glutamyl transpeptidase; HDL-c: High density lipoprotein-cholesterol, HOMA-IR: homeostasis model assessment-insulin resistance, IQR: interquartile range; LDL-c: low-density lipoprotein —cholesterol.

* $P < 0.05$ was considered significant. Statistical tests used were using Student's t-test, Mann Whitney U, and by chi-square test.

Table 3 Patients' characteristics and different genotypes in children with overweight/obesity and children with normal weight.

	Non-obese children N = 86				Children with overweight/obesity N = 143			
	GG N = 50	GA N = 30	AA N = 6	P value	GG N = 78	GA N = 51	AA N = 14	P value
Age in years	12.4 ± 2.5	12.7 ± 2	12.3 ± 1.4	0.9	11.7 ± 1.8	11.9 ± 2.1	12 ± 2.1	0.6
Sex; N (%)								
[•]*Male*	22 (44)	17 (56.7)	4 (66.7)	0.4	36 (46.2)	19 (39.6)	4 (28.6)	0.4
[•]*Female*	28 (56)	13 (43.3)	2 (33.3)		42 (53.8)	29 (60.4)	10 (71.4)	
Systolic blood pressure	108 ± 6.5	106.6 ± 6.9	106.3 ± 5.4	0.6	110.7 ± 6.6	113.4 ± 7.9	110.8 ± 8.7	0.1
Systolic blood percentile	66 ± 20.5	57.6 ± 24	58 ± 31	0.3	69.9 ± 21.3	75 ± 19.9	67.6 ± 25.7	0.3
Diastolic blood pressure	67.7 ± 5.5	68.2 ± 4	64.3 ± 3.4	0.2	68.3 ± 6.5	70.9 ± 9	69.5 ± 8.3	0.2
Diastolic blood percentile	66.6 ± 17.4	70.4 ± 11.9	59.7 ± 19	0.3	69.4 ± 17.3	72.5 ± 20.4	71.9 ± 22.5	0.7
Blood pressure values; N (%)								
[•]*Normal*	47(94)	29 (96.7)	6 (100)	0.9	59 (75.6)	31 (64.6)	9 (64.3)	0.1
[•]*Pre-hypertensive*	2 (4)	1 (3.3)	0		13 (16.7)	7 (14.6)	1 (7.1)	
[•]*Hypertensive*	1 (2)	0	0		6 (7.7)	10 (20.8)	4 (28.6)	
Weight in kg	38.2 ± 11.3	39.2 ± 5.9	38.7 ± 14.4	0.9	63.6 ± 14	62.5 ± 14	71.4 ± 18.3	0.1
Percentile; median (IQR)	39.4 (3.3–58.8)	26.5 (8.9–47.6)	9.3 (4.1–75.8)	0.9	97.6 (89.8–99.4)	96 (87.4–99)	98.7 (97.2–99.6)	0.2
Z score; median (IQR)	−0.3 (−1.8 to 0.2)	−0.6 (−1.4 to 0.06)	−1.3 (−1.7 to 0.7)	0.9	2 (1.3–2.5)	1.8 (1.2–2.3)	2.2 (1.9–2.7)	0.3
Height in cm	146.2 ± 12.2	146.3 ± 10	143.3 ± 12.6	0.8	144.3 ± 9.3	144.9 ± 10.4	145.9 ± 8.6	0.8
Percentile; median (IQR)	21.8 (7.8–44)	15.3 (1.4–46)	16.9 (2.4–35.9)	0.6	33.3 (10.9–55.9)	24.8 (9.6–49.5)	28.8 (16.8–54.9)	0.5
Z score; median (IQR)	−0.8 (−1.4 to 0.1)	−1 (−2 to −0.1)	−1 (−1.8 to −0.4)	0.6	−0.4 (−1.2 to 0.1)	−0.7 (−1.3 to −0.01)	−0.6 (−1 to 0.1)	0.5
BMI	17.8 ± 3.2	18.4 ± 1.8	18.3 ± 3.6	0.6	30.9 ± 5	29.9 ± 5.3	33 ± 6.4	0.1
Percentile; median (IQR)	41.8 ± 31.2	48.1 ± 26.4	41.6 ± 32	0.4	97.6 ± 3	96.4 ± 4.5	98.3 ± 2.3	0.2
Z score; median (IQR)	0.09 (−1.7 to 0.6)	−0.09 (−0.5 to 0.7)	−0.6 (−1 to 0.9)	0.3	2.3 (1.9–2.4)	2.2 (1.7–2.5)	2.3 (2.1–2.6)	0.3
Hemoglobin (g/dL)	11.9 ± 1.6	12 ± 1.1	11.3 ± 1.9	0.5	11.4 ± 1.5	11.7 ± 1.3	11 ± 1.5	0.4
Total leukocyte count ($\times 10^3/mm^3$)	6.9 ± 2.5	6.7 ± 2.3	7 ± 1.2	0.9	7.4 ± 2.4	8 ± 5.1	7.5 ± 2.4	0.6

