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LEPR Q223R Variant and Its Role in Type 2 Diabetes Susceptibility Among Iraqi Individuals

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ABSTRACT

Background: *Type 2 diabetes mellitus (T2DM) is a chronic metabolic disorder characterized by insulin resistance and impaired β-cell function. Recent studies have highlighted the contribution of genetic polymorphisms, particularly in the leptin receptor (LEPR) gene, to T2DM susceptibility. The LEPR rs1137101 (A>G) polymorphism, which leads to a Q223R amino acid substitution, may influence leptin signaling and insulin sensitivity, thereby affecting T2DM pathogenesis.* ***Methods:*** *A case-control study included 300 participants (150 T2DM patients and 150 healthy controls) recruited from Najaf, Iraq. Genotyping of LEPR rs1137101 was performed using allele-specific PCR, while biochemical parameters, including fasting blood glucose (FBG), insulin, lipid profile, HOMA-IR, and serum leptin levels, were measured using standard methods. Data were analyzed under different genetic inheritance models with adjustment for age, sex, and BMI.* ***Results:*** *The LEPR rs1137101 G allele was significantly associated with an increased risk of T2DM. Individuals with the GG genotype had a 4.9-fold increased risk ($p < 0.0001$; OR = 4.96, 95% CI = 2.26–10.90), and AG carriers had a 3.7-fold increased risk ($p < 0.0001$; OR = 3.70, 95% CI = 2.19–6.26) compared to AA homozygotes. The G allele also showed a strong association in dominant (OR = 3.95), over-dominant (OR = 2.47), and recessive models (OR = 2.48), indicating its robust link to T2DM susceptibility. Furthermore, GG carriers exhibited significantly elevated insulin, HOMA-IR, and leptin levels, suggesting a relationship with increased insulin resistance and altered leptin signaling. However, no significant differences were observed in BMI, FBG, or lipid profile between genotypes.* ***Conclusion:*** *The LEPR rs1137101 (A>G) polymorphism is strongly associated with T2DM in the Iraqi population.*

Keywords: LEPR Q223R, rs1137101SNP, T2DM.

Article Information

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a complex, multifactorial metabolic disease characterized by chronic hyperglycemia resulting from insulin resistance and β-cell dysfunction. It represents over 90% of diabetes cases worldwide and poses a significant public health challenge, particularly in low- and middle-income countries where prevalence rates are rising rapidly. [1&2] The disorder emerges from a combination of genetic, environmental, and behavioral factors such as sedentary lifestyle, poor diet, and obesity. [3] The clinical presentation of T2DM includes classic symptoms such as polyuria, polydipsia, and polyphagia, along with fatigue, visual disturbances, and increased susceptibility to infections. [4] If left untreated, the condition may progress into severe acute complications like diabetic such as hyperosmolar hyperglycemic state, or chronic complications such as cardiovascular disease, nephropathy, retinopathy, and neuropathy. [5&6]

The pathophysiology of T2DM primarily involves insulin resistance, which impairs glucose uptake in insulin-sensitive tissues like muscle and adipose tissue, and β-cell dysfunction, which reduces the pancreas's ability to produce adequate insulin. [7&8] This metabolic dysregulation is exacerbated by chronic low-grade inflammation and hormonal imbalances, including those involving adipokines like leptin.

Leptin is a hormone predominantly secreted by adipose tissue that regulates food intake and energy expenditure through its action on the hypothalamus. [9] It plays a significant role in glucose homeostasis, insulin sensitivity, and lipid metabolism. Leptin binds to the leptin receptor (LEPR), a member of the class I cytokine receptor family, which is widely expressed in several tissues including the hypothalamus. [10] Activation of LEPR initiates signaling cascades that modulate appetite and metabolic pathways via the JAK-STAT pathway, particularly through STAT3 phosphorylation.

