European Journal of Clinical and Experimental Medicine

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Article type: Original Article

Received: 10 July 2026

Accepted: 14 September 2026

Published online: 13 November 2026

eISSN: 2544-1361

Eur J Clin Exp Med

doi:10.15584/ejcem.2026.4.2

ONLINE FIRST Eur J Clin Exp Med

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Association of the *LEP* rs7799039 polymorphism with serum leptin and insulin levels in adults with obesity

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ABSTRACT

Introduction and aim. The single-nucleotide polymorphism (SNP) rs7799039 in the promoter region of the leptin (*LEP*) gene, which affects the expression and level of leptin, remains a significant area of research, especially concerning metabolic disorders like obesity and T2DM. Thus, the current study aims to investigate the relationship between the *LEP* rs7799039 SNP and elevated serum leptin and insulin in obese participants in Najaf, Iraq.

Material and methods. One hundred participants divided into four groups were including in this case-control study. Leptin and insulin levels were measured, and genotyping was performed by real-time polymerase chain reaction (RT-PCR).

Results. Real-time PCR analysis for the *LEP* rs7799039 SNP manifested a tri-allelic variation of the *LEP* rs7799039 polymorphism (G, A, C). The AA genotype has the highest prevalence within all groups (39%), while the GA genotype is the lowest (9%), and they are linked to elevated insulin levels in obese participants compared to controls, but not leptin levels. Regarding the CC genotype carriers, they exhibited the healthiest clinical profile among all participants, with a high percentage in the healthy group (40.9%).

Conclusion. The homozygous AA and heterozygous GA genotypes of the *LEP* rs7799039 variant are associated with elevated insulin levels in obese groups compared to homozygous GG and CC genotypes. Also, insulin rather than leptin was the key metabolic marker in the current study population.

Keywords. insulin resistance, Iraqi population, leptin, obesity, polymorphism

Introduction

Obesity is a chronic disorder recognized for its complexity and multifaceted nature. Obesity is defined as an excessive or abnormal accumulation of fat and categorized by a Body Mass Index (BMI) greater than or equal to 30 kg/m^2.[1] Obesity is multifactorial, resulting from a complex and dynamic interaction among environmental, behavioral, endocrine, and genetic factors, and exceeds the simplistic opinion of an energy imbalance.[2] While the primary mechanism remains a persistent excess of caloric intake over energy expenditure, genetic inheritance significantly influences the direction of this imbalance. Genome-Wide Association Studies (GWAS) have identified more than 100 loci linked with obesity, with genes playing critical roles in the regulation of appetite, satiety, and energy expenditure, such as the fat mass and obesity-associated (*FTO*) gene and polymorphisms in the leptin (*LEP*) and leptin receptor (*LEPR*) genes.[3]

The *LEP* gene, also called the obese gene (*ob*), is located on human chromosome 7 at locus 7q31.3 and is composed of three separated exons by two introns. The promoter region, located upstream of the transcription start site, contains binding sites for various transcription factors that regulate leptin expression in response to metabolic signals.[4,5] The *LEP* gene provides instructions for synthesizing the leptin hormone, an adipocyte-derived polypeptide of 167 amino acids, which results in a mature 16-kDa protein.[6]

The major physiological role of leptin is to act as a vital long-term indicator of the body's energy reserves to the central nervous system (CNS), particularly within the hypothalamus. The levels of circulating leptin correlate with the amount of adipose tissue, and its effects are mediated via the leptin receptor (LepRb), which activates the JAK-STAT signaling pathway in brain nuclei, especially the arcuate nucleus (ARC).[7,8]

Genetic variations or single-nucleotide polymorphisms (SNPs) in the *LEP* gene and its receptor are widely studied as prospective contributors to susceptibility to obesity and related metabolic diseases.[9] One of the most studied SNPs is rs7799039, a G-to-A/C change at position 2548 upstream of the ATG start codon in the *LEP* gene promoter region.

The A allele of rs7799039 is often associated with an increased risk of metabolic dysregulation. For instance, the A allele has been linked to hyperleptinemia, insulin resistance, and a higher risk of T2DM in various populations.[10-12] However, data on the association between *LEP* rs7799039 and obesity-related metabolic alterations in Iraqi populations remain limited, particularly regarding the simultaneous assessment of genotype distribution, circulating leptin, insulin, and insulin resistance across different classes of obesity. Therefore, evaluating these relationships in an Iraqi population may provide population-specific evidence regarding the metabolic relevance of this polymorphism. Therefore, the current study hypothesized that the *LEP* rs7799039 variation may contribute to changes in leptin levels and obesity-related phenotype in the study population.

Aim

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The current study aims to investigate the relationship between the *LEP* rs7799039 SNP and elevated serum leptin and insulin in obese participants in Najaf, Iraq.

Material and methods

Study subjects

The current case-control study was conducted in the department of biochemistry at the College of Medicine, University of Babylon, Iraq. 100 adult participants were recruited in this study from Najaf province, Iraq. The participants of the study were divided into 4 groups depending on the BMI score: healthy (n=22), obese class I (n=27), obese class II (n=28), and obese class III (n=23). This study was reported in accordance with the STROBE guidelines for case-control studies.

Inclusion and exclusion criteria

All the participants who were accepted to participate in the current study are obese and non-obese adults with an average age between 18 and 65 years old. The healthy group consisted of individuals with a BMI of 18–24.9 kg/m^2, while the obese group had a BMI of ≥30 kg/m^2, and was selected from friends, relatives, and medical staff. Exclusion criteria included chronic diseases, autoimmune disease, endocrine disorders, malignancy, pregnancy, surgery, and current use of weight-loss medications.

Ethics considerations

The current study was approved by the Scientific Research Ethics Committee at the College of Medicine, University of Babylon, Iraq (Ref. No. 5-72, on 18/09/2025). The Ethics Committee specifically approved the use of verbal informed consent. Prior to enrollment, the purpose of the study was clearly explained to all participants with their families, and verbal consent was obtained.

Biochemical parameter measurements

Blood samples were obtained after overnight fasting (10–12 hours). Five mL was drawn from each participant and distributed into 3 mL placed in a gel tube for biochemical tests and 2 mL placed in two EDTA tubes, one for HbA1c measurement and the other one for DNA extraction.