Table 3 (*Continued*)

	Non-obese children N = 86				Children with overweight/obesity N = 143			
	GG N = 50	GA N = 30	AA N = 6	P value	GG N = 78	GA N = 51	AA N = 14	P value
ALT (up to 37 IU/mL)	29.3 ± 6.3	30 ± 6.3	30 ± 5.4	0.9	30 ± 5.8	31 ± 5.6	32 ± 6.7	0.4
AST (up to 37 IU/mL)	30.6 ± 8.3	30.8 ± 7	31.3 ± 6.8	0.9	32.3 ± 7.7	35 ± 7.8	36 ± 9.6	0.08
Total bilirubin (up to 1 mg/dL)	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.7	0.7 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	0.2
Direct bilirubin (up to 0.25 mg/dL)	0.2 ± 0.06	0.2 ± 0.05	0.1 ± 0.05	0.8	0.1 ± 0.06	0.2 ± 0.06	0.1 ± 0.05	0.5
ALP (up to 150 IU/mL)	108.2 ± 38.9	209.7 ± 55	235.3 ± 30.8	0.002*	218 ± 85	233 ± 95.4	277.9 ± 147	0.1
GGT (up to 30 IU/mL)	37.5 ± 11	32 ± 6	37.7 ± 10.4	0.07	36.9 ± 10.5	37 ± 9.5	34.5 ± 6.7	0.7
Albumin (3.5–5.3 gm/L)	3.9 ± 0.2	3.9 ± 0.3	3.9 ± 0.3	0.8	3.8 ± 0.2	3.8 ± 0.2	3.8 ± 0.3	0.7
FBG (up to 100 mg/dL)	91 ± 6.6	92.5 ± 5.2	93 ± 4.6	0.5	101.3 ± 15	98.4 ± 15.7	108.9 ± 15.3	0.08
Cholesterol (mg/dL)	147.8 ± 15.4	152.5 ± 26.8	154.7 ± 10.8	0.5	172.4 ± 34.3	182.9 ± 37.6	174.4 ± 22.7	0.3
Triglycerides (mg/dL)	144 ± 15.3	147 ± 23.9	138 ± 12.9	0.5	159 ± 32	161.3 ± 33	159.7 ± 29.4	0.9
HDL-c (mg/dL)	46 ± 9	44.2 ± 6.8	43 ± 1.5	0.5	41.7 ± 8.8	39.4 ± 10	41.7 ± 8.3	0.4
LDL-c (mg/dL)	107.9 ± 11.9	106 ± 10	105.7 ± 5.7	0.7	116 ± 17	119.8 ± 21.5	126.6 ± 24.4	0.1
AFP (up 10 ng/mL); median (IQR)	5 (3.2–6.8)	6.2 (3.2–6.8)	4 (3.2–8)	0.7	6 (5–7.2)	6.2 (5–7.2)	6.4 (4.8–8)	0.7
Fasting insulin	18.8 ± 6.8	22.6 ± 7	26.7 ± 3	0.005*	23.2 ± 6.8	25 ± 6.4	23.9 ± 7.8	0.3
HOMA-IR HOMA IR ≥ 3.5; N (%)	4.2 ± 1.6 30 (60)	5.1 ± 1.6 23 (76.7)	6 ± 0.9 6 (100)	0.005* 0.07	5.8 ± 2 69 (88.5)	6 ± 1.8 45 (93.8)	6.4 ± 2 13 (92.9)	0.5 0.6
Hepatic insulin sensitivity	0.3 ± 0.1	0.2 ± 0.08	0.2 ± 0.03	0.008*	0.2 ± 0.07	0.2 ± 0.08	0.2 ± 0.06	0.6
Unhealthy metabolic status; N (%)	3 (6)	1 (3.3)	0	0.7	71 (91)	42 (87.5)	13 (92.9)	0.8
Leptin	3.7 (2.2–4)	3.7 (3–4)	3.9 (3.6–4.3)	0.6	18.5 (3.6–31.3)	27 (4–33.8)	29.5 (2.8–35.8)	0.04*

