Arch Biol Sci. 2021;73(1):39-45 https://doi.org/10.2298/ABS201213056M

Single nucleotide polymorphism rs7908486 of the *TCF7L2* gene is highly associated with obesity in the Iraqi population

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Received: December 13, 2020; **Revised**: December 20, 2020; **Accepted**: December 21, 2020; **Published online**: December 25, 2020

Abstract: This study was conducted to assess the possible association between polymorphisms of the transcription factor 7-like 2 (*TCF7L2*) gene and obese Iraqi adults. DNA was obtained from 158 obese subjects and 142 controls. Two specific PCR fragments were designed to incorporate two highly frequent single nucleotide polymorphism (SNP)s within *TCF7L2*, rs11196208 and rs7908486. Both amplified loci were genotyped by PCR-single-strand conformation polymorphism (SSCP) followed by sequencing. Logistic regression analysis was performed to detect the association between both genetic variants and obesity. Concerning rs11196208-based amplicons, PCR-SSCP genotyping showed homogeneous banding patterns for all investigated samples. In contrast with rs11196208, three SSCP banding patterns in the rs7908486-based amplicons, GG, AG and AA, were observed. Individuals with the AA genotype showed significantly higher ($P<0.05$) body mass index (BMI), waist circumference (WC), fasting blood glucose (FBG), and insulin values than those with either AG or GG genotypes. Association analysis revealed that individuals with the A allele exhibited a greater risk of obesity than individuals with the B allele. Data indicate that rs7908486 SNP exerted a tight association with obesity. The study suggests implementing *TCF7L2* rs7908486-based genotyping in the early detection of obesity.

Keywords: Obesity; *TCF7L2*; gene polymorphism; SNP; rs11196208; rs7908486

INTRODUCTION

More than 30% of the populace worldwide is obese, and cases of obesity have accumulated in different populations around the world, particularly in the Middle East. Within this part of the world, the Iraqi population suffers from a steady increase in obesity and its complications. The management of obesity-related traits by classical medical approaches has proven to be difficult due to the 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 [1]. This approach is based on the genotyping of several genetic loci to resolve their possible association with obesity. The transcription factor 7-like 2 (*TCF7L2*) gene has recently been suggested to play a crucial role in the development of obesity [2]. *TCF7L2* is located in chromosome 10q25.3, spanning a transcription element of 215.9 kb and containing seventeen exons, of which five are alternative splicing units [3]. This gene encodes a transcription factor that interacts with ß-catenin and functions through the Wingless type (Wnt) signaling pathway, thereby controlling cell morphology, motility, proliferation and oncogenesis [4]. The TCF7L2 protein has been considered as a key regulator for proper cellular activity and development [5]. Its variation may lead to several long-term outcomes in cellular metabolism and activity [6]. This gene and its many intronic variants have been reported to have a strong correlation with type-2 diabetes mellitus, impaired insulin secretion, synthesis, processing and modulation [7,8]. While polymorphism of *TCF7L2* is closely associated with the increased risk of diabetes mellitus [9], its precise role in obesity remains unclear [10,11].

Iraq is a Middle-Eastern country where the prevalence of overweight and obese individuals is on the increase [12], and the rates of increase in overweight and obese individuals is on the rise among adults [13].

Accordingly, it is may be possible to investigate the involvement of the genetic polymorphisms of the *TCF7L2* gene in obesity-related complications, taking into account the role of high-frequency SNPs in the Middle-Eastern population. Therefore, the study of *TCF7L2* genetic polymorphism can be informative when its high-frequency SNPs are targeted and analyzed alongside obesity-related indices. This study was conducted to investigate two high-frequency single nucleotide polymorphism (SNP)s of the *TCF7L2* coding sequences and to assess their possible association with obesity in Iraqi adults.

MATERIALS AND METHODS

Ethics statement

The study was conducted according to the Helsinki Declaration, following approval by the Institutional Review Board at the University of Babylon (1216/22-010-2019). Written informed consent was obtained from all participants prior to study participation.

