Arch Biol Sci. 2021;73(1):93-101 https://doi.org/10.2298/ABS210129005M

The single nucleotide polymorphisms rs11761556 and rs12706832 of the leptin gene are associated with type 2 diabetes mellitus in the Iraqi population

Karar N. J. Musafer[1], Fahrul Z. Huyop[2], Mufeed J. Ewadh[3], Eko Supriyanto[4], Tahreer M. Al-Thuwaini[5] and Mohammed Baqur S. Al-Shuhaib[5,*]

[1]*School of Biomedical Engineering and Health science Universiti Technologi Malaysia*, Jalan Iman, 81310 Skudai, Johor, Malaysia

[2]*Faculty of Science, Universiti Technologi Malaysia*, Jalan Hikmah, 81310 Skudai, Johor, Malaysia

[3]*Clinical Biochemistry Department, Faculty of Medicine, University of Babylon*, Hilla, Babil 51001, Iraq

[4]*School of Biomedical Engineering and Health Science, Universiti Technologi Malaysia*, Jalan Iman, 81310 Skudai, Johor, Malaysia

[5]*Department of Animal Production, College of Agriculture, Al-Qasim Green University*, Al-Qasim, Babil 51001, Iraq

***Corresponding author**: mohammed79@agre.uoqasim.edu.iq; baquralhilly_79@yahoo.com

Received: January 29, 2021; **Revised**: February 1, 2021; **Accepted**: February 2, 2021; **Published online**: February 2, 2021

Abstract: This study was conducted to assess the potential association between leptin (*LEP*) gene polymorphisms and type 2 diabetes mellitus (T2DM) in Iraqi patients. Genomic DNA was extracted from 120 diabetic subjects and 100 controls. Three specific PCR fragments were designed to flank three highly frequent single nucleotide polymorphism (SNP)s within *LEP*, rs11761556, rs12706832 and rs2167270. The amplified loci were genotyped by PCR-single-strand conformation polymorphism (SSCP) followed by Sanger sequencing for representative genotypes. Logistic regression analysis was performed to detect the association between the targeted genetic variants and T2DM. PCR-SSCP genotyping showed three banding patterns for all three targeted SNPs. Individuals with the AA genotype in both rs11761556 and rs12706832 SNPs showed significantly higher ($P<0.05$) body mass index (BMI), waist circumference (WC), fasting blood glucose (FBG), hemoglobin A1c (HbA1c), homeostatic model assessment for insulin resistance (HOMA-IR), insulin, low-density lipoprotein cholesterol (LDL-C) and triglyceride (TG) values than those with other genotypes. Association analysis revealed that individuals with the A allele exhibited a greater risk of T2DM. Data of the present investigation indicated that both rs11761556 and rs12706832 SNPs exerted a noticeable association with T2DM. The study suggests implementing both rs11761556 and rs12706832 SNPs in the early detection of T2DM.

Keywords: *LEP* gene; polymorphism; T2DM; rs11761556; rs12706832; rs2167270

INTRODUCTION

Type 2 diabetes mellitus (or T2DM) is a multifactorial complex disease, determined largely by variable environmental and genetic factors that possibly interact with each other to contribute to the onset and progression of this metabolic disorder. T2DM prevalence is increasing in an epidemic manner worldwide, with rapid expansion of affected persons in the last decades. In the Middle East, the Iraqi population suffers from a steady increase in T2DM and its complications, including several neurological, vascular and oral health issues [1,2]. The management of T2DM-related traits by medical approaches has proven to be difficult to control due to many multifactorial limitations associated with this epidemic health problem. A candidate gene-based approach has increasingly been reported in the literature with rapid and successful outcomes [3,4]. This approach is based on the genotyping of several genetic loci to resolve their possible association with T2DM. The leptin (*LEP*) gene has been suggested to play a crucial role in the development of T2DM and impaired insulin secretion [5]. The *LEP* gene is located in chromosome 7 within q32.1, spanning a transcription element of about 20 kb and containing three exons [6]. This gene encodes for leptin, a multifunctional

How to cite this article: Musafer KNJ, Huyop FZ, Ewadh MJ, Supriyanto E, Al-Thuwaini TM, Al-Shuhaib MBS. The single nucleotide polymorphisms rs11761556 and rs12706832 of the leptin gene are associated with type 2 diabetes mellitus in the Iraqi population. Arch Biol Sci. 2021;73(1):93-101.