The Q223R polymorphism (rs1137101) in the LEPR gene results from a single nucleotide substitution (A>G), leading to an amino acid change from glutamine (Q) to arginine (R) at position 223. This change occurs in exon 6 of the gene and may significantly affect receptor function and leptin signaling efficiency. [11] Studies have shown that the LEPR Q223R polymorphism may influence individual susceptibility to obesity, insulin resistance, and T2DM. [12] Mechanistically, the Q223R polymorphism may impair LEPR signaling, leading to reduced STAT3 activation and ineffective leptin signaling. This dysfunction can result in Hyperleptinemia—a hallmark of leptin resistance and a subsequent inability to regulate appetite and glucose metabolism properly. [13] Individuals with this polymorphism often exhibit higher serum leptin, insulin, and HOMA-IR values, suggesting a strong link to insulin resistance and metabolic dysregulation. [11]

METHODS

Study Design

This case-control study investigated the association between the LEPR Q223R (rs1137101) polymorphism and type 2 diabetes mellitus (T2DM) among an Iraqi population. A total of 300 individuals participated, comprising 150 T2DM patients (83 males and 67 females) and 150 apparently healthy controls (77 males and 73 females). The diabetic group included adults aged ≥35 years with a fasting blood glucose level ≥7 mmol/L (126 mg/dL), HbA1c ≥ 6.5% or greater. [14]
The controls were non-diabetics matched on age, sex, and body mass index, consisted of healthy individuals aged 35–65 years, selected randomly from the general population, and screened to exclude undiagnosed diseases. The study was conducted at the Department of Biochemistry, College of Medicine, and University of Kufa. Sample size was calculated using the OSSE online tool. The odds ratio (OR) from previous studies was 3.5-5, minor allele frequency (MAF) G =0.42, α-level was = 0.05, power of study was 97%. (osse.bii.a-star.edu.sg), ensuring statistical power and validity. [15]

Biochemical Measurements

Six milliliters of venous blood were collected from each participant after 8-12 hours of fasting. Blood was divided into three parts: A 2 ml in EDTA tubes for HbA1c measurement, 2 ml in gel tubes for serum separation and biochemical analyses, 2 ml in EDTA tubes for DNA extraction. Phenotypic evaluations included anthropometric measures (weight, height, BMI), and biochemical parameters such as fasting glucose, insulin, lipid profile (TC, TG, HDL-C, LDL-C, VLDL-C), The Manufacturer of HDL-C, TG, and Total cholesterol kits are BIOTEC/ Spain, and serum leptin. HDL-C was determined via precipitation and spectrophotometry, [16] while LDL-C and VLDL-C were calculated using the Friedwald formula. [17] Fasting serum insulin Catalogue number for insulin ELISA kit is

E0010Hu, Manufacturer is BT LAB and leptin concentrations Catalogue number for Leptin ELISA kit is E1559Hu, Manufacturer is BT LAB were measured using enzyme-linked immunosorbent assay (ELISA) kits following the sandwich technique. Insulin resistance was assessed using the Homeostatic Model Assessment for Insulin resistance (HOMA-IR).[18]

DNA Extraction

DNA was extracted from EDTA blood using the Geneaid DNA mini kit, based on silica membrane purification. DNA purity and concentration were assessed via UV spectrophotometry using a Nanodrop system, where an A260/A280 ratio between 1.7 and 2 indicated acceptable DNA purity. [19]

Genotyping

Genotyping for the LEPR rs1137101 (Q223R) polymorphism was performed using Allele-Specific Polymerase Chain Reaction (AS-PCR), a reliable and cost-effective method for SNP detection. [20&21] The PCR optimization was carried out using gradient annealing temperatures and varying primer concentrations. The primer sequences for rs1137101 were designed via Primer-BLAST and synthesized by Macrogen (Korea). The specific primers included:

forward primer (GGTTCCCCAAAAAGGCAGT)
Allele A (TGAACTGACATTAGAGGTGACT), and **Allele G (TGAACTGACATTAGAGGTGACC**), enabling the differentiation of homozygous and heterozygous genotypes through agarose gel electrophoresis.

PCR reactions were performed. The presence or absence of a specific PCR product indicates the presence or absence of the corresponding allele. Amplification was performed utilizing 2 PCR tubes in a total reaction volume of 25 µl in each tube as the following: tube1/ 12.5 µl GoTaq Green Master Mix (Promega, U.S.A), 2 µl of forward primer, 2 µl of allele A primer ,2.5 µl of nuclease free water, and 6µl genomic DNA solution as template. Tube2/ 12.5 µl GoTaq Green Master Mix (Promega, U.S.A), 2 µl of forward primer, 2 µl of allele G primer ,2.5 µl of nuclease free water, and 6µl genomic DNA solution as template.