Study population

A case-control study was conducted that involved 158 obese and 142 non-obese adults of Iraqi origin, aged 20-39. Obese patients were recruited from the Outpatients Clinic of the Obesity Treatment Center in Baghdad (OTCB), while the non-obese control participants were from the general Iraqi population. Both obese and control individuals were enrolled in this study between March and September 2019. All individuals with diabetes, chronic disease or pregnant women were excluded from this study. In addition to age, several data were considered for all the included participants. The body mass index (BMI) was calculated using the formula BMI= weight (kg)/height^2. The BMI of normal weight, obesity and morbid obesity were defined as 18.5-24.9 kg/m^2, 30-39.9 kg/m^2, and > 40 kg/m^2, respectively [14]. The waist circumference was measured at the narrowest point of the torso widthwise, usually just above the belly button, which is ≤102 cm in males and ≤88 cm in females [15]. Blood pressure was measured on the right arm in the sitting position using a digital sphygmomanometer after five minutes of rest. Three measurements were taken and the average reading was then computed.

Biochemical assays

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 on a spectrophotometer (UV-160, Shimadzu, Kyoto, Japan) according to the manufacturer's recommendations (Biolabo, Les Hautes Rives, France). LDL was calculated using Friedewald's formula [16]. Serum insulin concentration was determined using an ELISA kit (Elabscience, USA).

Sample collection and DNA extraction

Blood samples were collected from all participants (158 with obesity and 142 controls). Genomic DNA was extracted using a rapid salting-out method [17]. The purity of the isolated DNA was assessed by agarose gel electrophoresis and quantified by Nanodrop (Biodrop, UK).

PCR primer design

Based on the sequences of the *TCF7L2* (GenBank acc. no. NC_000010.11), two pairs of PCR primers pairs were designed. Both pairs of oligonucleotides were specifically designed to flank two intronic high frequent SNPs, rs11196208 and rs7908486, that are localized in introns 3 and 4, respectively (Fig. 1A). The referred PCR-specific primer pairs were designed by the NCBI primer BLAST server to incorporate the targeted SNPs in the middle of the designed fragments [18]. The lengths of both genetic fragments (242 bp and 337 bp) were made to suit the recommended amplicon lengths in the PCR-SSCP protocols [19]. Concerning rs11196208-based amplicons, the sequences of forward and reverse primers were 5'-GTTGGAGA-TAGTCTTCTGTCCCT-3' and 5'-CCTCAGACGAC-CACAAACCA-3', respectively. The targeted rs11196208 SNP was located at position 171 bp within the amplified 242 bp amplicon. The sequences of forward and reverse primers of the rs7908486-based amplicons were 5'-CGGGGCTCATCACACATTGAC-3' and 5'-TT-GATAATTCTTTGGGAGGGTGAGG-3', respectively. The targeted rs7908486 SNP was located at position 156 bp within the amplified 337 bp amplicons.