polypeptide hormone of 16 KDa that is secreted into the blood circulatory system by adipocytes [7]. Leptin interacts with many metabolic activities throughout the body, thereby controlling food intake, energy metabolism, body weight, appetite and reproduction [8]. Leptin has been considered as a key regulator for severe insulin resistance and the diabetes phenotype of genetic disorders that impair adipogenesis [9]. Furthermore, the administration of leptin leads to reduced food intake, increased energy expenditure and weight loss [10]. Its variation may lead to several long-term outcomes in cellular metabolism and activity [11,12]. Defects or dysfunction in leptin signaling may contribute to the etiology of diabetes and raise the possibility that either leptin or downstream targets of leptin may expose the body to a variety of deleterious consequences [13-15]. Advances in genomic technology have initiated a myriad of novel genetic discoveries including many *LEP*-based common variants contributing to the risk of complex etiology of T2DM. Each year new candidate SNPs within the *LEP* gene are tested for their possible contributions to the development of T2DM. This gene has many high-frequency variants that have been reported to exert a strong correlation with the onset of T2DM, impaired insulin secretion, synthesis and obesity [16-18]. While polymorphism of *LEP* is closely-associated with the increased risk of diabetes mellitus (DM) [19], variation in only a small portion of the *LEP* gene has only been explained in the total heritability of diabetes, leaving many more variants to be resolved. Iraq is a Middle Eastern country in which the prevalence of diabetic individuals is on the increase, and the rate of increase is on the rise among adults [20,21]. It has been shown that distinct variations in *LEP* are associated with a predisposition to several metabolic dysfunctions, such as obesity [22], lupus erythematosus [23] and coronary artery disease [24]. However, the roles of these variations in the onset of T2DM have not been fully resolved in Middle Eastern populations. Accordingly, it is of interest to investigate the involvement of the genetic polymorphisms of the *LEP* gene in T2DM-related complications, taking into account the role of high-frequency SNPs in the Middle Eastern population.

The study of the *LEP* genetic polymorphism can be informative when its high-frequency SNPs are targeted and analyzed alongside T2DM-related indices. This study aimed to investigate whether an association exists between three high-frequency single nucleotide polymorphism (SNP) variants of the *LEP* gene and T2DM in Iraqi adults. Accordingly, a three-SNP (rs2167270, rs12706832 and rs11761556)-based case-control study was conducted to assess this potential association.

MATERIALS AND METHODS

Ethics statement

The study was conducted following the Helsinki Declaration after it was approved by the Institutional Review Board at the University of Babylon (number 652, dated 28.01.2019). Written informed consent was obtained from all participants prior to study participation.

Study population

A case-control study was conducted that involved 120 diabetic subjects and 100 non-diabetic controls of Iraqi origin, aged 35-50 years. T2DM patients were recruited from the T2DM treatment centers in Al-Hussein Medical City and Al-Imam Al-Hujja Hospital, while the control participants were from the general Iraqi population. Both subjects and controls were enrolled in the study from January to November 2019. All individuals with type 1 DM, gestational DM, a previous history of thyroid diseases, liver and muscle diseases along with other chronic illnesses, except for blood pressure, were excluded from this study. In addition to age, several data were considered for all included participants. The body mass index (BMI) was calculated using the formula BMI= weight (kg)/height^2. The BMI values of the normal weight, T2DM and morbid T2DM were defined as 18.5-24.9 kg/m^2, 30-39.9 kg/m^2 and >40 kg/m^2, respectively, according to the standards provided in [25]. Blood pressure was measured on the right arm in the sitting position using a digital sphygmomanometer after 5 min of rest. Three measurements were taken and the average reading was then computed.

Biochemical assays

Hemoglobin A1c (HbA1c) was measured based on the fluorescence immunoassay technology using i-CHROMA™ Reader (Boditech Med Inc., Gang-won-do,