Biochemical tests included fasting serum insulin, fasting blood sugar (FBS), total cholesterol (Ch), high density lipoprotein (HDL), triglycerides (TG), and glycated hemoglobin (HbA1c) measured by enzymatic colorimetric methods on the Mindray BS-230 and CL-900i analyzer (Mindray BioMedical, China). Very low-density lipoprotein (VLDL) and low-density lipoprotein (LDL) levels were calculated using the Friedewald formula. Leptin level was measured using commercial ELISA kits (cat. No. SL1052Hu, SunLong, China), following the manufacturer's instructions. This assay has sensitivity of 12 pg/ml, a detection range of 36–2000 pg/ml, and inter- and intra-assay coefficients of variation (CV) of <12% and

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<10%, respectively. The Homeostasis Model Assessment of Insulin Resistance (HOMA-IR) index was used to assess insulin resistance following this equation: Fasting Glucose (mg/dL) × Fasting Insulin (μlU/mL) /405. Anthropometric data, including waist circumference (WC) and height and weight, were collected to calculate BMI.

DNA extraction and genotype analysis

The DNA was extracted from whole blood by using the Magen® Hi-Pure Universal DNA kit (cat. No. D301802, Magen, China) and following the manufacturer's instructions. Genotype analysis was conducted using the real-time polymerase chain reaction (RT-PCR). The primer and probe sequences used in the present study are shown in Table 1. The PCR mixture was executed in 20 μL volumes comprising from 10 μl master mix (GoTaq® Green Master Mix, Promega, USA), 2 μl DNA template, 2 μl from each primer, and 2 μL from each probe. Thermal cycling was performed on a real-time PCR Mx3005 STRATAGENE/USA thermocycle. Real-time PCR optimization was done as a first step by determining the optimum annealing temperature (58°C). After that, the following program was set in the thermocycler to amplify the target DNA fragments as shown in Table 2. The G and A alleles were detected through direct specific primers and probes, while the C allele was detected indirectly via a probe drop-out or negative selection mechanism, where individuals with the C allele showed a complete lack of fluorescence signaling (flat amplification curves) in both the HEX and FAM points, despite strong amplification, which was confirmed by the internal reference control. Furthermore, the C allele is documented in the NCBI dbSNP database but remains less studied compared to the A allele.[13] However, our results should be considered preliminary and interpreted with caution until Sanger sequencing or another independent genotyping method is available.

Table 1. Primer and probe characteristics

Characteristics	Sequence (5′ - 3′)	Length	Tm (°C)	GC content (%)	Reporter/ quencher
Forward primer	AGGGCCAGGTAGAAAACA	18	58.9	50%	-
Reverse primer	TCTCAGCACTTAGGGAGACT	20	59.6	50%	-
Probe I	AGGATCAGCGCAACCCTG	18	63.1	61	FAM-G/ BHQ1
Probe II	AGGATCAGTGCAACCCTGT	19	63.3	53	HEX-A/ BHQ1

Table 2. PCR thermocycler conditions

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PCR steps	Temperature	Time	Cycles
Initial denaturation	94 °C	10 min	1
Denaturation	94 °C	20 sec	40
Annealing	58 °C	1 min	
Extension	72 °C	20 sec	
Final extension	72 °C	5 min	1

Statistical analysis

JASP version 0.98.1 and SPSS version 26.0 software for windows were used to perform statistical analysis. The mean and standard deviation were used to express data. The normality of data distribution was tested by using Shapiro-Wilk tests. A p-value (<0.05) was considered statistically significant. Because the data were normally distributed, a one-way ANOVA test and standard post-Hoc test Tukey-correction comparisons were used, respectively. Also, homogeneity of variance was evaluated using Levene's test. The post-hoc power analyses were calculated by G*Power software (version 3.1.9.7). For insulin, the omega squared (ω^2) of ANOVA test was 0.15, which compatible to Cohen's f=0.42, and the resultant power was 94%, indicating the study was sufficiently powered to detect insulin differences in groups. While the leptin omega squared was 0.05, which is compatible with Cohen's f=0.23, and the resultant power was 45%, indicating the study was underpowered to detect small effects. Pearson correlation analysis was conducted to determine the association between variables. Additionally, separate binary logistic regression was conducted to compare healthy vs. each obesity class (I-III) to determine whether the biomarkers retained independent predictive value after adjustment. Age and sex were included as covariates in all binary logistic regression analyses to account for their potential confounding effect. Genetic Analysis in Excel (GenAlEx) software version 6.5 from the Australian National University, Australia, was used to calculate the LEP G2548A SNP allele and genotype frequencies. The odds ratio was calculated with a 95% confidence interval. A p-value<0.05 was considered statistically significant.

Results

Anthropometric and biochemical data analysis

Anthropometric and biochemical data are shown in Table 3. The results of the chi-square test showed that sex distribution did not significantly differ across groups (X^2=7.4, p=0.06). Obese individuals manifested higher BMI, WC, insulin, and HOMA-IR levels compared to healthy subjects, while there was no statistically significant difference in leptin and FBS levels between study groups (p=0.2) with the small effect size of leptin (ω^2=0.05, 95% CI [0.001–0.181]), indicating limited ability to detect differences of this magnitude. Regarding lipid profiles, obese individuals had low HDL and high TG, Ch, LDL, and VLDL levels compared to healthy controls (p<0.01). The distribution of the history of obesity among the

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participants is illustrated in Figure 1. A chi-square test result manifested that history of obesity distribution did significantly differ among groups (X^2=42.5, p<0.01).