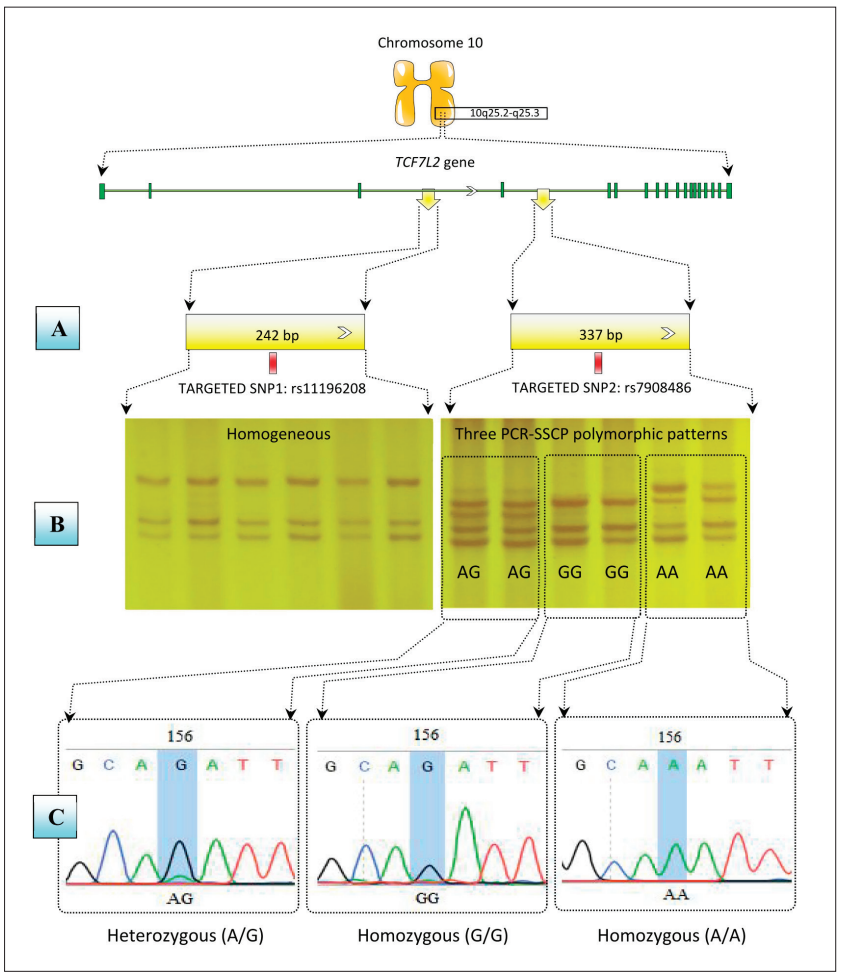

Fig. 1. A schematic diagram of the *TCF7L2* gene-based PCR-single strand conformation polymorphism (SSCP)-sequencing strategy. **A** – PCR design of two PCR specific primers pairs for the amplification of 242 bp and 337 bp containing rs11196208 and rs7908486 SNPs, respectively. **B** – Post-PCR genotyping using SSCP in which rs7908486 showed three patterns of nucleic acid variations. **C** – Sequencing results of the three detected PCR-SSCP genotypes in which three patterns of AA/AG/GG were identified in the targeted SNP.

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 premix. Then, 10-30 ng in 3 µL of the genomic DNA template was added and the final PCR volume to 20 µL. The annealing temperatures were determined empirically by using a gradient PCR thermocycler (Mastercycler® nexus, Eppendorf, Germany). The PCR experiment was conducted as follows: one cycle of initial denaturation at 94°C for 5 min, 30 cycles of denaturation at 94°C for 30 s, annealing (at 59.9°C and 60.9°C for rs11196208-based and rs7908486-based amplicons, respectively) for 30 s, elongation at 72 °C for 30 s, and a final extension at 72°C for 5 min. The conducted PCR experiments were verified by electrophoresis on a 1.5% (w/v) agarose gel in parallel with a DNA marker before being exposed to downstream genotyping reactions.

Post-PCR genotyping

Genotyping experiments were conducted by PCR-single strand conformation polymorphism (PCR-SSCP) as described [20]. The PCR products were heat-denatured at 95 °C for 7 min and chilled on ice for 10 min. Denatured samples were loaded on neutral polyacrylamide gels. Electrophoresis was conducted at constant conditions as follows: 8% gel, room temperature, 4 h 200 V, 100 mA. SSCP gels were stained using a rapid silver staining protocol [21]. Each detected PCR-SSCP banding pattern was sent for sequencing using both forward and reverse primers in separate reactions. Sequencing protocols were performed as recommended by Macrogen sequencing laboratories (Macrogen Inc., South Korea). Only clear electropherograms of Applied Biosystem sequence files were considered for further annotations. The chromatogram sequencing files were processed using the BioEdit tool, ver. 7.1 (DNASTAR; Madison, USA). The sequences of the PCR-SSCP patterns were aligned alongside the deposited referring sequences of the *TCF7L2* gene using the GenBank accession number NC_000010.11.