Data are shown as mean ± SD unless otherwise mentioned. AFP: Alfa-fetoprotein, ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index, FBG: Fasting blood Glucose; GGT: gamma glutamyl transpeptidase; HDL-c: High density lipoprotein-cholesterol, HOMA-IR: homeostasis model assessment- insulin resistance, IQR: interquartile range; LDL-c: low-density lipoprotein –cholesterol.

* $P < 0.05$ was considered significant. Tests used were one-Way ANOVA, and Kruskal–Wallis.

Table 4 The presence of GG/GA versus AA and patients' characteristics in children with overweight/obesity and children with normal weight.

	Non-obese children N = 86			Children with overweight/obesity N = 143		
	GG/GA N = 80	AA N = 6	P value	GG/GA N = 129	AA N = 14	P value
Age in years	12.4 ± 2.5	12.3 ± 1.4	0.9	11.9 ± 2.1	12 ± 2.1	0.6
Sex; N (%)						
[•]*Male*	39 (48.8)	4 (66.7)	0.4	55 (43.7)	4 (28.6)	0.3
[•]*Female*	41 (51.3)	2 (33.3)		71 (56.3)	10 (71.4)	
Blood pressure values; N (%)						
[•]*Normal*	76 (95)	6 (100)	0.8	90 (71.4)	9 (64.3)	0.2
[•]*Pre-hypertensive*	3 (3.8)	0		20 (15.9)	1 (7.1)	
[•]*Hypertensive*	1 (1.3)	0		16 (12.7)	4 (28.6)	
Systolic blood pressure	110.5 ± 7	106.3 ± 5.4	0.5	113.4 ± 7.9	110.8 ± 8.7	0.1
Systolic blood percentile	66.9 ± 21.3	58 ± 31	0.5	75 ± 19.9	67.6 ± 25.7	0.3
Diastolic blood pressure	69 ± 7.6	64.3 ± 3.4	0.3	70.9 ± 9	69.5 ± 8.3	0.2
Diastolic blood percentile	67.8 ± 19	59.7 ± 19	0.3	72.5 ± 20.4	71.9 ± 22.5	0.7
Weight in kg	38.6 ± 9.6	38.7 ± 14.4	0.9	63.2 ± 14	71.4 ± 18.3	0.1
Percentile; median (IQR)	31.6 (5.4–50)	9.3 (4.1–75.8)	0.8	97 (89.8–99.2)	98.7 (97.2–99.6)	0.6
Z score; median (IQR)	−0.5 (−1.6 to 0)	−1.3 (−1.7 to 0.7)	0.8	1.9 (1.3–2.4)	2.2 (1.9–2.7)	0.4
Height in cm	146.2 ± 11.4	143.3 ± 12.6	0.6	144.6 ± 9.7	145.9 ± 8.6	0.8
Percentile; median (IQR)	18.8 (7.2–44.3)	16.9 (2.4–35.9)	0.6	30.7 (10.7–55.6)	28.8 (16.8–54.9)	0.4
Z score; median (IQR)	−0.9 (−1.5 to 0.1)	−1 (−1.8 to −0.4)	0.6	−0.5 (−1.2 to 0.1)	−0.6 (−1 to 0.1)	0.8
BMI	17.8 ± 3.2	18.3 ± 3.6	0.6	29.9 ± 5.3	33 ± 6.4	0.1
Percentile; median (IQR)	41.8 ± 31.2	41.6 ± 32	0.4	96.4 ± 4.5	98.3 ± 2.3	0.2
Z score; median (IQR)	0.09 (−1.7 to 0.6)	−0.6 (−1 to 0.9)	0.3	2.2 (1.7–2.5)	2.3 (2.1–2.6)	0.3
Hemoglobin (g/dL)	11.9 ± 1.6	11.3 ± 1.9	0.5	11.7 ± 1.3	11 ± 1.5	0.4