Table 3. Anthropometric and biochemicals data of study groups*

Parameters	Obese sub-classes				
	Healthy (n=22)	**Class I (n=27)**	**Class II (n=28)**	**Class III (n=23)**	**p**
Sex (M/F)	12/10	17/10	15/14	6/17	0.06
Age (y)	27.7±6.17	27.9±4.08	35.82±10.09	39.26±10.99	<0.01
BMI (kg/m^2)	22.54±1.51	32.0±1.44	35.91±0.59	41.94±2.19	<0.01
WC (cm)	81.91±8.19	105.15±9.04	109.6±10.7	119.1±15.59	<0.01
FBS (mg/dL)	92.16±7.72	89.88±10.94	97.19±14.3	94.62 ± 16.1	0.22
Insulin (µIU/mL)	8.47±2.54	15.26±7.78	15.05±7.01	16.33±7.34	<0.01
HOMA-IR	1.92±0.61	3.6±2.03	4.1±2.14	4.01±2.05	<0.01
HbA1c (%)	5.50±0.29	5.72±0.46	5.83±0.62	6.04±0.47	<0.01
Ch (mg/dL)	144.8±19.4	170.63±35.45	167.31±25.56	179.2±37.6	<0.01
HDL (mg/dL)	45.9±11.45	41.38±7.71	36.74±6.08	36.6±9.13	<0.01
LDL (mg/dL)	81.43±16.74	104.3±19.07	109.81±20.55	118.9±30.7	<0.01
VLDL (mg/dL)	15.97±6.74	20.91±9.6	17.47±4.38	24.86±11.9	<0.01
TG (mg/dL)	79.8±33.7	104.5±47.9	87.36±21.9	124.3±59.5	<0.01
Leptin (pg/ml)	687.1±195.6	790.2±163.9	748.3±175.6	767.07±166.7	0.22

* Obese sub-classes: class I (BMI: 30–34.9 kg/m^2), class II (BMI: 35–39.9 kg/m^2), class III (BMI ≥40 kg/m^2); BMI – body mass index, WC – waist circumference, FBS – fasting blood sugar, Ch – total cholesterol, HDL – high-density lipoprotein, LDL – low-density lipoprotein, VLDL – very low-density lipoprotein, TG – triglyceride

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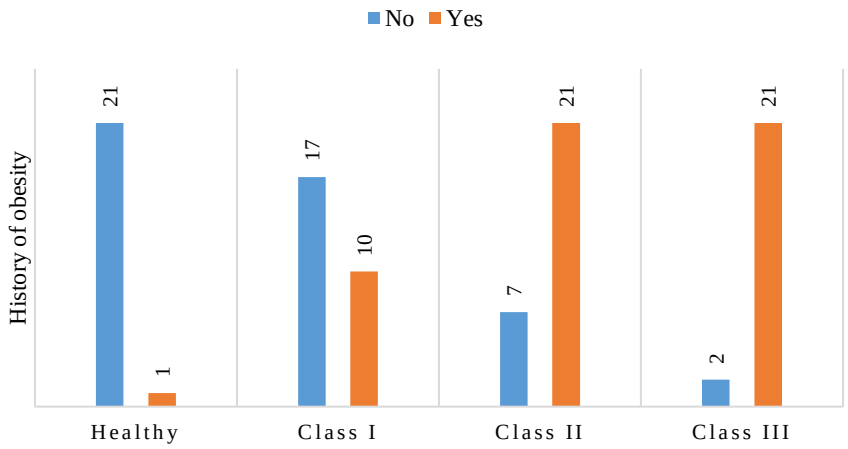

Fig. 1. The distribution of family history in the study groups

Pearson correlation analyses

Table 4 presents Pearson correlation analyses between leptin and insulin and obesity-related biomarkers. Leptin shows a weak positive correlation with other markers, while there is a conflicted correlation between insulin and other markers. A strong positive correlation between insulin and HOMA-IR (r=0.89, p<0.01) and a moderate with BMI and WC. While a negative correlation has been shown between insulin and age, HDL, and HbA1c. Furthermore, a very weak correlation is present between leptin and insulin (r=0.004, p=0.97).

Table 4. Pearson correlation analysis of the leptin and insulin*

Variables	Insulin r (p)	Leptin r (p)
Age	−0.07 (0.47)	0.04 (0.70)
BMI	0.37*** (<0.01)	0.18 (0.07)
WC	0.39*** (<0.01)	0.15 (0.13)
LDL	0.02 (0.85)	0.05 (0.62)
VLDL	0.18 (0.08)	0.004 (0.96)
HDL	−0.2# (0.05)	0.17 (0.09)
Ch	0.02 (0.83)	0.07 (0.49)
TG	0.18 (0.08)	0.004 (0.96)
HOMA-IR	0.89*** (<0.01)	−0.05 (0.65)
FBS	0.03 (0.75)	−0.03 (0.73)
HbA1c	−0.004 (1.0)	0.02 (0.84)
Insulin	–	0.004 (0.97)

* # – p <0.05, *** – p <0.001

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Figure 2 illustrates the correlation analyses through scatter plots with fitted regression lines. The plots demonstrate a moderate positive correlation between BMI and insulin ($p<0.01$) and a weak positive correlation with leptin ($p=0.07$). Also, the plot demonstrates a strong positive association between insulin and HOMA-IR. While there is no correlation between insulin and leptin in this study ($p=0.97$).