Statistical analysis

Statistical analysis of the data was performed using SPSS, ver. 23.0 (IBM, NY, USA). Data were expressed as the mean±standard deviation (SD), least-square

means±standard error (LSM±SE), and percentage. The allele and genotype frequencies were analyzed using PopGen32 software, ver. 1.31 [22]. Deviation from the Hardy-Weinberg equilibrium (HWE) between the case and control samples in the investigated population was calculated by the χ^2 test. Logistic regression analysis was used to examine the relationship between *TCF7L2* polymorphisms and obesity. Comparison of the baseline variables of the study population was analyzed using the Student's t-test. The significant effect of genotype on the different obesity-related parameters studied was analyzed using one-way ANOVA. Multiple pairwise comparisons between main factors were performed using the Tukey-Kramer method, which is statistically significant at a level of P<0.05.

RESULTS

The characteristics of the studied population

Table 1 shows the baseline variables of the study population, which consisted of 142 non-obese and 158 obese individuals. The prevalence of obesity in females was higher than in males (P<0.0001). Age, BMI, WC, FBG and insulin concentrations were significantly higher (P<0.01) in obese subjects when compared to non-obese subjects.

Table 1. Baseline characteristics of the study population

Characteristic	Non-obese N=142	Obese N= 158	P-value
Gender (M/F)	90/52	51/107	**<0.0001**
Age (year)	27±3.54	30±4.23	**0.02**
BMI (kg/m2)	23.10±2.45	3.3136	**<0.0001**
WC (cm)	84.50±7.43	114±10.53	**<0.0001**
SBP (mmHg)	11.80±0.84	12.50±0.93	0.14
DBP (mmHg)	7.90±0.71	8.25±0.78	0.39
FBG (mmol/L)	4.35±0.23	6.10±0.13	**0.01**
Insulin (ng/mL)	22.28±4.36	29.40±5.32	**<0.0001**
TC (mmol/L)	4.10±0.37	5.10±0.41	0.31
HDL-C (mmol/L)	1.22±0.03	1.16±0.01	0.26
LDL-C (mmol/L)	2.50±0.30	2.90±0.32	0.46
TG (mmol/L)	1±0.05	1.30±0.03	0.82

Values are expressed as the mean±standard deviation (SD). 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.

Genetic association analysis

In this study, two high-frequency intronic SNPs, rs11196208 and rs7908486, were targeted within the *TCF7L2* gene. Specific primers were designed to flank each of these SNPs in each fragment individually. Unlike the targeted rs11196208 SNP that showed no polymorphic pattern in the amplified 242 bp fragments, the polymorphic nature of the rs7908486 SNP was confirmed in the investigated population as it was observed in the 337 bp fragments. Three clear PCR-SSCP banding patterns were detected in the analyzed rs7908486 SNP-based 337 bp amplicons, two homogeneous (GG and AA) and one heterogeneous (AG) (Fig. 1B). These three identified PCR-SSCP banding patterns were confirmed by the conducted sequencing experiments in the investigated 337 bp amplicons. Accordingly, three patterns of nucleic acid variations (A/G, G/G and A/A) were validated in the targeted rs7908486 SNP (Fig. 1C). Another confirmation for the observed homogenous PCR-SSCP banding patterns of the rs11196208-based 242 bp amplicons was made from sequencing experiments that showed no genetic polymorphism in this targeted locus.

Genetic diversity of the polymorphic rs7908486 SNP was examined to assess whether the investigated population was in HWE. The chi-squared test of the studied genotypes revealed that the rs7908486 (A/G) SNP deviated from HWE in both obese (χ^2 27.34, P-value 0.001) and non-obese (χ^2 5.14, P-value 0.023) populations (Table 2).