Table 4 (*Continued*)

	Non-obese children		N = 86	Children with overweight/obesity		N = 143
	GG/GA N = 80	AA N = 6	P value	GG/GA N = 129	AA N = 14	P value
Total leukocyte count ($\times 10^3/mm^3$)	6.9 ± 2.5	7 ± 1.2	0.9	8 ± 5.1	7.5 ± 2.4	0.6
ALT (up to 37 IU/mL)	29.3 ± 6.3	30 ± 5.4	0.9	31 ± 5.6	32 ± 6.7	0.4
AST (up to 37 IU/mL)	30.6 ± 8.3	31.3 ± 6.8	0.9	35 ± 7.8	36 ± 9.6	0.08
Total bilirubin (up to 1 mg/dL)	0.7 ± 0.2	0.7 ± 0.2	0.7	0.8 ± 0.2	0.8 ± 0.2	0.2
Direct bilirubin (up to 0.25 mg/dL)	0.2 ± 0.06	0.1 ± 0.05	0.8	0.2 ± 0.06	0.1 ± 0.05	0.5
ALP (up to 150 IU/mL)	108.2 ± 38.9	235.3 ± 30.8	0.02*	233 ± 95.4	277.9 ± 147	0.05*
GGT (up to 30 IU/mL)	37.5 ± 11	37.7 ± 10.4	0.07	37 ± 9.5	34.5 ± 6.7	0.7
Albumin (3.5–5.3 gm/L)	3.9 ± 0.2	3.9 ± 0.3	0.8	3.8 ± 0.2	3.8 ± 0.3	0.7
FBG (up to 100 mg/dL)	91 ± 6.6	93 ± 4.6	0.5	98.4 ± 15.7	108.9 ± 15.3	0.04*
Cholesterol (mg/dL)	147.8 ± 15.4	154.7 ± 10.8	0.5	182.9 ± 37.6	174.4 ± 22.7	0.3
Triglycerides (mg/dL)	144 ± 15.3	138 ± 12.9	0.5	161.3 ± 33	159.7 ± 29.4	0.9
HDL-c (mg/dL)	46 ± 9	43 ± 1.5	0.5	39.4 ± 10	41.7 ± 8.3	0.4
LDL-c (mg/dL)	107.9 ± 11.9	105.7 ± 5.7	0.7	119.8 ± 21.5	126.6 ± 24.4	0.1
AFP (up 10 ng/mL); median (IQR)	5 (3.2–6.8)	4 (3.2–8)	0.7	6.2 (5–7.2)	6.4 (4.8–8)	0.7
Fasting insulin	20.2 ± 7	26.7 ± 3	0.03*	25 ± 6.4	23.9 ± 7.8	0.3
HOMA-IR	4.6 ± 1.7	6 ± 0.9	0.03*	6 ± 1.8	6.4 ± 2	0.5
HOMA IR > 3.5; N (%)	35 (66.3)	6 (100)	0.09	114 (90.5)	13 (92.9)	0.8
Hepatic insulin sensitivity	0.3 ± 0.1	0.2 ± 0.03	0.05*	0.2 ± 0.06	0.2 ± 0.07	0.5
Unhealthy metabolic status; N (%)	4 (5)	0	0.6	113 (89.7)	13 (92.9)	0.7
Leptin level	3.7 (2.2–4)	3.9 (3.6–4.3)	0.6	27 (4–33.8)	29.5 (2.8–35.8)	0.4

Data are shown as mean ± SD unless otherwise mentioned. AFP: Alfa-fetoprotein, ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index, FBG: Fasting blood Glucose; GGT: gamma glutamyl transpeptidase; HDL-c: High density lipoprotein-cholesterol, HOMA-IR: homeostasis model assessment- insulin resistance, IQR: interquartile range; LDL-c: low-density lipoprotein –cholesterol.