Amplification of PCR was performed using a thermal cycler (Biometra, Germany) and GoTaq® G2 Green Master Mix (Promega, USA), which includes all necessary PCR components and tracking dyes (blue and yellow dye) for gel analysis. [22] Thermocycling conditions included initial denaturation at 95°C for 5 minutes, 35 cycles of denaturation at 95°C for 30 second, annealing at 57°C for 30 second, and extension at 72°C for 1minute, and final extension at72°C for 5 minutes. The resulting PCR products were visualized under UV light using a gel documentation system.

Statistical Analysis

Statistical analysis was performed using SPSS version 26. Numerical data were presented as mean ± standard deviation (SD) and compared using Student's t-test or ANOVA, as appropriate. Genotype and allele frequencies were compared using the Chi-square test. Hardy-Weinberg equilibrium (HWE) was assessed to ensure allele distribution consistency in the control group. [23] Genetic association models—codominant, dominant, recessive, additive, and allelic—were evaluated using multinomial logistic regression. Adjusted odds ratios (OR), 95% confidence intervals (CI), and p-values were reported to estimate the strength of association between genotypes and T2DM risk. [24]

RESULTS

The demographic and biochemical characters of the study groups were illustrated in **Table (1)**. Significant differences between T2DM and control groups were recorded for lipid profile parameters, FBS, insulin, and HOMA-IR, as well as leptin (P<0.001). While, both age and BMI were well matched between the two groups. Genotype frequencies revealed significant associations between rs1137101 and T2DM risk were observed in **Table (2)**. Under the co-dominant model, the frequency of the GG genotype was significantly higher in T2DM patients (18%) compared to controls (8%). Carriers of the GG genotype had nearly five folds increased risk of developing T2DM (OR = 4.96; 95% CI = 2.26–10.90; p < 0.0001) compared to those with the AA genotype. Similarly, individuals with the AG genotype also showed a significantly elevated risk of T2DM (OR = 3.70; 95% CI = 2.19–6.26; p < 0.0001). When applying the dominant model (AG + GG vs. AA), carriers of the G allele had about four folds increased risk of T2DM (OR = 3.95; 95% CI = 2.40–6.50; p < 0.0001), indicating a strong dominant effect of the G allele on disease susceptibility.

In the over-dominant model (AG vs. AA + GG), the heterozygous AG genotype also presented a significant association with increased disease risk (OR = 2.47; 95% CI = 1.53–3.99; p = 0.0002). Under the recessive model (GG vs. AA + AG), the GG genotype independently conferred a 2.48-fold risk of T2DM (OR = 2.48; 95% CI = 1.20–5.15; p = 0.012). Additionally, minor allele frequency (G allele) was substantially higher in the diabetic group (46%) compared to the control group (24.7%), reflecting a statistically significant association with disease status (OR = 4.06; 95% CI = 2.44–6.79; p < 0.000).

The genetic power of the LEPR Q223R (rs1137101) to estimate a significant difference at a level of 0.05 was well-powered (97%), which indicates a true and strong association with T2DM. Genotyping for LEPR Q223R (rs1137101) polymorphism achieved via allele-specific PCR (AS-PCR), the products were electrophoresed on 2.5% agarose gel electrophoresis. Amplification of rs1137101 SNP resulted in the production of three genotypes; AA, AG, and GG. The expected product sizes of the rs rs1137101 (A > G) variant were one band 168 bp for both wild homozygous (AA) and mutant homozygous (GG), while, the heterozygous (AG) revealed two bands (168,168 bp) one for allele A and one for allele G as shown in **Figure (1).**