The association between *TCF7L2* and obesity was calculated by comparing the allele and genotype frequencies of rs7908486 (A/G) in the 158 obese patients and 142 healthy controls using logistic regression analysis (Table 3). In the obese group, the frequencies of the A

Table 2. Hardy-Weinberg equilibrium (HWE) for the *TCF7L2* gene between the two groups (obese vs non-obese).

Genotypes	Obese		Non-obese	
	Observed	Expected	Observed	Expected
AA	74	58	49	42
AG	44	75	57	70
GG	40	24	36	29
$\chi 2$	27.34		5.14	
P-value	0.001		0.023	

Deviation from HWE expectations were determined using the chi-squared test. The *P*-value with statistical significance is indicated in bold numbers.

Table 3. Association analysis of *TCF7L2* rs7908486 (A/G) polymorphism with obesity.

rs7908486 (A/G)	Obese	Non-obese	Logistic Regression analysis	
Alleles	**N (%)**	**N (%)**	**Odds ratio (95% Cl)**	***P*-value**
A	96 (61)	78 (55)	2.15 (0.41-3.04)	**0.02**
G	62 (39)	64 (45)	Reference	
Total	158	142		
Genotypes				
AA	74 (46.9)	49 (34.5)	2.96 (0.65-3.87)	**0.03**
AG	44 (27.8)	57 (40.1)	1.96 (0.65-3.87)	0.15
GG	40 (25.3)	36 (25.4)	Reference	
Total	158	142		

The P-value with statistical significance is in bold. CL – confidence interval.

and G alleles were 61% and 39% respectively, higher than in the non-obese group (A and G allele frequencies were 55% and 45%, respectively). When comparing the obese and non-obese groups, it was noted that obese individuals showed a significantly higher frequency of homozygote genotype AA (P-value=0.03, odds ratio 2.96). This observation indicated that individuals with the A allele showed a greater risk of obesity (P-value=0.02, odds ratio 2.15) than individuals with the G allele, suggesting a strong association of obesity with the increased frequency of this risk allele.

Genotype-phenotype correlation

A significant association between the observed genotypes of the rs7908486 SNP-based *TCF7L2* variation and obesity were observed. Association analysis indicated that individuals with the AA genotype exhibited significantly higher BMI, WC, FBG and insulin concentrations than those with AG and GG genotypes (P<0.05) (Table 4). However, no noticeable association was observed in the other traits measured (SBP, DBP, TC, HDL-C, TG and LDL-C) as the interactions among the AA, AG, and GG genotypes did not approach statistical significance.

Table 4. Association analysis of genetic variants of the *TCF7L2* gene with obesity-related parameters.

Indices	Genotypes of the *TCF7L2* gene (LSM±SE)			*P*-value
	AA (123)	**AG (101)**	**GG (76)**	
BMI (kg/m^2)	39.37±3.89	34.16±3.69	33.84±3.69	**0.001****
WC (cm)	117.12± 12.41	111.93±11.94	102.22±9.27	**0.001****
SBP (mmHg)	12.41±0.81	12.01±0.92	11.18±1.02	0.07
DBP (mmHg)	8.35±0.62	8.10±0.70	7.31±0.65	0.08
FBG (mmol/l)	6.16±0.71	4.83±0.64	4.36±0.81	**0.02***
Insulin(ng/ml)	33.02±6.70	29.61±4.19	27.75±5.67	**0.03***
TC (mmol/l)	5.23±0.76	5.22±0.68	5.31±0.65	0.93
HDL-C (mmol/l)	1.16±0.04	1.18±0.06	1.23±0.07	0.64
TG (mmol/l)	1.31±0.03	1.27±0.02	1.36±0.01	0.55
LDL-C (mmol/l)	2.94±0.04	2.81±0.06	2.94±0.07	0.98

LSM±SE – least square means±standard error, *(P<0.05), **(P<0.01). 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.