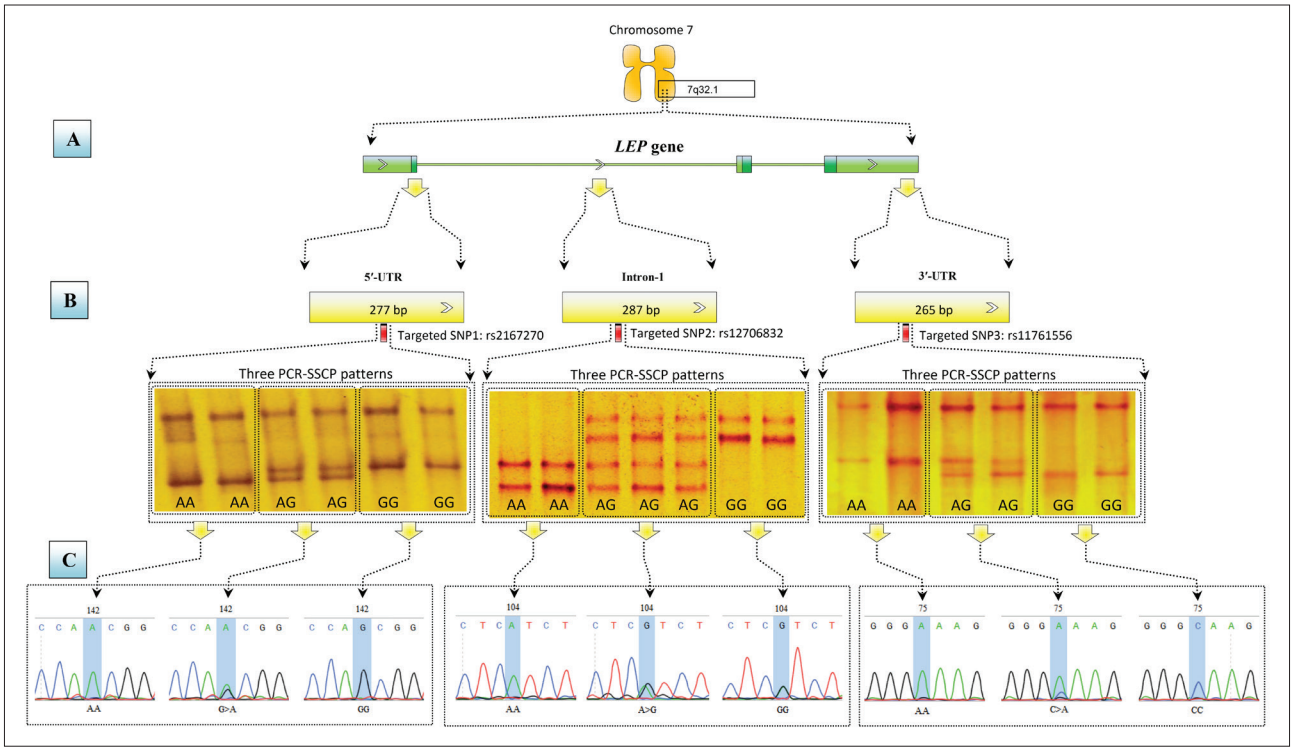

Fig. 1. A schematic diagram of the *LEP* gene-based PCR-single-strand conformation polymorphism (SSCP)-sequencing strategy. **A** – PCR design of three PCR specific primer pairs for the amplification of 277 bp, 287 bp and 265 bp containing rs2167270, rs12706832 and rs11761556 SNPs, respectively. **B** – Post-PCR genotyping using SSCP showed three patterns of nucleic acid variations in each designed fragment. **C** – Sequencing results of the three detected PCR-SSCP genotypes in which three patterns were identified in the targeted SNP.

Korea). Insulin levels were measured by Sandwich immune-luminometric assay using a Maglumi 800 analyzer (Shenzhen, 518057 China). Fasting blood glucose (FBG), total cholesterol (TC), triglycerides (TG), high-density lipoprotein in cholesterol (HDL-C HDL) and low-density lipoprotein in cholesterol (LDL-C HDL) were measured based on the enzymatic colorimetric method using an autoanalyzer (Mindray BS-Clinical Chemistry Analyzer, Guangzhou Shihai Medical Equipment Co, Guangdong, China). The LDL was calculated using Friedewald's formula [26].

Sample collection and DNA extraction

Blood samples were collected from all patients and control individuals (120 with T2DM and 100 controls). Genomic DNA was extracted using an improved salting-out protocol as outlined [27]. The purity and integrity of the isolated DNA were verified by agarose gel electrophoresis. Quantification of the extracted DNA was performed using a Nanodrop spectrophotometer (Biodrop, UK).

PCR primer design

Based on the sequences of the *LEP* (GenBank acc. no. NC_000007.14), three sets of PCR primer pairs were designed to amplify three genomic DNA fragments using the NCBI primer BLAST server [28]. These fragments were constructed to flank three high-frequency SNPs, rs2167270, rs12706832 and rs11761556, that are localized in the 5'-untranslated region (UTR), intron 1 and 3'-UTR, respectively (Fig. 1A). The lengths of these three genetic fragments (277 bp, 287 bp and 265 bp) were made to suit the recommended amplicon lengths in the PCR-SSCP protocols [29]. The sequences of the forward and reverse primers, annealing temperature and other details are described in Supplementary Table S1. The targeted rs2167270, rs12706832 and rs11761556 SNPs were located at positions 142 bp, 104 bp and 75 bp within the amplified 277 bp, 287 bp and 265 bp amplicons.

PCR

The PCR experiments were conducted using a lyophilized PCR PreMix (Cat # K-2012, Bioneer, South Korea). One μL of both forward and reverse primers were added (10 pmol each) to the PCR pre-mix. Then, 10-30 ng of the genomic DNA template was added in 3 μL to bring the final PCR volume to 20 μL. The annealing temperatures were determined empirically by using a gradient PCR thermocycler (Mastercycler nexus, Eppendorf, Germany). After PCR reactions, the amplicons were verified by electrophoresis on a 1.5% (w/v) agarose gel in parallel with a 100-bp DNA marker before being exposed to downstream genotyping reactions.

Genotyping

Genotyping experiments were conducted by PCR-single-strand conformation polymorphism (PCR-SSCP) following the protocol recommended [30]. The PCR products were heat-treated at 95 °C for 7 min and chilled on ice for at least 10 min. Denatured samples were loaded onto 8% neutral polyacrylamide gels. Electrophoresis was conducted at room temperature, 4 h 199 V, 99 mA. The gels were stained by silver nitrate according to the described standard procedure [31]. To confirm the observed PCR-SSCP results, each banding pattern was sent for sequencing using both forward and reverse primers in separate reactions. Sequencing reactions were performed as recommended by the manufacturers' protocols (Macrogen Inc., South Korea). Electropherograms and sequencing files were analyzed using the sequencing analysis suit of the SnapGene Viewer ver. 4.0.4. (Insightful Science, Canada). The sequences of the PCR-SSCP patterns were aligned alongside with their corresponding reference sequences of the *LEP* gene using the BioEdit tool, ver. 7.1 (DNASTAR; Madison, USA).