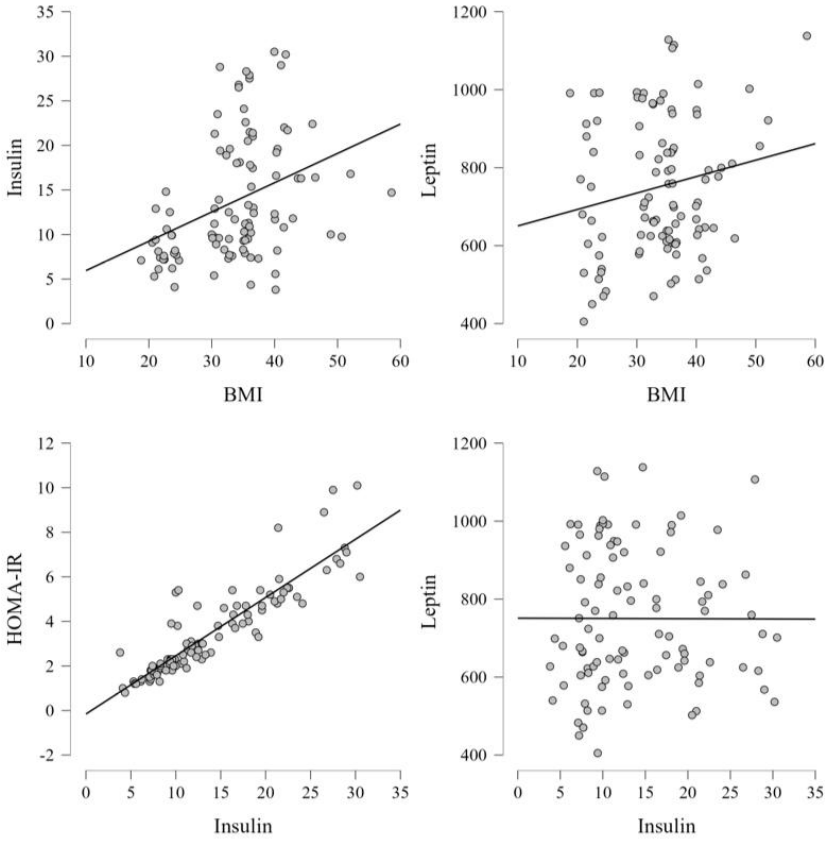

Fig. 2. Correlation plots of leptin and insulin

Multivariable logistic regression of leptin and insulin

The binary logistic regression analysis was conducted to assess the independent predictive value of leptin and insulin for obesity classes versus the healthy group, as shown in Table 5. Insulin has appeared as a consistent and significant predictor across all obesity classes ($p<0.01$). Also, age was significant in the comparison of obesity class III versus the healthy group ($p=0.024$). In contrast, leptin was not significant in any models ($p>0.13$). Variance inflation factors (VIFs) were all <5, indicating no multicollinearity concerns. BMI was not included as an adjustment factor in regression models due to groups' classification being defined by BMI thresholds.

Table 5. Multivariable logistic regression of leptin and insulin*

Healthy vs. class I				
Variables	**Beta**	**OR (95% CI)**	**p**	**VIF**
Leptin	0.003	1.003 (0.999–1.007)	0.160	1.054
Insulin	0.402	1.495 (1.107–2.019)	0.009[#]	1.523
Age	0.051	1.052 (0.971–1.141)	0.216	1.116
Sex (M)	1.474	4.365 (0.710–26.826)	0.112	1.56
Healthy vs. class II				
Variables	**Beta**	**OR (95% CI)**	**p**	**VIF**
Leptin	0.003	1.003 (0.999–1.007)	0.137	1.093
Insulin	0.318	1.374 (1.105–1.709)	0.004	1.039
Age	0.070	1.073 (0.992–1.160)	0.079	1.101
Sex (M)	0.241	1.272 (0.298–5.424)	0.745	1.025
Healthy vs. class III				
Variables	**Beta**	**OR (95% CI)**	**p**	**VIF**
Leptin	0.0003	1.000 (0.995–1.005)	0.906	1.181
Insulin	0.393	1.481 (1.111–1.974)	0.007	1.493
Age	0.115	1.122 (1.015–1.240)	0.024	1.490
Sex (M)	-0.860	0.423 (0.065–2.747)	0.367	1.102

* [#] – $p<0.05$, OR – odds ratio, CI – confidence interval, VIF – variance inflation factor

Genotype and allele frequencies of LEP G2548A polymorphism

Real-time PCR analysis for the *LEP* rs7799039 SNP manifested a tri-allelic variation of the *LEP rs7799039* polymorphism (G, A, C), resulting in six expected and four observed genotypes within the study population (wild-homozygous GG, mutant-homozygous AA, mutant-homozygous CC, and heterozygous GA). The genotype and allele frequencies are shown in Table 6 and 7 and in Figure 3 and 4. The homozygous AA genotype shows the highest total proportion in all groups (39%), while the heterozygous GA shows the lowest total proportion (9%). The heterozygous GA genotype was absent in the healthy group, which requires Haldane-Anscombe correction to allow odds ratio estimation.

Regarding the C allele in the present study, it was highly prevalent in healthy controls (40.9%) and progressively decreased in class III obesity (17.4%). Additionally, the C allele variant has a lower OR in both class I and II obesity compared to controls, with significantly reduced frequency in class III (OR=0.23, p=0.01). Thus, this inverse association progressively strengthened with increasing BMI, as shown in Figure 4.

The detection of the C allele was carried out indirectly via a probe drop-out or negative selection mechanism, where individuals with the CC genotype showed a complete lack of fluorescence signaling in

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both the HEX and FAM points, despite strong amplification, which was confirmed by the internal control. Furthermore, the C allele is documented in the NCBI dbSNP database but remains less studied compared to the A allele.[13] However, our results should be considered preliminary and interpreted with caution until confirmatory sequencing is available.

Table 6. Genotype frequencies of the *LEP* re7799039 SNP among study groups*

Groups	Genotypes			
	GG	**AA**	**GA**	**CC**
	No. (%); OR (95% CI); p	**No. (%); OR (95% CI); p**	**No. (%); OR (95% CI); p**	**No. (%); OR (95% CI); p**
Healthy (n=22)	5 (22.73%)	8 (36.36%)	0	9 (40.91%)
Class I (n=27)	6 (22.22%)	9 (33.33%) 0.94 (0.20–4.29); 1.00	4 (14.81%); 7.50 (0.32–175.7); 0.23	8 (29.63%); 0.74 (0.16–3.39); 1.00
Class II (n=28)	6 (21.43%)	14 (50.0%); 1.46 (0.34–6.35); 0.71	2 (7.14%); 4.17 (0.16–108.6); 0.48	6 (21.43%); 0.56 (0.12–2.68); 0.69
Class III (n=23)	8 (34.78%)	8 (34.78%); 0.62 (0.14–2.76); 0.71	3 (13.04%); 4.38 (0.18–103.8); 0.50	4 (17.39%); 0.28 (0.05–1.41); 0.23
Total (100)	25 (25%)	39 (39%)	9 (9%)	27 (27%)

***** The odds ratio for the GA genotype utilizes a Haldane-Anscombe correction due to the zero-count in healthy control group, resulting in a wide confidence interval



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Fig. 3. Percentage of genotype frequencies among the study groups

Table 7. Allele frequencies of the *LEP* rs7799039 SNP among the study groups

Groups	Alleles		
	G	**A**	**C**
	No. (%); OR (95% CI); p	**No. (%); OR (95% CI); p**	**No. (%); OR (95% CI); p**
Healthy (n=22)	10 (22.7%)	16 (36.3%)	18 (40.9%)
Class I (n=27)	16 (29.6%)	22 (40.7%); 0.86 (0.31–2.38); 0.8	16 (29.6%); 0.56 (0.20–1.57); 0.3
Class II (n=28)	14 (25.0%)	30 (53.5%); 1.34 (0.49–3.69); 0.61	12 (21.4%); 0.48 (0.16–1.42); 0.27
Class III (n=23)	19 (41.3%)	19 (41.3%); 0.62 (0.23–1.72); 0.4	8 (17.4%); 0.23 (0.08–0.73); 0.01
Total (n=100)	59 (29.5%)	87 (43.5%)	54 (27%)