* $P < 0.05$ was considered significant. Statistical tests used were using Student's t-test, Mann Whitney U, and by chi-square test.

Table 5 Leptin level in children with overweight/obesity and children with normal weight.

	Non-obese children N = 86			Children with overweight/obesity N = 140		
	Low leptin <3.7 N = 40	High leptin ≥3.7 N = 46	P value	Low leptin <23 N = 65	High leptin ≥23 N = 75	P value
Age in years	12.4 ± 2.5	12.7 ± 2	0.7	11.7 ± 1.8	11.9 ± 2.1	0.6
Sex; N (%)						
[•]*Male*	22 (44)	17 (56.7)	0.7	25 (38.5)	34 (45.3)	0.4
[•]*Female*	28 (56)	13 (43.3)		40 (61.5)	41 (54.7)	
Systolic blood pressure	107.4 ± 6	107.5 ± 7.1	0.9	110.6 ± 7.6	112.5 ± 7.6	0.1
Systolic blood percentile	57.7 ± 20.2	66.8 ± 24	0.06	69.2 ± 23.3	73.3 ± 19.4	0.3
Diastolic blood pressure	66.3 ± 4.4	68.8 ± 5.2	0.02*	67.5 ± 6.1	70.9 ± 8.6	0.01*
Diastolic blood percentile	64.7 ± 14.4	69.8 ± 16.8	0.1	67.8 ± 17.6	73.3 ± 19.7	0.09
Blood pressure values; N (%)						
[•]*Normal*	40 (100)	42 (91.3)	0.2	51 (78.5)	48 (64)	0.2
[•]*Pre-hypertensive*	0	3 (6.5)		7 (10.8)	14 (18.7)	
[•]*Hypertensive*	0	1 (2.2)		7 (10.8)	13 (17.3)	
Weight in kg	38.3 ± 9.8	38.9 ± 10	0.8	57.3 ± 13	69.8 ± 13.5	0.000*
Percentile; median (IQR)	32.3 (6.4–58.9)	28 (4–50)	0.9	92.4 (81.2–97.7)	98.8 (97–99.6)	0.000*
Z score; median (IQR)	−0.5 (−1.5 to 0.2)	−0.6 (−1.7 to 0.01)	0.9	1.4 (0.9–2)	2.3 (1.9–2.7)	0.000*
Height in cm	146.4 ± 10	145.6 ± 12.5	0.7	144 ± 8.7	145.3 ± 10.3	0.4
Percentile; median (IQR)	21 (9.5–44.9)	17.4 (5.6–44)	0.6	20.6 (8–50.4)	37.8 (17–56.7)	0.04*
Z score; median (IQR)	−0.8 (−1.3 to 0.1)	−0.9 (−1.6 to −0.2)	0.6	−0.8 (−1.4 to 0.01)	−0.3 (−0.9 to 0.2)	0.04*
BMI	17.8 ± 3.2	18.4 ± 1.8	0.6	30.9 ± 5	29.9 ± 5.3	0.000*
Percentile; median (IQR)	41.8 ± 31.2	48.1 ± 26.4	0.4	97.6 ± 3	96.4 ± 4.5	0.000*
Z score; median (IQR)	0.09 (−1.7 to 0.6)	−0.09 (−0.5 to 0.7)	0.3	2.3 (1.9–2.4)	2.2 (1.7–2.5)	0.000*
Hemoglobin (g/dL)	11.9 ± 1.6	12 ± 1.1	0.5	11.4 ± 1.5	11.7 ± 1.3	0.4
Total leukocytic count ($\times 10^3/mm^3$)	6.9 ± 2.5	6.7 ± 2.3	0.9	7.4 ± 2.4	8 ± 5.1	0.6