Statistical analysis

Statistical analysis of the data was performed using SPSS ver. 23.0 (Statistical Package for Social Sciences, Inc., Chicago, IL, USA). Data were expressed as the mean and standard deviation (SD) of at least four independent experiments. The case and control difference for the allele and genotype frequencies was analyzed using PopGen32 software ver. 1.31 [32]. The significance of deviation from the Hardy-Weinberg equilibrium (HWE) between the case and control samples in the investigated population was calculated by the χ^2 test. Pairwise linkage disequilibrium (LD) between SNPs was calculated by r^2 and D' values using SHEsis software [33]. Logistic regression analysis was used to examine the relationship between *LEP* polymorphisms with T2DM. Comparison of the clinical variables of the study population was analyzed using the Student's t-test. The significant effect of genotype on different T2DM-related parameters studied was analyzed using one-way ANOVA. Unless otherwise stated, multiple pairwise comparisons between main factors were performed using Tukey-Kramer, which is statistically significant at a level of $P<0.05$.

RESULTS

The characteristics of the studied population

The baseline variables of the study population, which consisted of 100 non-diabetic individuals and 120 diabetic patients, are presented in Supplementary Table S2. The prevalence of T2DM in males was higher than in females ($P<0.02$). Age, BMI, WC, FBG, HbA1c, HOMA-IR, insulin, LDL-C and TG levels were significantly higher ($P<0.01$) in T2DM subjects compared with healthy controls, whereas both TC and HDL-C concentrations showed non-significant differences between non-diabetic and type 2 diabetic patients.

Genetic association analysis

Three high-frequency SNPs, rs2167270, rs12706832 and rs11761556, were targeted within three different positions of the *LEP* gene. Specific primers were designed to flank each of these SNPs in each fragment individually. The expected three polymorphic patterns of the rs2167270, rs12706832 and rs11761556 SNPs were confirmed in the investigated population. Three clear PCR-SSCP banding patterns were detected in the analyzed SNPs, two homogeneous and one heterogeneous (Fig. 1B). These three identified PCR-SSCP banding patterns were confirmed by the conducted sequencing experiments in the investigated 277 bp, 287 bp and 265 bp amplicons. Accordingly, three

Table 1. Hardy-Weinberg equilibrium (HWE) for the *LEP* gene between the two groups (type 2 diabetic vs non-diabetic).

SNP ID	Genotype	Type 2 diabetic		Non-diabetic	
		Observed	Expected	Observed	Expected
rs11761556 (C/A)	C/C	22	17	46	39
	C/A	46	56	34	47
	A/A	52	47	20	14
χ^2		3.98		7.32	
***P*-value**		**0.001***		**0.001***	
rs12706832 (A/G)	AA	54	46	26	15
	AG	40	57	26	48
	GG	26	17	48	37
χ^2		10.43		20.57	
***P*-value**		**0.001***		**0.001***	
rs2167270 (G/A)	GG	30	19	26	17
	GA	34	57	30	48
	AA	56	44	44	35
χ^2		19.72		14.43	
***P*-value**		0.92		0.83	

Deviation from HWE expectations were determined using the chi-squared test.
* Significant at P<0.05, ** Significant at P<0.001.

Table 2. Association analysis of *LEP* polymorphism with the risk of T2DM.

SNP ID	Diabetic (120)	Non-diabetic (100)	Logistic Regression analysis	
rs11761556 (C/A)	**N (%)**	**N (%)**	**Odds ratio (95% Cl)**	***P*-value**
C allele	45 (37.5)	63 (36)	Reference	
A allele	75 (62.5)	37 (37)	1.93 (1.17-3.17)	**0.008***
C/C	22 (18.3)	46 (46.0)	Reference	
C/A	46 (38.3)	34 (34.0)	1.36 (0.62-2.99)	0.68
A/A	52 (43.4)	20 (20.0)	4.58 (1.47-14.29)	**0.01***
rs12706832 (A/G)				
A allele	74 (61.7)	39 (39)	1.75 (1.04-2.95)	**0.006***
G allele	46 (38.3)	61 (61)	Reference	
A/A	54 (45.0)	26 (26.0)	2.38 (1.15-4.98)	**0.01***
A/G	40 (33.3)	26 (26.0)	1.48 (0.60-3.68)	0.17
G/G	26 (21.7)	48 (48.0)	Reference	
rs2167270 (G/A)				
G allele	47 (39.1)	41 (41)	Reference	
A allele	73 (60.9)	59 (59)	0.82 (0.43-1.54)	0.54
G/G	30 (23.3)	26 (24.0)	Reference	
G/A	34 (25.0)	30 (28.0)	0.68 (0.26-1.78)	0.71
A/A	56 (51.7)	44 (48.0)	0.75 (0.19-2.98)	0.73

*Significant at P<0.05; ** Significant at P<0.001. CL – confidence interval.

patterns of nucleic acid variations were validated in these targeted SNPs (Fig. 1C).

Genetic diversity analyses of the polymorphic SNPs were performed to assess whether the investigated population was in HWE. The chi-squared test of the observed genotypes revealed that the variations of both rs11761556 (C/A) and rs12706832 (A/G) SNPs deviated from HWE in both T2DM and healthy populations (P=0.001) (Table 1). The variation of rs2167270 (G/A) SNP did not exhibit any significant deviation from HWE for both T2DM subjects (P=0.92) and non-diabetic controls (P=0.83). The observed LD values of rs11761556 and rs12706832 SNPs indicated that the D' and r^2 values were between 0.48-0.23, signifying there was no coinheritance between these two SNPs.