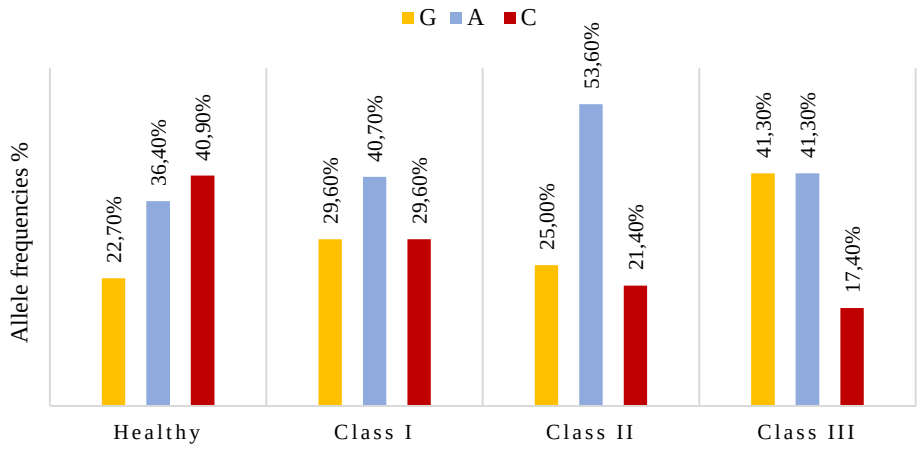

Fig. 4. Percentage of allele frequencies among the study groups

Hardy-Weinberg Equilibrium (HWE) Analysis of rs7799039 SNP

The HWE analysis of the tri-allelic *LEP* rs7799039 SNP has shown significant deviation in all study groups, as illustrated in Table 8. High consanguineous marriage rates in several Iraqi populations and population stratification often reduce heterozygous frequency and increase homozygous genotype and may plausibly

contribute to the observed pattern,[14,15] although the data available does not permit determination of the specific mechanism responsible for the deviation. Consequently, the genetic findings should be interpreted with caution and require validation in independent cohorts using confirmatory genotyping approaches. The absence of GA heterozygous genotype in the healthy group may therefore reflect underlying genetic patterns in the Iraqi population rather than solely methodological error.

However, the predominance of the A allele across groups suggests enrichment in the Iraqi cohort, consistent with regional studies reporting leptin variants as contributors to metabolic dysregulation. Thus, while HWE deviation in controls warrants caution, interpretation of these results within the regional genetics landscape strengthens the relevance of the present findings.[16,17]

Table 8. HWE analysis of the *LEP* rs7799039 SNP*

Groups	Genotype counts (Observed)				Allele counts (Observed)			HWE test metrics	
	GG	GA	AA	CC	G	A	C	Chi-Square (x^2)	p
Healthy (n=22)	5	0	8	9	10	16	18	44.0	<0.001
Class I (n=27)	6	4	9	8	16	22	16	35.7	<0.001
Class II (n=28)	6	2	14	6	14	30	12	45.5	<0.001
Class III (n=23)	8	3	8	4	19	19	8	33.7	<0.001

* The heterozygous GC and AC genotypes were not observed in this study, deviations are noted as p<0.05.

Anthropometric and biochemical features among LEP rs7799039 SNP genotypes

Table 9 shows the anthropometric and biochemical features across the genotypes of the *LEP* rs7799039 SNP. Each parameter is examined separately across genotypes, and the p-value of a one-way ANOVA is obtained. There was no statistically significant association between the genotypes of the *LPE* rs7799039 SNP and waist circumference, leptin, FBS, HbA1c, HOMA-IR, LDL, VLDL, HDL, Ch, and TG levels. While there is a significant association between genotypes and BMI and insulin (p=0.02 and p=0.04, respectively). Clinically, individuals with the AA and GA genotypes exhibited higher levels of leptin, insulin, HOMA-IR, BMI, and WC compared to individuals with the GG and CC genotypes. While there were no differences between other parameters.

Table 9. Clinical and biochemical characteristics among the *LEP* genotypes*

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Parameters	GG (n=25)	AA (n=39)	GA (n=9)	CC (n=27)	p
Age	34.5±9.6	31.2±8.8	37.4±13.5	36.6±10.8	0.12
WC	105.36±17.05	106.8±15.6	105.5±10.3	98.4±18.1	0.22
BMI	34.3±7.45	34.9±3.86	35.7±4.29	30.3±6.98	0.02
Leptin	726.8±165.6	742.7±166.9	754.2±135.04	724.4±169.6	0.95
Insulin	12.75±5.76	14.58±8.22	16.77±3.36	10.45±3.87	0.04
HOMA-IR	3.1±1.3	3.42±2.07	4.4±1.24	2.7±1.35	0.06
FBS	92.5±8.75	91.86±11.14	97.4±21.47	91.82±9.96	0.63
HbA1c	5.92±0.48	5.61±0.5	5.9±0.62	5.66±0.29	0.06
LDL	104.83±24.4	101.9±24	111.6±16.94	102.42±24.84	0.72
VLDL	18.56±6.41	17.86±7.36	24.14±8.58	18.24±8.24	0.26
HDL	37.2±6.42	40.21±8.84	38.76±10.3	40.05±9.5	0.6
Ch	164.6±30.09	162.8±30.06	168.13±32.77	165.3±30.07	0.97
TG	92.8±32.05	89.3±36.78	120.7±42.9	91.2±41.2	0.26

***** Data are presented as mean (±SD), as all parameters are normally distributed, a one-way ANOVA test with a p-value <0.05 is considered significant

Discussion

The rs7799039 polymorphism of the human leptin promoter region has recently been associated with several metabolic issues, like obesity, T2DM, hyperleptinemia, and reproductive disorders.[18,19] Data about the rs7799039 SNP differ among the populations studied, the size of the sample, gender, and age, therefore, the present study aimed to investigate the effect of the *LEP* rs7799039 SNP on leptin and insulin levels in obese individuals.