Table 5 (*Continued*)

	Non-obese children N = 86			Children with overweight/obesity N = 140		
	Low leptin <3.7 N = 40	High leptin ≥3.7 N = 46	P value	Low leptin <23 N = 65	High leptin ≥23 N = 75	P value
ALT (up to 37 IU/mL)	29.3 ± 6.3	30 ± 6.3	0.9	30 ± 5.8	31 ± 5.6	0.4
AST (up to 37 IU/mL)	30.6 ± 8.3	30.8 ± 7	0.9	32.3 ± 7.7	35 ± 7.8	0.08
Total bilirubin (up to 1 mg/dL)	0.7 ± 0.2	0.8 ± 0.2	0.7	0.7 ± 0.2	0.8 ± 0.2	0.2
Direct bilirubin (up to 0.25 mg/dL)	0.2 ± 0.06	0.2 ± 0.05	0.8	0.1 ± 0.06	0.2 ± 0.06	0.02*
ALP (up to 150 IU/mL)	108.2 ± 38.9	209.7 ± 55	0.002*	218 ± 85	233 ± 95.4	0.02*
GGT (up to 30 IU/mL)	37.5 ± 11	32 ± 6	0.07	36.9 ± 10.5	37 ± 9.5	0.02*
Albumin (3.5–5.3 gm/L)	3.9 ± 0.2	3.9 ± 0.3	0.8	3.8 ± 0.2	3.8 ± 0.2	0.7
FBG (up to 100 mg/dL)	90 ± 6.3	93.1 ± 5.4	0.02*	98.4 ± 15.7	101.3 ± 15	0.08
Cholesterol (mg/dL)	147.8 ± 15.4	152.5 ± 26.8	0.5	172.4 ± 34.3	182.9 ± 37.6	0.000*
Triglycerides (mg/dL)	144 ± 15.3	147 ± 23.9	0.5	159 ± 32	161.3 ± 33	0.000*
HDL-c (mg/dL)	46 ± 9	44.2 ± 6.8	0.5	41.7 ± 8.8	39.4 ± 10	0.1
LDL-c (mg/dL)	107.9 ± 11.9	106 ± 10	0.7	116 ± 17	119.8 ± 21.5	0.000*
AFP (up 10 ng/mL); median (IQR)	5 (3.2–6.8)	6.2 (3.2–6.8)	0.7	6 (5–7.2)	6.2 (5–7.2)	0.06
Fasting insulin	18.8 ± 6.8	22.6 ± 7	0.005*	23.2 ± 6.8	25 ± 6.4	0.000*
HOMA-IR	4.2 ± 1.6	5.1 ± 1.6	0.005*	5.8 ± 2	6 ± 1.8	0.000*
HOMA IR > 3.5; N (%)	30 (60)	23 (76.7)	0.8	53 (81.5)	74 (98.7)	0.000*
Hepatic insulin sensitivity	0.3 ± 0.1	0.2 ± 0.09	0.4	0.3 ± 0.08	0.2 ± 0.05	0.000*
Unhealthy metabolic status; N	0	4 (8.7)	0.06	58 (89.2)	68 (90.7)	0.5

The median of leptin level for each group was used as cut off to stratify groups. Data are shown as mean ± SD unless otherwise mentioned. AFP: Alfa-fetoprotein, ALT: alanine amino-transferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index, FBG: Fasting blood Glucose; GGT: gamma glutamyl transpeptidase; HDL-c: High density lipoprotein-cholesterol, HOMA-IR: homeostasis model assessment- insulin resistance, IQR: interquartile range; LDL-c: low-density lipoprotein –cholesterol.

* $P < 0.05$ was considered significant. Statistical tests used were using Student's t-test, Mann Whitney U, and by chi-square test.

Table 6 Correlation between Leptin levels with BMI categories and different Genotypes.