Further analysis was performed to evaluate the impact of rs1137101 polymorphism on key metabolic parameters among T2DM patients. Under co-dominant inheritance models, statistically significant associations were observed with insulin levels, HOMA-IR, and serum leptin. In the co-dominant model, mean insulin levels and HOMA-IR increased progressively with the presence of the G allele, reaching the highest values in GG carriers (Insulin: 14.13 ± 6.17 µU/L, HOMA-IR: 7.50 ± 3.27, p = 0.001). Similarly, serum leptin concentrations showed a genotype-dependent increase (AA: 3.48 ± 1.68 ng/ml, AG: 4.46 ± 2.41 ng/ml, GG: 6.01 ± 2.81 ng/ml; p = 0.001) as showed in **Table (3).**

In contrast, no significant differences were observed across genotypes for other metabolic markers including BMI, fasting blood glucose, and lipid profile components (LDL, HDL, TG, VLDL, TC) under either inheritance model.

Table 1: Demographic and biochemical features of study participants.

Parameters	Control (n= 150)	T2DM (n= 150)	P-Value
No (M/F)	150 (77/73)	150 (83/67)	
Age (y)	52.12 ± 6.71	53.36 ± 6.74	0.111
BMI (Kg/m2)	27.51 ± 2.46	28.02 ± 2.32	0.071
TG (mg/dl)	138.16 ± 16.69	241.61 ± 22.02	<0.001
TC (mg/dl)	159.16 ± 16.01	230.83 ± 17.36	<0.001
VLDL-C (mg/dl)	27.63 ± 3.34	48.26 ± 4.41	<0.001
LDL-C (mg/dl)	85.33 ± 13.51	142.19 ± 15.97	<0.001
HDL-C (mg/dl)	46.19 ± 4.53	40.35 ± 3.01	<0.001
FBG (mg/dl)	92.37 ± 7.66	222.15 ± 29.23	<0.001
HbA1C %	5.10 ± 0.394	9.53 ± 1.46	<0.001
Insulin (µU/L)	5.40 ± 2.92	10.10 ± 5.33	<0.001
HOMA-IR	1.24 ± 0.69	5.50 ± 2.88	<0.001
Leptin (ng/ml)	2.02 ± 0.869	4.49 ± 2.45	<0.001

NO., Number of participants; BMI, Body mass index; TG, triglyceride; TC, total cholesterol, VLDL-C, very low density lipoprotein; HDL-C, high density lipoprotein; FBG fasting blood glucose; HOMA-IR, Homeostasis Model Assessment for Insulin Resistance; P<0.05 significant**.**

