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Association of *GHRL* and *GHSR* Gene Polymorphisms with Type 2 Diabetes Mellitus Susceptibility and Metabolic characteristics: A case-control study in an Iraqi population

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Abstract

Type 2 diabetes mellitus (T2DM) is multifactorial, genetically predisposes and influenced by environment. The ghrelin (*GHRL*) and ghrelin receptor (*GHSR*) genes are related to appetite, insulin release, glucose metabolism and may be associated with susceptibility to T2DM. The aim of this study was the relationship between the *GHRL* rs3755777 and the *GHSR* rs495225 and rs572169 polymorphisms and T2DM risk and biochemical parameters in Iraqi population. This case–control study comprised 100 T2DM patients and 51 healthy controls. TaqMan™ SNP Genotyping was used for genotyping. The genotype and allele frequencies, Hardy–Weinberg equilibrium, genetic models, and multivariable logistic regression adjusted for age and sex were analyzed. There was difference in the genotype and allele distribution of the loci rs3755777 and rs495225. The T allele of rs3755777 showed a protective association under the dominant (OR = 0.22, 95% CI: 0.09–0.51) and allelic (OR = 0.43, 95% CI: 0.27–0.70) models. The allelic association of rs572169 was weak and didn't show an independent association in the multivariable model. Within the T2DM group, the differences in biochemical parameters by genotype weren't statistically significant following correction for multiple comparisons, the nominal association between rs495225 genotype and HDL-C was explored. The BMI adjusted sensitivity analysis revealed a high association of BMI and T2DM, while the individual SNP associations were reduced. The results indicate that genetic polymorphisms of *GHRL* and *GHSR* may play a role in the susceptibility to T2DM in this Iraqi population, but the results need to be confirmed in larger and independent population.

Keywords

diabetes, ghrelin, receptor, polymorphisms, metabolism

Introduction

Type 2 diabetes mellitus (T2DM) is one of the most common metabolic diseases worldwide and is characterized by elevated blood glucose levels over a prolonged period of time resulting from insulin secretion defects, insulin resistance and/or a combination of these (DeFronzo et al., 2015; Zheng et al., 2018; American Diabetes Association, 2024). It is a complex disease in that there are several genes and environmental factors that play a role in the disease, including obesity, diet and inactivity. While lifestyle factors are important in the onset of disease, there is also an important role for genetic variation in the individual susceptibility and disease progression. Pancreatic β-cell function, insulin sensitivity and a number of endocrine pathways are involved in glucose homeostasis in a coordinated manner. The 28-amino-acid peptide hormone ghrelin is encoded by the *GHRL* gene and primarily synthesized in the stomach and is well known for its role in appetite and growth hormone secretion, but has also been demonstrated to be involved in glucose metabolism and energy homeostasis (Tschöp et al., 2000; Kojima & Kangawa, 2005; Sun et al., 2007; Pradhan et al., 2013; Müller et al., 2015). The biological actions of ghrelin are thought to be mediated by a G protein coupled receptor, the growth hormone secretagogue receptor (GHSR), which is expressed in the hypothalamus, pancreatic islets and several other peripheral tissues. Experimental studies have demonstrated that the activation of ghrelin–GHSR signaling pathway has an effect on pancreatic β-cell activity, insulin secretion and glucose homeostasis. In humans, ghrelin also has an inhibitory effect on insulin secretion in response to glucose, indicative of a role in the control of metabolism (Broglio et al., 2001; Tong et al., 2010). Additionally, the interaction of ghrelin, GHSR and the endogenous antagonist LEAP2 have been shown to be important regulators of glucose metabolism and insulin sensitivity (Holst & Schwartz, 2004; Gehlert, 2004; Mani et al., 2014; Wellman & Abizaid, 2015; Geary et al., 2022). Variations in the genes encoding GHRL and GHSR can affect either the expression of the genes or the activity of the GH receptor or the downstream signaling pathways and therefore the susceptibility to metabolic diseases. Association studies in humans have shown conflicting results about the association of ghrelin related polymorphisms and T2DM. A meta-analysis from Li et al. (2012) indicated that different ethnic groups might have different genetic effects and Rivera-León et al. (2020) found that *GHRL* variants were associated with protection for T2DM in a Mexican population. Additionally, there has been a number of studies on the association between GHSR polymorphisms and glucose regulation and insulin secretion, with inconsistent results across different populations (Geiger et al., 2012; Lindqvist et al., 2017; Wang et al., 2020; Villanueva-Paz et al., 2021; Selçuk et al., 2022; Oğuz et al., 2022). Although numerous genetic studies have been carried out across the world, data on the association of genetic polymorphisms of *GHRL* and *GHSR* with susceptibility to T2DM are scarce in Middle Eastern populations especially in Iraq. Specific markers for a population could be used to increase the understanding of susceptibility to disease and metabolic variation. Therefore, the current study was designed to evaluate the association of the *GHRL* polymorphisms (rs3755777) and *GHSR* polymorphisms (rs495225 and rs572169) with the susceptibility to T2DM in Iraqi case–control population. Moreover, the study assessed the associations of these genetic polymorphisms with biochemical parameters in T2DM patients.