	Genotypes		BMI categories	
	GG/GA N = 206	AA N = 20	Normal N = 86	Overweight/obese N = 140
Age	NS	NS	NS	NS
Weight in kg	0.6 (0.000)*	0.6 (0.01)*	NS	0.4 (0.000)*
Weight Z score	0.5 (0.000)*	0.6 (0.004)*	NS	0.5 (0.000)*
Weight percentile	0.5 (0.000)*	0.6 (0.009)*	NS	0.4 (0.000)*
Height in cm	NS	NS	NS	NS
Height Z score	NS	NS	NS	NS
Height Percentile	NS	NS	NS	NS
BMI	0.7 (0.000)*	0.7 (0.002)*	NS	0.5 (0.000)*
BMI Z score	0.5 (0.000)*	0.6 (0.004)*	NS	0.5 (0.000)*
BMI Percentile	0.4 (0.000)*	0.5 (0.02)*	NS	0.4 (0.000)*
Systolic blood pressure	0.2 (0.01)*	NS	NS	0.2 (0.04)*
Systolic blood Percentile	0.2 (0.01)*			NS
Diastolic blood pressure	0.2 (0.009)*	NS	NS	0.2 (0.007)*
Diastolic blood Percentile	0.2 (0.02)*			0.2 (0.03)*
Hemoglobin	NS	NS	NS	NS
Total leukocyte count	NS	NS	NS	NS
ALT	NS	NS	NS	NS
AST	NS	NS	NS	NS
Total bilirubin	NS	NS	NS	NS
Direct bilirubin	NS	NS	NS	NS
ALP	0.2 (0.000)*	NS	NS	0.2 (0.01)*
GGT	0.2 (0.02)*	NS	NS	0.2 (0.02)*
Albumin	NS	NS	NS	NS
FBG	0.2 (0.01)*	NS	NS	NS
Cholesterol	0.6 (0.000)*	0.6 (0.01)*	NS	0.6 (0.000)*
Triglycerides	0.3 (0.000)*	NS	NS	0.3 (0.001)*
HDL-c	−0.2 (0.003)*	NS	NS	NS
LDL-c	0.4 (0.000)*	NS	NS	0.3 (0.000)*
AFP	NS	NS	NS	NS
Fasting insulin	0.4 (0.000)*	NS	NS	0.5 (0.000)*
HOMA-IR	0.4 (0.000)*	0.5 (0.01)*	NS	0.5 (0.000)*
Hepatic insulin sensitivity	−0.4 (0.000)*	−0.5 (0.02)*	NS	−0.4 (0.000)*

Data are shown as r: correlation coefficient (*P. value*). AFP: Alfa-fetoprotein, ALT: alanine aminotransferase; ALP: alkaline phosphatase; AST: aspartate aminotransferase; BMI: body mass index, FBG: Fasting blood Glucose; GGT: gamma-glutamyl transpeptidase; HDL-c: High density lipoprotein-cholesterol, HOMA-IR: homeostasis model assessment- insulin resistance, IQR: interquartile range; LDL-c: low-density lipoprotein −cholesterol, SD: standard deviation.

* $P < 0.05$ was considered significant. The test used was Person correlations.

ing the leptin level, we excluded those with diabetes mellitus receiving insulin, hormonal therapies, or taking long-term steroids. Also, we fix a similar timing for sampling to avoid diurnal variation and uniform the fasting period. Similarly, Eldosouky et al. [27] reported higher leptin levels among their studied children with obesity than in non-obese children. Adult studies showed that subjects with exogenous ''diet-induced'' obesity had high leptin levels than did lean subjects [28,30]. Having high serum leptin in obese subjects usually reflects leptin resistance, based on the fact that leptin decreases food intake. The association between high values of leptin and individuals' BMI was [31].

Among the study groups, no association was detected between leptin and the gender or age of participants. The ages of controls ranged from 5 to 15 years, while ages of cases from 7 to 16 years. In adults, strong correlations were established between leptin levels with the age and gender of cases, which were attributed to its reflection to adiposity and strong correlation with BMI [32,33]. Usually, females have higher leptin than males due to differences in the body-fat distribution and sex hormones [34]. The situation is different in children with considerably variable BMI according to their pubertal stage, age, and sex. Only late in adolescence, the youth with obesity can have similar correlations as adults [35].