The association between the *LEP* gene and T2DM was calculated by comparing the allele and genotype frequencies of all three investigated SNPs in the T2DM patients and healthy controls using logistic regression analysis (Table 2). Concerning rs11761556 (C/A) SNP, it was observed that the frequencies of the C and A alleles were 37.5% and 62.5%, respectively, of the T2DM group, which were higher than in the non-diabetic group (C and A allele frequencies were 36% and 37%, respectively). When comparing the T2DM and non-diabetic groups, it was noted that obese patients showed a significantly higher frequency of homozygote genotype AA with a higher risk to develop T2DM (P=0.01, odds ratio 4.58). This observation showed that individuals with the A allele showed a greater risk of T2DM (P=0.008, odds ratio 1.93) than individuals with the C allele. Concerning rs12706832 (A/G) SNP, it was observed that the frequencies of the A and G alleles were 61.7% and 38.3%, respectively, of the T2DM group, which were higher than that found in the non-diabetic group (A and G allele frequencies were 39% and 61%, respectively). When comparing the T2DM to non-diabetic groups, it was noted that T2DM patients showed a significantly higher frequency of homozygote genotype AA with a higher risk to develop T2DM (P=0.001, odds ratio 2.38). As in the case of rs11761556 SNP, the polymorphism of

Table 3. Association analysis of genetic variants of rs11761556 and rs12706832 SNPs within the *LEP* gene with T2DM-related parameters.

Indices	rs11761556 (LSM ± SE)			*P*-value	rs12706832 (LSM ± SE)			*P*-value
	C/C (68)	C/A (80)	A/A (72)		G/G (74)	A/G (66)	A/A (80)	
BMI (kg/m²)	29.74 ± 3.12	31.11 ± 4.10	36.37 ± 3.69	**0.001***	28.81 ± 4.11	32.34 ± 3.21	37.13 ± 4.31	**0.001***
WC (cm)	101.34 ± 10.21	107.94 ± 9.34	118.36 ± 13.23	**0.001***	103.24 ± 11.53	112.61 ± 12.31	117.22 ± 10.69	**0.001***
FBG (mg/dL)	83.66 ± 5.93	97.23 ± 10.71	158.43 ± 8.21	**0.001***	78.72 ± 8.91	100.34 ± 9.67	160.36 ± 12.44	**0.001***
HbA1c (%)	4.12 ± 0.51	5.93 ± 1.11	8.93 ± 1.22	**0.001***	4.46 ± 0.74	5.63 ± 1.93	8.92 ± 2.21	**0.001***
HOMA-IR index	2.31 ± 0.31	3.92 ± 0.21	5.20 ± 1.11	**0.001***	2.33 ± 0.13	3.35 ± 0.97	4.51 ± 1.41	**0.003***
Insulin(ng/mL)	19.02 ± 3.03	24.75 ± 5.76	30.01 ± 4.33	**0.02***	18.42 ± 3.61	22.61 ± 4.11	29.74 ± 4.19	**0.04***
TC (mg/dL)	210.10 ± 18.93	212.67 ± 22.36	220.43 ± 20.31	0.74	209.32 ± 20.59	210.49 ± 24.57	215.93 ± 23.31	0.28
HDL-C (mg/dL)	35.61 ± 3.45	34.05 ± 2.28	31.92 ± 3.13	0.24	34.59 ± 2.41	33.18 ± 3.81	30.94 ± 3.62	0.51
LDL-C (mg/dL)	87.56 ± 9.80	102.57 ± 10.08	117.53 ± 11.81	**0.001***	90.33 ± 9.89	111.51 ± 10.42	120.45 ± 11.41	**0.003***
TG (mg/dL)	179.78 ± 18.86	210.18 ± 20.89	224.26 ± 21.31	**0.001***	182.81 ± 20.54	214.41 ± 19.16	222.44 ± 20.14	**0.001***

LSM±SE – least-square means±standard error, * Significant at P<0.05, ** Significant at P<0.001. BMI – body mass index, WC – waist circumference, SBP – systolic blood pressure, DBP – diastolic blood pressure, FBG – fasting blood glucose, TC – total cholesterol, HDL-C – high-density lipoprotein cholesterol, LDL-C – low-density lipoprotein cholesterol, TG – triglycerides. The P-value with statistical significance is in bold.

rs12706832 SNP also revealed that individuals with the A allele showed a greater risk of T2DM (P-value 0.006, odds ratio 1.75) than individuals with the G allele. In both SNP cases, these observations suggested a strong association of T2DM with an increased frequency of this risky allele. In contrast to both rs11761556 and rs12706832 SNPs, no significant differences were observed in the case of rs2167270 SNP between T2DM and control groups, which indicated no possible association between this SNP and the risk of T2DM.

Genotype-phenotype correlation

A significant association between the observed genotypes of both rs11761556 and rs12706832 SNP-based *LEP* variation and T2DM were observed. Association analysis of both rs11761556 and rs12706832 SNPs indicated that individuals with the homozygous AA genotype exhibited significantly higher BMI, WC, FBG, HbA1c, HOMA-IR, insulin, LDL-C and TG levels than those with the other heterozygous and homozygous genotypes (P<0.05) (Table 3). However, no noticeable association was observed in TC and HDL-C traits (the interaction between the observed genotypes did not approach statistical significance).