Material and methods

Study design and participants

The relationship between selected *GHRL* and *GHSR* gene polymorphisms and the susceptibility to T2DM in an Iraqi population was investigated using a case–control study. A total of 151 subjects were included in the study, of which 100 were patients with T2DM diagnosed based on the diagnostic criteria (American Diabetes Association, 2024) and 51 were apparently healthy subjects without a previous history of diabetes or any major metabolic disorders. People with conditions that might affect glucose metabolism were excluded. Demographic data (age and sex) were obtained for all subjects and anthropometric and biochemical parameters were determined to characterize metabolic differences between the groups.

The study protocol was approved by the Research Committee of Nineveh Health Directorate, Training and Human Development Center, Mosul, Iraq (Decision No. 292; Protocol No. 2025292; approved on 3 December 2025). All participants provided written informed consent prior to participation. The study procedures were carried out in accordance with the ethical principles of the Declaration of Helsinki.

Biochemical measurements

All subjects had peripheral blood samples taken for biochemical analysis. Fasting blood glucose (FBG) and glycated hemoglobin (HbA1c) were obtained as markers of glycemic status. The lipid profile parameters, including total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and very-low-density lipoprotein cholesterol (VLDL-C), were measured. To assess metabolic changes associated with T2DM, biochemical parameters of the kidney, including urea and creatinine, were also measured. Standard laboratory procedures for biochemical analysis were used.

DNA extraction and genotyping

Genomic DNA was isolated from peripheral blood samples using the Genomic DNA Mini Kit (Blood/Cultured Cell; Geneaid Biotech Ltd., Taiwan) according to the manufacturer's instructions. Three single nucleotide polymorphisms (SNPs)—rs3755777 in the *GHRL* gene and rs495225 and rs572169 in the *GHSR* gene—were genotyped using pre-designed TaqMan™ SNP Genotyping Assays (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA). The corresponding assay IDs were C__27512105_10 for rs3755777, C__1079488_1_ for rs495225, and C__1079489_20 for rs572169.

The selected variants were investigated because of their potential relevance to GHRL/GHSR signaling and their reported or predicted relationship with metabolic regulation. All samples were amplified and allelic discrimination was performed using a real-time PCR instrument according to the manufacturer's recommended protocol. Ten samples were independently re-genotyped as a quality-control check, and complete concordance (100%) was observed between the original and repeat genotype calls.

SNP annotation

Genomic annotation and predicted functional characteristics of the investigated variants were obtained from established public genomic

databases, including dbSNP and Ensembl, and are summarized in Table 1. The functional descriptions represent predicted or reported biological relevance and were not experimentally validated in the present study.