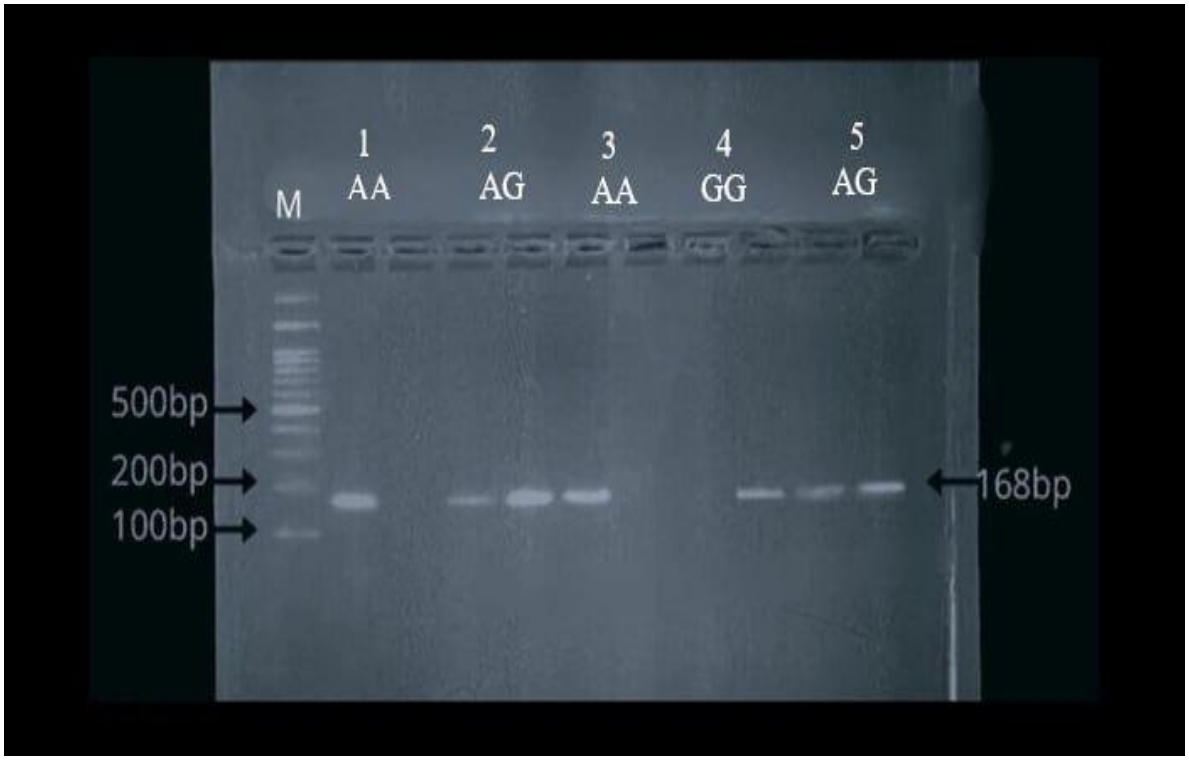

Figure 1: AS-PCR-Technique genotype of LEPR gene rs1137101A>G on 2.5% agarose gel electrophoresis. M; DNA ladder. Lanes 1&3 (AA) genotype. Lanes 2&5 indicates (AG) genotype. Lane 4 (GG) genotype**.**

Table 2: Genotype and allele frequency of rs1137101 SNP of LEPR gene in T2DM patients and controls.

Model	Genotype/Allele	T2DM (n=150) No. (%)	Control (n=150) No. (%)	Adjusted OR (95% CI)	P-value
Codominant	AA	39 (26%)	88 (58.66%)	Ref.	
	AG	84 (56%)	50 (33.34%)	3.70 (2.19 - 6.26)	<0.0001
	GG	27 (18%)	12 (8%)	4.96 (2.26 - 10.90)	<0.0001
Dominant	AG + GG	111 (74%)	62 (41%)	3.95 (2.40 - 6.50)	<0.0001
Over Dominant	AA + GG	66 (44%)	100 (66.6%)	2.47 (1.53 - 3.99)	0.0002
	AG	84 (56%)	50 (33.345)		
Recessive	AA + AG	123 (82%)	138 (92%)	2.48 (1.20 - 5.15)	0.012
	GG	27 (18%)	12 (8%)		
Allele	A	162 (54%)	226 (75.4%)	4.06 (2.44 - 6.79)	0.000
	G	138 (46%)	74 (24.6%)		

OR adjusted for age, BMI, and sex.

Table 3: Biochemical characteristics of T2DM patients in revelence to the genotypes of rs1137101 polymorphism under codominant model.

Parameters	AA=39 (Mean ±SD)	AG=8 (Mean ±SD)	GG=27 (Mean ±SD)	P value
BM (Kg/m2)	27.84 ±2.61	27.97 ±2.22	28.43 ±2.21	0.568
TG (mg/dl)	240.28±18.89	241.76±24.94	243.04±16.33	0.820
TC (mg/dl)	230.43±16.73	230.85±16.81	231.32±20.38	0.982
VLDL (mg/dl)	48.06 ±3.75	48.32 ±5.00	48.39 ±3.30	0.922
LDL-(mg/dl)	141.70±15.90	142.48±15.07	141.99±19.13	0.965
HDL (mg/dl)	40.69±2.98	40.08 ±3.02	40.70 ±3.06	0.474
Leptin (ng/ml)	3.48±1.68	4.46± 2.41	6.01±2.81	0.001
FBG (mg/dl)	218.33±27.57	225.90±31.48	216±22.70	0.167
HbA1C %	9.13± 1.31	9.57± 1.50	9.97± 1.43	0.054
Insulin(µU/L)	8.34±4.92	9.62±4.59	14.13±6.17	0.001
HOMA-IR	4.45±2.46	5.35±2.63	7.50±3.27	0.001

DISCUSSION

This current study evaluated the association of the LEPR rs1137101 (A>G) polymorphism with T2DM risk and related metabolic parameters among Iraqi population. The findings observed that LEPR Q223R gene polymorphism rs1137101 (A>G) is as an important marker for greater risk of developing Type 2 Diabetes Mellitus (T2DM) among Iraqi subjects and the G allele of this variant is linked to increased insulin resistance and Hyperleptinemia, supporting the role of leptin receptor dysfunction in T2DM pathophysiology.