DISCUSSION

The *LEP* gene is one of the most commonly associated loci clinically reported in T2DM in most parts of the world, and many SNPs have been recognized to constitute a risk factor for T2DM patients. However, the potential risk of specific alleles of many genetic fragments to T2DM subjects is not clear. This study described the association between *LEP* variations with T2DM-related traits in Iraqi adults. This association was based on the genotyping of three high-frequency SNPs positioned in three different regions within the *LEP* gene. PCR-SSCP was used instead of other commonly used genotyping methods because of its low-cost and high sensitivity for large-scale samples [29]. Concerning the rs2167270 SNP, the observed genetic polymorphism detected in this locus was not associated with any traits measured in the analyzed samples, which suggests the absence of any noticeable correlation with the onset of T2DM in the investigated population. Irrespective of the highly reported involvement of the rs2167270 SNP in many metabolic syndromes [34-37], this study showed no noticeable role of the SNP in T2DM in the analyzed population. In contrast to the rs2167270 SNP, both rs11761556 and rs12706832 SNPs showed obvious association with T2DM in the investigated population. This observation may be explained by the presence of a potential role of these variants with the clinical traits observed in the T2DM patients. Our study showed the association of both rs11761556 and rs12706832 SNPs with the BMI, WC, FBG, HbA1c, HOMA-IR, insulin, LDL-C and TG variables. These *LEP* variants were previously reported to be associated with appetite regulation, BMI and obesity that may be due to the role of *LEP* in the energy balance homeostasis [38-41]. In both rs11761556 and rs12706832 SNPs, individuals with

the AA genotype exhibited more T2DM association than those with the other two genotypes. This point of adiposity association of both SNPs can be manifested by the important role played by the A allele in the progression of T2DM. The significantly elevated values of most T2DM-related traits in individuals with the A allele have given multiple confirmations for the role of this allele in the onset of this chronic disease. Thus, this study suggested a tight association of rs11761556 and rs12706832-based AA genotypes with the clinical and biological traits of T2DM.

Whereas no previous study has clearly related these SNPs with the onset of T2DM, the *LEP* polymorphism in such variable loci may be a causative factor for several effects associated with clinical and biological traits of T2DM [42]. In contrast to rs12706832 SNP, the rs11761556 SNP has been reported in the ClinVar database (a freely available archive of relationships among medical variants and phenotypes) [43]. However, our study observed a correlation between both loci and distinct T2DM-related traits in the study population. It is noteworthy that rs11761556 SNP was identified to be associated with T2DM in the Korean population [44]. Furthermore, this 3'-UTR SNP has recently been associated with the onset of obesity in the Tunisian population [45]. However, rs12706832 SNP has not been directly associated with the etiology of T2DM. Apart from our findings, an association between rs11761556 and rs12706832 SNPs and a variety of other clinical and biological traits of T2DM has been reported, such as autism, cancer and hypertension [40,46,47]. Although the relationship of the rs2167270 SNP-based polymorphism was not found in this study, our results suggest that the rs11761556 and rs12706832 SNP-based polymorphism of the *LEP* gene presents susceptible loci for T2DM in the study population. The pathophysiological mechanism of the *LEP* gene in susceptibility to T2DM remains to be established.

CONCLUSIONS

The genotyping of three high-frequency SNPs within *LEP* gene polymorphism demonstrated a significant association of rs11761556 and rs12706832 SNPs with T2DM in the investigated Iraqi population. Individuals with the AA genotype exhibited more divergent BMI, WC, FBG, HbA1c, HOMA-IR, insulin, LDL-C and TG values than individuals with the other homozygous and heterozygous genotypes, which points to the association of a risk factor connected with allele A and T2DM. This study confirms the presence of an interesting correlation between the polymorphism of the *LEP* gene and T2DM, which can serve as a marker for the assessment of several T2DM-related parameters in the Iraqi population.

Funding: This work was supported by the University of Babylon, Babil province, Iraq (No. UOB-688, UOB-01-19).

Acknowledgments: The authors thank the T2DM Treatment Center in Al-Hussein Medical City and Al-Imam Al-Hujja Hospital, Karbala, for their help and support in the experimental procedures.

Author contributions: KNJM developed the work, helped in the design and the experimentation, and contributed to writing the draft. FZH, MJE, and ES contributed to the supervision of the work and analysis of data. TMA performed statistical analyses. MBSA analyzed the data and wrote the manuscript.

Conflict of interest disclosure: No conflict of interest is reported by the authors