Statistical analysis

Normality of continuous variables was assessed using the Shapiro–Wilk test, and appropriate parametric or non-parametric tests were used according to the distribution of the data. Continuous variables between patients and controls were compared using Welch's t-test; the Mann–Whitney U test was used for non-normally distributed variables. Categorical variables were compared using the chi-square test. Genotype and allele frequencies were compared between patients and controls using chi-square tests. Hardy–Weinberg equilibrium was assessed in the control group. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to assess associations between polymorphisms and T2DM risk under different genetic models. The associations between genotype and biochemical parameters among patients with T2DM were evaluated using one-way analysis of variance (ANOVA). Because multiple genotype–biochemical comparisons were performed, a Bonferroni-adjusted significance threshold of $p < 0.0024$ was applied to the 21 comparisons in Table 6. Multivariable logistic regression was performed to assess the independent associations of age, sex and genetic factors with T2DM risk. The primary model was adjusted for age and sex. Because BMI differed substantially between patients and controls and could produce instability or separation in the conventional model, BMI was additionally examined in a sensitivity analysis using Firth penalized logistic regression. The BMI-adjusted model included age, sex, BMI and the three SNPs modeled per allele. The Hosmer–Lemeshow test was used to assess goodness of fit, and Nagelkerke R^2 was used to describe model explanatory performance. For the primary analyses, a p-value < 0.05 was considered statistically significant. Where multiple testing was specifically applicable, the corresponding Bonferroni-adjusted threshold was used. The study was exploratory, and no *a priori* power calculation was performed.

Results and discussion

Demographic and biochemical characteristics

Demographic and biochemical parameters of the T2DM patients and healthy controls are summarized in Table 2. Significant differences were observed in age, BMI, glycemic parameters, lipid parameters and renal markers between the two groups. Patients with T2DM had higher BMI, HbA1c, FBG, total cholesterol, triglycerides, LDL-C, VLDL-C, urea and creatinine levels, while HDL-C was lower than in controls. Sex distribution did not differ significantly between the groups. Metabolic patterns of this study reflect the typical pattern of type 2 diabetes (mainly glucose disturbance and dyslipidaemia). We also observed a significant difference in BMI between the patients and controls. As body weight is one of the major factors contributing to insulin resistance and metabolic problems we ensured that this was accounted for by performing a sensitivity analysis using Firth's penalized logistic regression.

Association of GHRL and GHSR polymorphisms with T2DM susceptibility

The genotypic and allelic frequencies of the polymorphisms studied are presented in Table 3,

Table 1. Genomic annotation and predicted functional characteristics of the investigated *GHRL* and *GHSR* polymorphisms

SNP ID	Gene	Genomic feature	Variant consequence	Potential biological relevance
rs3755777	*GHRL*	Regulatory region	C>T substitution	May influence *GHRL* expression or transcriptional regulation
rs495225	*GHSR*	Coding region	Synonymous (p. Gly57=)	May affect mRNA processing or translation efficiency
rs572169	*GHSR*	Coding region	Synonymous (p. Arg159=)	May influence *GHSR* expression or represent linkage with functional variants

Table 2. Demographic and biochemical characteristics of the study groups polymorphisms

Variable	Patients (n=100)	Controls (n=51)	p-value
Age (years)	54.17 ± 7.61	49.98 ± 8.83	0.0049
BMI (kg/m²)	29.86 ± 2.39	23.10 ± 0.90	<0.0001
HbA1c (%)	8.40 ± 0.89	5.00 ± 0.33	<0.0001
FBG (mg/dL)	171.97 ± 26.39	88.06 ± 6.27	<0.0001
Total cholesterol (mg/dL)	228.86 ± 26.42	165.47 ± 11.81	<0.0001
Triglycerides (mg/dL)	238.86 ± 61.61	108.57 ± 11.29	<0.0001
HDL-C (mg/dL)	38.74 ± 6.40	55.75 ± 4.72	<0.0001 †
LDL-C (mg/dL)	140.78 ± 22.50	93.33 ± 11.40	<0.0001
VLDL-C (mg/dL)	46.68 ± 13.52	20.48 ± 2.96	<0.0001
Urea (mg/dL)	29.90 ± 6.95	21.57 ± 3.13	<0.0001
Creatinine (mg/dL)	1.03 ± 0.19	0.82 ± 0.08	<0.0001
Sex, male / female, n (%)	59 (59.0) / 41 (41.0)	27 (52.9) / 24 (47.1)	0.477 ‡

Continuous variables were compared with Welch's t-test; † Mann–Whitney U test (non-normal distribution in controls); ‡ chi-square test.