In the current study, the FBG and insulin levels were significantly higher among the group with obesity than controls, with a more often unhealthy metabolic state. Our results follow previous reports in children and adults with obesity [28].

In our study group, the systolic blood pressure percentiles were significantly higher among children with obesity. Leptin

values were positively correlated with both systolic and diastolic percentiles in children having GG/GA alleles regardless of their BMI and in obese children as well. Hypertension is a significant risk for the development of cardiovascular diseases in children [7]. Leptin was incriminated in the pathophysiology of cardiovascular disease in cases with obesity, being the cause of inflammation in the adipose tissue [36].

From our results, non-obese children having AA alleles had more insulin resistance and less hepatic insulin sensitivity than those with GG/GA alleles, which was not observed in overweight/obese children. When the association of leptin levels was studied in different alleles regardless of the BMI of children, insulin resistance was significantly associated with high leptin levels in all alleles. In obese children, higher leptin was associated with less hepatic insulin sensitivity, linking together high leptin levels to insulin resistance. Insulin resistance had been observed in subjects with excess serum leptin, a metabolic derangement usually occurring in leptin-deficiency status. Leptin's feedback regulatory pathways [37], its pro-inflammatory functions, its effects on insulin-sensitization, and its regulatory impact on beta-cell mass [38], could explain this phenomenon. Differently, Aboelros et al. [28] reported significant positive correlations between the allele G and studied metabolic disturbances in adults.

Among our studied group, cases with higher leptin levels had significantly higher lipid profiles. Leptin levels were highly correlated with abnormal lipid profile values in children carrying the GG/GA alleles of the rs7799039 SNPs regardless of their BMI and in children with obesity/overweight. Similarly, Aboelros et al. [28] reported significant positive correlations between the allele G and dyslipidemia in adults. On the other hand, Okada et al. [39] reported a lack of associations between lipid profile and leptin/leptin receptor gene polymorphisms in their studied children with obesity. Interestingly, a study on pregnant women reported that the AA allele of G2548A was associated with high TG values than GA/GG [40].

Pediatrics studies regarding leptin gene SNPs were few, and those for the leptin receptor were mainly concerned about Q223R gene polymorphism. Pyrzak et al. [41] reported an association of leptin receptor 223 gene polymorphism and obesity. A Mexican study that included normal and obese adolescents said that GG or GA alleles had higher leptin levels and cardiovascular complications, which was not related to their BMI [4]. Another study for the same SNP reported that GG alleles were a risk factor for obesity in children, and it was statistically correlated with anthropometric parameters and leptin levels [42]. A meta-analysis of adult studies regarding Q223R reported controversial results [12].

Our study limitations included being a single center with a limited number of participants. The power of our study comes from being one among few which study the leptin gene rs7799039 SNPs in children, with its association with the phenotype and metabolic status of the children. Further studies are recommended to study the role of leptin in cases of obesity.

In conclusion: The genotypes distribution of leptin gene rs7799039 SNP was comparable between children with obesity and lean children. Higher leptin levels denoting resistance were significantly associated with higher blood pressure, abnormal lipid profile, and increased insulin resistance, less hepatic insulin, and sensitivity, i.e., unhealthy metabolic status.

Conduction site

The National Research Institute.

Ethical approval

Research ethics committee of the National Research Institute.

Author contributions

- Amal Ahmed Mohamed: idea, study design, share in carrying-out the experiments
- Hoda H. Ahmed: data collection, patient selection
- Sanaa M. ElSadek: prepared the scientific materials
- Rasha S. Mohamed: data collection, literature search
- Reham Y. El-Amir: literature search
- Wafaa Salah: patient selection
- Eman sultan: patient selection
- Dalia M. Abd El-Hassib: share in carrying-out the experiments
- Hanan M. Fouad: data analysis and interpretation, writing the draft of the manuscript

All authors were involved in writing the paper and had final approval of the submitted and published versions.

Competing interests

All authors have no conflict to declare

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