REFERENCES

1. Mohammed SM, Hasan AS, Al-Hindy HAAM, Mousa MJ. C-Reactive Protein is Associated with the Severity of Periodontal Disease—An Observational Study Among Acute Myocardial Infarction Patients. Sys Rev Pharm. 2020;11:252-7.
2. Al-Mmumin A, Al-Hindy HA-AM. Hyperuricemia Has a Deleterious Role in Patients with Acute Coronary Syndrome Presented with Poor Oral Hygiene. Int J Pharm Sci Res. 2020;12(sp1):193.
3. Coppari R, Bjørbæk C. Leptin revisited: its mechanism of action and potential for treating diabetes.Nat Rev Drug Discov. 2012;11:692-708.
4. Wang DX, Kaur Y, Alyass A, Meyre D. A Candidate-Gene Approach Identifies Novel Associations Between Common Variants in/Near Syndromic Obesity Genes and BMI in Pediatric and Adult European Populations. Diabetes. 2019;68:724-32.
5. Qadir MI. Role of green tea flavonoids and other related contents in cancer prevention. Crit Rev Eukaryot Gene Expr. 2017;27.
6. Zhang F, Chen Y, Heiman M, DiMarchi R. Leptin: structure, function and biology. Vitam Horm. 2005;71:345-72.
7. Londraville RL, Prokop JW, Duff RJ, Liu Q, Tuttle M. On the molecular evolution of leptin, leptin receptor, and endospanin. Front Endocrinol. 2017;8:58.
8. Park H-K, Ahima RS. Physiology of leptin: energy homeostasis, neuroendocrine function and metabolism. Metabolism. 2015;64:24-34.
9. Joffe BI, Panz VR, Raal FJ. From lipodystrophy syndromes to diabetes mellitus. The Lancet. 2001;357:1379-81.

10. Pelleymounter MA, Cullen MJ, Baker MB, Hecht R, Winters D, Boone T, Collins F. Effects of the obese gene product on body weight regulation in ob/ob mice. Science. 1995;269(5223):540-3.
11. Ramos-Lobo AM, Donato Jr J. The role of leptin in health and disease. Temperature. 2017;4:258-91.
12. Lyssenko V, Lupi R, Marchetti P, Del Guerra S, Orho-Melander M, Almgren P, Sjögren M, Ling C, Eriksson KF, Mancarella R, Berglund G. Mechanisms by which common variants in the TCF7L2 gene increase risk of type 2 diabetes. J Clin Invest. 2007;117(8):2155-63.
13. Al-Shuhaib MBS. D76V, L161R, and C117S are the most pathogenic amino acid substitutions with several dangerous consequences on leptin structure, function, and stability. Egypt J Med Hum Genet. 2019;20(32):1-12.
14. Musafer KNJ, Zaman Huyop F, J Ewadh M, Supriyanto E, Rava M. A Systematic Mapping Study on the Risk Factors Leading to Type II Diabetes Mellitus. KIJOMS. 2020;6:6.
15. Al-Shuhaib M. A comprehensive in silico prediction of the most deleterious missense variants in the bovine LEP gene. BioTechnologia. 2019;100:429-39.
16. Paracchini V, Pedotti P, Taioli E. Genetics of leptin and obesity: a HuGE review. A J Epidemiol. 2005;162:101-14.
17. Gonzalez-Sanchez JL, Martinez-Larrad MT, Zabena C, Perez-Barba M, Serrano-Rios M. Association of variants of the TCF7L2 gene with increases in the risk of type 2 diabetes and the proinsulin: insulin ratio in the Spanish population. Diabetologia. 2008;51:1993-7.
18. Alami FM, Ahmadi M, Bazrafshan H, Tabarraei A, Khosravi A, Tabatabaiefar MA, Samaei NM. Association of the TCF7L2 rs12255372 (G/T) variant with type 2 diabetes mellitus in an Iranian population. Genet Mol Biol. 2012;35(2):413-7.
19. Roszkowska-Gancarz M, Kurylowicz A, Polosak J, Mossakowska M, Franek E, Puzianowska-Kuźnicka M. Functional polymorphisms of the leptin and leptin receptor genes are associated with longevity and with the risk of myocardial infarction and of type 2 diabetes mellitus. Endokrynol Pol. 2014;65:11-6.
20. Al Busaidi N, Shanmugam P, Manoharan D. Diabetes in the Middle East: government health care policies and strategies that address the growing diabetes prevalence in the Middle East. Curr Diabetes Rep. 2019;19:8.
21. 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;8:156.
22. Wasim M, Awan FR, Najam SS, Khan AR, Khan HN. Role of leptin deficiency, inefficiency, and leptin receptors in obesity. Biochem Genet. 2016;54:565-72.
23. Li HM, Zhang TP, Leng RX, Li XP, Wang DG, Li XM, Ye DQ, Pan HF. Association of leptin and leptin receptor gene polymorphisms with systemic lupus erythematosus in a Chinese population. J Cell Mol Med. 2017;21:1732-41.
24. Wang H, Wang C, Han W, Geng C, Chen D, Wu B, Zhang J, Wang C, Jiang P. Association of leptin and leptin receptor polymorphisms with coronary artery disease in a North Chinese Han population. Rev Soc Bras Med Trop. 2020;53:e20190388.
25. Association AD. Standards of medical care in diabetes—2013. Diabetes Care. 2013;36:S11-66.
26. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem. 1972;18:499-502.
27. Hashim HO, Al-Shuhaib MBS. A Novel DNA Extraction Protocol from Frozen Blood of Normal Individuals and Patients Who Received Systemic Chemotherapy 2020. 2020090683 [Preprint]. 2020 [cited 2020 Sep 18]. Available from: https://doi.org/10.20944/preprints202009.0683.v1
28. Ye J, Coulouris G, Zaretskaya I, Cutcutache I, Rozen S, Madden TL. Primer-BLAST: a tool to design target-specific primers for polymerase chain reaction. BMC Bioinformatics. 2012;13:134.
29. Hashim HO, Al-Shuhaib MBS. Exploring the potential and limitations of PCR-RFLP and PCR-SSCP for SNP detection: A review. J Appl Biotechnol Rep. 2019;6:137-44.
30. Mohammed AK, Al-Thuwaini TM, Al-Shuhaib MBS. Single nucleotide polymorphism rs7908486 of the tcf7l2 gene is highly associated with obesity in the Iraqi population. Arch Biol Scie. 2020:56.
31. Byun SO, Fang Q, Zhou H, Hickford JGH. An effective method for silver-staining DNA in large numbers of polyacrylamide gels. Anal Biochem. 2009;385:174-5.
32. Yeh FC, Yang RC. POPGENE version 1.31, Microsoft window-based freeware for population genetic analysis. University of Alberta and Tim Boyle, Centre for International Forestry Research. Ftp Microsoft Com/Softlib/Slfiles/Hpgl Exe. 1999.
33. Yong Y, He L. SHEsis, a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res. 2005;15:97.
34. Duan D, Jhang J, Wu S, Teng M, Hsu L, Ko Y. Modification effect of sex and obesity on the correlation of LEP polymorphisms with leptin levels in Taiwanese obese women. Mol Genet Genomic Med. 2020;8:e1113.
35. Daniels TE, Sadovnikoff AI, Ridout KK, Lesseur C, Marsit CJ, Tyrka AR. Associations of maternal diet and placenta leptin methylation. Mol Cell Endocrinol. 2020;505:110739.
36. Li L, Wang J, Ping Z, Li Y, Wang C, Shi Y, Zhou W, Zhang L. Interaction analysis of gene variants of TCF7L2 and body mass index and waist circumference on type 2 diabetes. Clin Nutr. 2020;39:192-7.
37. Dallel M, Sghaier I, Finan RR, Douma Z, Hachani F, Letaifa DB, Mahjoub T, Almawi WY. Circulating leptin concentration, LEP gene variants and haplotypes, and polycystic ovary syndrome in Bahraini and Tunisian Arab women. Gene. 2019;694:19-25.
38. Labayen I, Ruiz JR, Moreno LA, Ortega FB, Beghin L, DeHenauw S, Benito PJ, Diaz LE, Ferrari M, Moschonis G, Kafatos A. The effect of ponderal index at birth on the relationships between common LEP and LEPR polymorphisms and adiposity in adolescents. Obesity. 2011;19:2038-45.
39. Lombard Z, Crowther NJ, Van der Merwe L, Pitamber P, Norris SA, Ramsay M. Appetite regulation genes are associated with body mass index in black South African adolescents: a genetic association study. BMJ Open. 2012;2.
40. Nurmi EL, Spilman SL, Whelan F, Scahill LL, Aman MG, McDougle CJ, Arnold LE, Handen B, Johnson C, Sukhodolsky