DISCUSSION

The *TCF7L2* gene is one of the most common associated loci clinically reported in type 2 diabetes in most parts of the world, and many SNPs have been recognized to constitute a risk factor for type 2 diabetic patients [23-26]. However, the potential risk of a specific allele of its genetic fragment with obese subjects is not clear. Our study describes the association between *TCF7L2* variations and obesity-related traits in Iraqi adults. This association was based on the genotyping of two high-frequency SNPs positioned in introns 3 and 4. PCR-SSCP was used in post-PCR genotyping because of its low cost and high sensitivity for large-scale samples [19]. Concerning the intron 3 SNP (rs11196208), no genetic polymorphism was detected in the investigated samples. Irrespective of the reported high frequency of the rs11196208 SNP in the *TCF7L2* database [27], the absence of any detectable genetic variation in the analyzed rs11196208 variant might be due to the possible absence of any noticeable role of the association of this SNP with obesity in the analyzed population. In contrast to the rs11196208 SNP, three genotypes were observed in the intron 4 SNP (rs7908486). Thus, rs7908486-based genotyping yielded distinctly different genetic patterns of the utilized fragment. Three patterns of GG/AG/AA might be associated with the potential role of these variations with demographic, clinical and biological traits. We observed an association of rs7908486-based GG/AG/AA patterns with the BMI, WC, FBG and insulin variables. *TCF7L2* variants were

previously reported to be associated with BMI, WC and body fat composition in type 2 diabetes that may be due to the role of *TCF7L2* in adipocyte differentiation [28]. *TCF7L2* is an important transcription factor involved in the Wnt signaling pathway that affects the expression of proglucagon, influencing blood glucose regulation and insulin [29]. Individuals with the AA genotype exhibited more obesity-associated values than those with AG and GG genotypes. This points to an important role of the A allele in the onset of obesity. In addition to BMI values, the effects on WC, FBG and insulin also correlated with the genotypes. This suggested a clear association of rs7908486-based genotypes with the clinical and biological traits measured. Whereas no previous study has clearly linked this SNP to either the onset of obesity or diabetes, it is possible that *TCF7L2* polymorphism is a causative factor for several effects associated with the clinical and biological traits of type 2 diabetes [30]. While the rs7908486 SNP has not been reported in the ClinVar database and is scarcely mentioned in the literature [31], this study observed a link between this locus and distinct obesity-related traits. Apart from our finding, an association between the *TCF7L2* gene and a variety of clinical and biological traits has been reported [11,32,33]. Although a relationship between the rs11196208 SNP-based polymorphism and clinical and biological traits was not found in this study, our results suggest that the rs7908486 SNP-based polymorphism of the *TCF7L2* gene presents a susceptibility locus for obesity in Iraq. The pathophysiological mechanism of the *TCF7L2* gene in susceptibility to obesity remains to be established.

CONCLUSIONS

The genotyping experiments of the *TCF7L2* gene polymorphism demonstrated variations in the rs7908486 SNP in the investigated group of adults in Iraq. Individuals with the AA genotype exhibited more divergent BMI, WC, FBG and insulin values than individuals with AG and GG genotypes, which points to the association of a risk factor connected with allele A and obesity. *TCF7L2* gene variation has an interesting correlation with obesity and can serve as a marker for the assessment of several obesity-related parameters in the Iraqi population.

Funding: This work was partially supported by the Obesity Treatment Center, Baghdad Province, Iraq (No. OTCB-1243, OCT-13-18).

Acknowledgments: The authors thank the Obesity Treatment Center, Baghdad, for the help and support in the experimental procedures.

Author contributions: AKM developed the work and helped in the design and experimentation. TMA contributed to the experiments and performed statistical analyses. MBSA analyzed the data and wrote the manuscript.

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

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