Generally, obese individuals have more fat mass (high BMI), resulting in high circulating leptin levels, which acts as a long-term energy-storage signal.[20] In the present study, leptin levels were different between groups, with 687.1±195.6 in healthy individuals and 767.07±166.7 in class III obese individuals, as shown in Table 3. This difference did not attain statistical significance (p=0.2), which may be interpreted as a speculative observation attributable to small sample size or variability within groups. Furthermore, the small effect size of leptin (ω^2=0.05, 95% CI [0.001–0.181]) indicates limited ability to detect differences of this magnitude; thus, the absence of statistical significance for leptin cannot be taken as evidence of no difference between groups; therefore, future studies with larger samples are needed to clarify the true role of leptin.

Our results revealed that insulin, HOMA-IR, and HbA1c levels were high in obese groups compared to controls, suggesting a clear state of insulin resistance, a key contributor to the pathophysiology of obesity.[21-23]

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Although sex distribution did not differ significantly between groups (p=0.06), the leptin levels differ clinically between men and women. This increase of leptin levels is due to the quantity and distribution of adipose tissue, as well as the nature of the hormonal state in females and males.[24] Women typically have more body fat than men and more stored fat in subcutaneous tissue compared to visceral fat in men. Consequently, there is a high circulating level of leptin, two to three times in women compared to men.[25] Furthermore, estradiol stimulates leptin levels in women, while testosterone inhibits them in men.[26]

The absence of a significant linear correlation between serum leptin and insulin levels (r=0.004, p=0.97) was important finding of the current study. Although leptin and insulin participate in reciprocal metabolic signaling and elevated leptin is associated with insulin resistance, circulating levels of these hormones are regulated by multiple overlapping but non-identical mechanisms.[27,28] Leptin level is strongly influenced by adipose tissue mass, sex, nutritional status, and leptin sensitivity, whereas fasting insulin reflects the balance between insulin secretion and insulin sensitivity.[29-31] Consistent with the different mechanisms of leptin-insulin interaction with adiposity, BMI in our study correlated significantly with insulin (r=0.37, p<0.01), but its correlation with leptin was weak and did not reach statistical significance (r=0.18, p=0.07). Furthermore, leptin levels did not differ among the genotype groups (p=0.95), whereas insulin concentrations differed significantly (p=0.04). Therefore, the absence of a leptin–insulin correlation may reflect the complex regulation of these hormones and the fact that fasting serum concentrations do not necessarily represent tissue-level leptin sensitivity or insulin action.[28,31]

Our regression analysis indicates that insulin is the metabolic variable most consistently associated with obesity status in the current study. Insulin was independently associated with higher odds of class I, II, and III obesity, with ORs ranging from 1.374 to 1.495, whereas leptin was not significantly associated with any obesity class. This finding was consistent with the significant increase in insulin levels across obesity classes and the positive correlation between BMI and insulin observed in our study; in return, leptin did not differ significantly across groups and showed a weak association with BMI. Therefore, insulin demonstrated a more consistent association with obesity status in the present cohort.

Clinically, the homozygous AA genotype carriers showed severe physical adiposity, recording the highest mean of BMI (34.9±3.86 kg/m^2), waist circumference (106.8±15.6 cm), and exhibited high levels of leptin (742.7±166.9 pg/mL), insulin (14.58±8.22 μIU/mL), and HOMA-IR (3.42±2.07) compared to the homozygous GG genotype carriers, which massively increases the risk for insulin resistance and cardiovascular disease.[10,32] El-Shoura confirmed that the mutant A allele is a primary driver of disrupted insulin signaling and increasing the risk of T2DM.[35] Other studies confirmed that the *LEP* rs7799039 SNP association with hyperleptinemia, hyperinsulinemia, and insulin resistance, which agrees with the results of the present study.[34-37] Also, Iraqi population studies reported an association of *LEP* and *LEPR* polymorphisms with serum leptin levels and risk of T2DM development.[17,18] On the other hand, several studies conflicted with these results, where the GG genotype of the *LEP* rs7799039 SNP was associated

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with a high leptin level compared to the AA genotype.[33,38] These discrepancies may reflect differences in population structure, ethnic background, sample size, or genotyping methodology.

Despite the depletion of the heterozygous GA genotype in the present study which may result from many factors like the high prevalence of consanguineous marriages in Iraqi society, population stratification, or the small sample size, individuals with the heterozygote GA genotype show highest mean levels of fasting serum leptin, insulin, FBS, TG, VLDL, LDL, HOMA-IR, and BMI when compared to other genotypes. Post-hoc Tukey-corrected comparisons showed that GA genotype individuals have higher insulin levels and HOMA-IR score compared to CC genotype individuals ($p=0.05$). These results consistent with many studies that have investigated the relationship between AA and GA genotypes and elevated leptin, insulin, and other biomarkers.[11,35,36] However, the absence of the GA genotype in the healthy group produced sparse data and wide confidence intervals, which highlight the instability of the data; thus, the results must be interpreted with caution until a study with a larger sample size is performed to confirm the results related to allele distribution and HWE deviation.

Individuals with the homozygous CC pattern demonstrate the favorable clinical profiles across all groups. These individuals exhibited the lowest mean BMI (30.3±6.98 kg/m^2), WC (98.4±18.1 cm), fasting insulin (10.45±3.87 μIU/mL), HOMA-IR (2.7±1.35), and serum leptin (724.4±169.6 pg/mL). Furthermore, the emergence of the CC genotype may suggest a unique structural variant within the Iraqi gene pool. These findings strongly warrant future validation through whole-genome sequencing or targeted Sanger sequencing to map this putative genetic architecture in the broader Iraqi population.