DG, Posey DJ. Moderation of antipsychotic-induced weight gain by energy balance gene variants in the RUPP autism network risperidone studies. Transl Psychiat. 2013;3:e274-e274.
41. Ovsyannikova IG, White SJ, Larrabee BR, Grill DE, Jacobson RM, Poland GA. Leptin and leptin-related gene polymorphisms, obesity, and influenza A/H1N1 vaccine-induced immune responses in older individuals. Vaccine. 2014;32:881-7.
42. Kasim NB, Huri HZ, Vethakkan SR, Ibrahim L, Abdullah BM. Genetic polymorphisms associated with overweight and obesity in uncontrolled Type 2 diabetes mellitus. Biomark Med. 2016;10:403-15.
43. Landrum MJ, Lee JM, Riley GR, Jang W, Rubinstein WS, Church DM, Maglott DR. ClinVar: public archive of relationships among sequence variation and human phenotype. Nucleic Acids Res. 2014;42:D980-5.
44. Han HR, Ryu HJ, Cha HS, Go MJ, Ahn Y, Koo BK, Cho YM, Lee HK, Cho NH, Shin C, Shin HD. Genetic variations in the leptin and leptin receptor genes are associated with type 2 diabetes mellitus and metabolic traits in the Korean female population. Clin Genet. 2008;74:105-15.
45. Nesrine Z, Haithem H, Imen B, Fadoua N, Asma O, Fadhel NM, Ali B. Leptin and Leptin receptor polymorphisms, plasma Leptin levels and obesity in Tunisian volunteers. Int J Clin Exp Pathol. 2018;99:121-30.
46. Nyante SJ, Gammon MD, Kaufman JS, Bensen JT, Lin DY, Barnholtz-Sloan JS, Hu Y, He Q, Luo J, Millikan RC. Common genetic variation in adiponectin, leptin, and leptin receptor and association with breast cancer subtypes. Breast Cancer Res Tr. 2011;129:593-606.
47. Ma D, Feitosa MF, Wilk JB, Laramie JM, Yu K, Leiendecker-Foster C, Myers RH, Province MA, Borecki IB. Leptin is associated with blood pressure and hypertension in women from the National Heart, Lung, and Blood Institute Family Heart Study. Hypertension. 2009;53:473-9.

Supplementary Material

The Supplementary Material is available at: http://www.serbiosoc.org.rs/NewUploads/Uploads/Musafer%20et%20al_6202.pdf