Leptin works through receptors in the beta cells of the pancreas to prevent the release and synthesis of insulin. In the meantime, insulin stimulates the release of leptin from adipose tissue. The "adipo-insular axis" connects the pancreas with adipocytes and controls body metabolism. [25] Disruption of the adipo-insular axis may be a contributing factor for the development of insulin resistance and type 2 diabetes mellitus due to leptin resistance in the pancreatic cell. Leptin resistance is a well-known contributor to obesity and insulin resistance, both of which are key elements in T2DM pathogenesis. [11&26]

The G allele (mutant variant) of Q223R SNP was found at a significantly higher frequency among T2DM patients compared to healthy controls (46% vs 24.6%, respectively) as shown in Table 2, consistent with previous findings from meta-analyses and independent population studies. [27&28] The data also demonstrated that Individuals who carrying the AG and GG genotypes had a notably increased risk of developing T2DM compared to AA carriers, suggesting a dominant genetic effect of the G allele on disease susceptibility.

The results of this study are consistent with those reported in other ethnic groups, such as the Kashmiri, Mexican, and Chinese populations, where the G allele of rs1137101 was linked to increased T2DM risk. [29&30] Nonetheless, some previous studies reported no significant association between LEPR rs1137101 and T2DM, such as those conducted in the Chinese Han population and Iranian populations. [31&32] These discrepancies could be due to differences in ethnic backgrounds, environmental exposures, sample sizes, or methodological variations.

Among T2DM patients, it was shown that those with the GG genotype of the LEPR Q223R had substantially higher fasting serum insulin, leptin, and HOMA-IR index than those with the AA genotype as summarized in Table (3) Other investigations' results were in agreement with ours. Ziablitsev et al. (2018) discovered that the Q223R polymorphism was linked to insulin and leptin resistance in T2DM patients who were Ukrainian Participants. [33] According to recent research, individuals with the GG homozygote of the Q223R variant in the LEPR gene had significantly higher levels of Hyperleptinemia, hyperinsulinemia, and HOMA-IR index than those with the other genotypes among obese and type 2 diabetic patients. These studies were carried out among populations in Tunisia, Indonesia, and India. [34&35]

Moreover, the significant rise in HbA1c levels among G allele carriers under the dominant model suggests that this polymorphism may also affect long-term glycemic control. However, no significant differences were observed regarding BMI, fasting blood glucose, or lipid profiles across genotypes, implying that the primary metabolic effects of LEPR rs1137101 are more closely linked to insulin sensitivity rather than general adiposity or dyslipidemia. [33]

Overall, the findings suggest that the LEPR rs1137101 polymorphism plays a significant role in the pathogenesis of T2DM. Considering the rising prevalence of diabetes in Iraq and

globally, understanding such genetic influences can help identify individuals at high risk and inform targeted prevention strategies. Future studies with larger sample sizes and diverse ethnic groups are needed to further validate these results and to explore potential gene-environment interactions affecting T2DM susceptibility.

There are few limitations to this study, including the incomplete data due to loss of compliance also the current result may not be generalizable to other populations due to geographic, ethnic or demographic difference. In spite of the small sample size of the study population, the power of the study was achieved above 90% Therefore, studies with a large sample size involving other governorates required for future studies to confirm current finding.

CONCLUSIONS

The current study found that T2DM was significantly correlated with the Q223R (rs 1137101) of LEPR gene.

Recommendations

Larger sample size is demanded to validate our findings. To show the prevalence and/or correlation with type 2 diabetes in the Iraqi population, more SNPs in the LEPR gene need to be investigated.

Ethical approval

The present study Which is conducted by (NNM, AAA, ZNF) was approved by the local Department of Biochemistry and Kufa ethical committee.

Statement of Permission and Conflict of Interests

The authors declare that they have no competing interests.

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