Study limitations

The current study has many limitations. First, the relatively small sample size (n=100, divided into four groups) reduced the statistical power to detect small effects, particularly for leptin differences. Therefore, the non-significant leptin results should be interpreted cautiously, and there is an important necessity to validate the results in larger and multi-center groups. Second, group classification was based on BMI thresholds, and BMI was not included as an adjustment factor in regression models because it defines the outcome categories and would introduce collinearity. Also, despite age being statistically adjusted for regression models, the study groups were not age-matched, and residual confounding cannot be fully excluded. Third, the reported C allele is preliminary and was not validated by Sanger sequencing or another method. While such validation would strengthen confidence in the genotyping results, time and cost constraints prevented additional sequencing; subsequently, the findings related to this allele should be interpreted with caution until confirmatory sequencing is available. Fourth, genotype distributions deviated significantly from HWE, raising concerns about genotyping error, population stratification, or selection bias. Also, the absence of heterozygotes for the C allele further restricts interpretation of allele effects. These limitations highlight that our genetic findings are preliminary and require replication in larger,

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independent cohorts with validated methods. Fifth, our study did not collect data on physical activity, dietary habits, or socioeconomic status. These unmeasured variables are important determinants of obesity and may influence genotype–phenotype associations. Finally, the case-control design restricts causal inference; therefore, prospective studies are required to clarify temporal relationships between biomarkers, genetic variants, and obesity progression.

Conclusion

Insulin emerges as the most consistent metabolic predictor of obesity in the current study, showing significant variation across genotypes. The homozygous AA and heterozygous GA genotypes of the *LEP* rs7799039 variant are associated with elevated insulin levels in obese groups compared to homozygous GG and CC genotypes. Leptin concentration did not differ significantly among genotypes, underscoring insulin rather than leptin as the key metabolic marker in the current study population. The emerging C allele was associated with comparatively lower values in many clinical and metabolic parameters; however, these observations should be considered preliminary and require confirmation using validated genotyping approaches. Furthermore, future studies need to include an expanded sample size to strengthen the statistical power and generalizability of findings related to the *LEP* rs7799039 polymorphism and obesity, additionally, to explore novel leptin SNPs in the Iraqi population.

Declarations

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

Conceptualization, A.A.S. and M.F.S.; Methodology, A.A.S, M.F.S. and S.A.A.; Software, A.A.S.; Validation, A.A.S, M.F.S. and S.A.A.; Formal Analysis, A.A.S.; Investigation, A.A.S. and S.A.A.; Resources, A.A.S.; Data Curation, A.A.S.; Writing – Original Draft Preparation, A.A.S.; Writing – Review & Editing, A.A.S, M.F.S. and S.A.A.; Visualization, A.A.S; Supervision, M.F.S. and S.A.A.; Project Administration, M.F.S. and S.A.A.

Ethics approval

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The current study was approved by the Scientific Research Ethics Committee at the College of Medicine, University of Babylon, Iraq (Ref. No. 5-72, on 18/09/2025).

Data availability

The datasets generated during and analyzed during the current study are available from the corresponding author on reasonable request.

Use of AI and AI-assisted technologies in the writing process

The AI tools have been used to improve language and check grammar where necessary.

References

1. Salam MM, Yousuf R, Salam MW, Haque M. Obesity and overweight: A global public health issue. *Adv Hum Bio*. 2023;13(1):154-156. doi:10.4103/aihb.aihb_106_22
2. Masood B, Moorthy M. Causes of obesity: a review. *Clin Med (Lond)*. 2023;23(4):284-291. doi:10.7861/clinmed.2023-0168
3. Sun C, Kovacs P, Guiu-Jurado E. Genetics of Obesity in East Asians. *Front Genet*. 2020;11:575049. doi:10.3389/fgene.2020.575049
4. Dornbush S, Aeddula NR. Physiology, leptin. *PubMed*. https://www.ncbi.nlm.nih.gov/books/NBK537038/. Updated April 10, 2023. Accessed July 10, 2026.
5. Casado ME, Collado-Pérez R, Frago LM, Barrios V. Recent advances in the knowledge of the mechanisms of leptin physiology and actions in neurological and metabolic pathologies. *Int J Mol Sci*. 2023;24(2):1422-1422. doi:10.3390/ijms24021422
6. Ortega-López GI, Reyes-Espinosa F, López-Y-López VE, Benítez-Cardoza CG. Bioinformatic Analysis of the Leptin–Ob-R Interface: Structural Modeling, Thermodynamic Profiling, and Stability in Diverse Microenvironments. *Int J Mol Sci*. 2025;26(14):6955. doi:10.3390/ijms26146955
7. Liu Z, Xiao T, Liu H. Leptin signaling and its central role in energy homeostasis. *Front Neurosci*. 2023;17:1238528. doi:10.3389/fnins.2023.1238528
8. LeDuc CA, Skowronski AA, Rosenbaum M. The role of leptin in the development of energy homeostatic systems and the maintenance of body weight. *Front Physiol*. 2021;12:789519. doi:10.3389/fphys.2021.789519
9. Penes ON, Weber B, Pop AL, et al. Gene Polymorphisms LEP, LEPR, 5HT2A, GHRL, NPY, and FTO-Obesity Biomarkers in Metabolic Risk Assessment: A Retrospective Pilot Study in

ONLINE FIRST Eur J Clin Exp Med

Overweight and Obese Population in Romania. *Cardiogenetics*. 2024;14(2):93-105. doi:10.3390/cardiogenetics14020008

10. Sabi EM, Bin Dahman LS, Mohammed AK, Sumaily KM, Al-Daghri NM. -2548G> A LEP polymorphism is positively associated with increased leptin and glucose levels in obese Saudi patients irrespective of blood pressure status. *Medicina*. 2022;58(3):346. doi:10.3390/medicina58030346
11. Mohsin NN, Aljanabi AA. The Association of LEP G2548A Polymorphism with Type 2 Diabetes Mellitus in the Iraqi Population. *Int J Med Health Res*. 2025;18;3(3):73-80. doi:10.61424/ijmhr.v3i3.395
12. Martínez-Lara CM, Casas-Avila L, Ramos-García ML, et al. Associations and interactions of clinical and genetic variables for type 2 diabetes mellitus in the Mexican population. *Hum Nutr Metab*. 2025;40:200301. doi:10.1016/j.hnm.2025.200301
13. Reference SNP (rs) Report: rs7799039. Nih.gov. https://www.ncbi.nlm.nih.gov/snp/rs7799039#clinical_significance. Accessed 10 July 2026.
14. Hawi ZA, Pugsley K, Namipashaki A, Samarrai W, Hawi Z. A review of consanguinity in the Iraqi population: A call to action to prevent a future health catastrophe. *Ibn AL-Haitham Journal for Pure and Applied Sciences*. 2024;37(2):64-70. doi:10.30526/37.2.3472
15. El Goundali K, Chebabe M, Zahra LF, Hilali A. The Determinants of Consanguineous Marriages among the Arab Population: A Systematic Review. *Iran J Public Health*. 2022;51(2):253-265. doi:10.18502/ijph.v51i2.8679
16. Almyah MK, Albadran AI. Screening of LEP gene polymorphisms as a risk factor for obesity and type 2 diabetes in Iraqis. *Mol Biol Res Commun*. 2019;8(4):156-165. doi:10.22099/mbrc.2019.34274.1423
17. Amhe NH, Al-Fahdawi FH, Gheisarzadeh A, Hatamnia AA. The Polymorphism Study of LEP and LEPR Genes in Association of its Serum Level in Iraqi Patients with Type 2 Diabetic. *Pakistan Journal of Medical and Health Sciences*. 2022;16(4):926. doi:10.53350/pjmhs22164926
18. Picó C, Palou M, Pomar CA, Rodríguez AM, Palou A. Leptin as a key regulator of the adipose organ. *Rev Endocr Metab Disord*. 2022;23(1):13-30. doi:10.1007/s11154-021-09687-5
19. Kiyani-Afzali E, Forghani F, Jahantigh D, Ghazaey-Zidanloo S, Rezaei M, Taheri M. Leptin G2548 A and Leptin Receptor Q223R Gene Polymorphisms are Associated with an Increased Risk of Endometriosis. *Bratisl Lek Listy*. 2025;126(8):2024-2036. doi:10.1007/s44411-025-00186-6
20. Misch M, Puthanveetil P. The head-to-toe hormone: leptin as an extensive modulator of physiologic systems. *Int J Mol Sci*. 2022;23(10):5439. doi:10.3390/ijms23105439
21. Kong Y, Yang H, Nie R. Obesity: pathophysiology and therapeutic interventions. *Mol Biomed*. 2025;6(1):25. doi:10.1186/s43556-025-00264-9

ONLINE FIRST Eur J Clin Exp Med

22. Ullah MI, Tamanna S. Obesity: clinical impact, pathophysiology, complications, and modern innovations in therapeutic strategies. *Medicines*. 2025;12(3):19. doi:10.3390/medicines12030019
23. Wondmkun YT. Obesity, insulin resistance, and type 2 diabetes: associations and therapeutic implications. *Diabetes Metab Syndr Obes*. 2020;13:3611-3616. doi:10.2147/dmso.s275898
24. Nikolettos K, Vlahos N, Pagonopoulou O, et al. The association between leptin, adiponectin levels and the ovarian reserve in women of reproductive age. *Front Endocrinol*. 2024;15:1369248. doi:10.3389/fendo.2024.1369248
25. Salem AM. Variation of Leptin During Menstrual Cycle and Its Relation to the Hypothalamic–Pituitary–Gonadal (HPG) Axis: A Systematic Review. *Int J Womens Health*. 2021;13:445-458. doi:10.2147/ijwh.s309299
26. Ahima RS, Flier JS. Leptin: 30 Years Later. *Annu Rev Physiol*. 2025;88(1):229-250. doi:10.1146/annurev-physiol-042324-100259
27. Schwartz MW, Prigeon RL, Kahn SE, et al. Evidence That Plasma Leptin and Insulin Levels are Associated with Body Adiposity via Different Mechanisms. *Diabetes Care*. 1997;20(9):1476-1481. doi:10.2337/diacare.20.9.1476
28. Liu J, Lai F, Hou Y, Zheng R. Leptin signaling and leptin resistance. *Med Rev*. 2022;2(4):363-384. doi:10.1515/mr-2022-0017
29. Galiniak S, Biesiadecki M, Podgórski R, et al. Serum leptin and its relation to body composition, puberty, and metabolism in severe obesity. *Endocr Connect*. 2025;14(7). doi:10.1530/ec-25-0194
30. Dobrzycka M, Karczewska-Kupczewska M. White adipose tissue expansion as the driver of insulin resistance and secondary endocrine disturbances in obesity- a narrative review. *Diabetol Metab Syndr*. 2026;18(1). doi:10.1186/s13098-026-02185-1
31. Sidhu S, Parikh T, Burman K D. Endocrine Changes in Obesity. MDText.com, Inc.; 2017. Accessed August 12, 2026. https://www.ncbi.nlm.nih.gov/books/NBK279053/
32. Patial R, Batta I, Thakur M, Sobti RC, Agrawal DK. Etiology, pathophysiology, and treatment strategies in the prevention and management of metabolic syndrome. *Arch Intern Med Res*. 2024;7(4):273. doi:10.26502/aimr.0184
33. Mohamed AA, Abo-Elmatty DM, Wahba AS. Leptin Rs7799039 polymorphism is associated with type 2 diabetes mellitus Egyptian patients. *Arch Physiol Biochem*. 2024;130(6):742-754. doi:10.1080/13813455.2023.2265078
34. Abdullah G. Association of leptin promoter 2548G/A variant with serum leptin, lipid profile and type 2 diabetes mellitus. *Diyala J Med.* 2020;19(1):60-67. doi:10.26505/djm.19015300311
35. Ali LA, Jemon K, Ab Latif N, Bakar SA, Alwi SSS. LEP G2548A polymorphism is associated with increased serum leptin and insulin resistance among T2DM Malaysian patients. *Biomedicine*. 2022;12(3):31-39. doi:10.37796/2211-8039.1326

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36. Bains V, Kaur H, Badaruddoza B. Association analysis of polymorphisms in LEP (rs7799039 and rs2167270) and LEPR (rs1137101) gene towards the development of type 2 diabetes in North Indian Punjabi population. *Gene*. 2020;754:144846. doi:10.1016/j.gene.2020.144846
37. Manju SK, Anilkumar TR, Vysakh G, et al. A Case-Control Study of the Association of Leptin Gene Polymorphisms with Plasma Leptin Levels and Obesity in the Kerala Population. *J Obes.* 2022;2022:11. doi:10.1155/2022/1040650
38. Song Y, Zhang Y, Liu X, et al. The rs7799039 variant in the leptin gene promoter drives insulin resistance through reduced serum leptin levels. *Front Endocrinol*. 2025;16:1589575. doi:10.3389/fendo.2025.1589575

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