Table 3. Genotype and allele frequencies of *GHRL* and *GHSR* polymorphisms in patients and controls

SNP	Genotype/Allele	Patients n (%)	Controls n (%)	Genotype p	Allele p
rs3755777	CC / CT / TT	46 (46.0) / 39 (39.0) / 15 (15.0)	8 (15.7) / 30 (58.8) / 13 (25.5)	0.0011	—
	C / T allele	131 (65.5) / 69 (34.5)	46 (45.1) / 56 (54.9)	—	0.0007
rs572169	AA / AG / GG	50 (50.0) / 44 (44.0) / 6 (6.0)	18 (35.3) / 25 (49.0) / 8 (15.7)	0.0733	—
	A / G allele	144 (72.0) / 56 (28.0)	61 (59.8) / 41 (40.2)	—	0.0318
rs495225	CC / CT / TT	34 (34.0) / 52 (52.0) / 14 (14.0)	9 (17.6) / 23 (45.1) / 19 (37.3)	0.0027	—
	C / T allele	120 (60.0) / 80 (40.0)	41 (40.2) / 61 (59.8)	—	0.0011

and the functional consequences of the polymorphisms in Table 2. The genotype distributions for all three variants were in Hardy–Weinberg state in the control group (Table 4) indicating the internal consistency of the genotype distributions. The rs3755777 polymorphism showed a significant difference in genotype distribution between patients and controls (p = 0.0011), and the allele distribution also differed significantly. The T allele was less frequent among patients than controls and was associated with lower odds of T2DM under the dominant and allelic models (Table 5). The dominant model showed an OR of 0.22 (95% CI: 0.09–0.51), while the allelic comparison showed an OR of 0.43 (95% CI: 0.27–0.70). The results indicate that this population may have a protective association between the T allele of the rs3755777 and susceptibility to T2DM. The biological meaning of this result must be taken with a pinch of salt, however. The position of rs3755777 and its predicted regulatory role indicate that genetic variation may affect the expression or regulation of *GHRL*, but it was not tested in the current study. The results are thus correlative and not necessarily evidence of a direct functional mechanism. Previous studies of *GHRL* variants have also yielded conflicting results. Ethnic background is a potential confounder for associations between *GHRL* polymorphisms and T2DM as found by Li et al (2012). Likewise, Rivera-León et al. (2020) found a protective association of a *GHRL* variant with T2DM in a Mexican population. Another significant difference between the genotype and allele frequencies of patients and controls was seen for the rs495225 polymorphism in the *GHSR* gene. The recessive and allelic models showed that the T allele was linked to decreased risk of T2DM (Table 5). More importantly, rs495225 remained independently associated with T2DM after adjustment for age and sex in the primary multivariable model (Table 7), with an adjusted OR of 0.469 (95% CI: 0.267–0.824, p = 0.0085). This finding identifies rs495225 as the strongest independent genetic association observed in the present analysis. The possible biological relevance of rs495225 may be related to its location within the coding region of *GHSR*. Although the variant is synonymous, synonymous variants can potentially influence mRNA processing, stability or translation efficiency, or may be linked to another functional variant. However, these possibilities were not tested experimentally in this study and therefore should not be interpreted as established mechanisms. The rs572169 variant showed weaker evidence of association. Its allele distribution differed between patients and controls (p = 0.0318), but the genotype distribution did not reach conventional statistical significance (p = 0.0733), and the variant was not an independent predictor in the multivariable model. Thus, the rs572169 finding should be regarded as exploratory rather than as evidence of an independent susceptibility marker.

Genetic models, biochemical associations, and multivariable analysis

Different inheritance models may produce different estimates of the contribution of genetic variants to disease susceptibility (Table 5). For rs3755777, the dominant and allelic models showed significant protective associations, whereas the recessive model was not significant. This pattern suggests that the observed association is more compatible with a dominant or allele-related effect than with a recessive effect. In the case of rs495225, the TT genotype and the allelic and recessive models were significantly associated with reduced risk of T2DM.

Table 4. Hardy–Weinberg equilibrium analysis in the control group

SNP	Genotype (O)	Observed genotype frequencies	Expected genotype frequencies	χ^2	p-value	Result
rs3755777	CC / CT / TT	8 / 30 / 13	10.37 / 25.25 / 15.37	1.8004	0.1797	HWE
rs572169	AA / AG / GG	18 / 25 / 8	18.24 / 24.52 / 8.24	0.0196	0.8887	HWE
rs495225	CC / CT / TT	9 / 23 / 19	8.24 / 24.52 / 18.24	0.1959	0.6581	HWE

Table 5. Association of *GHRL* and *GHSR* polymorphisms with risk of T2DM under different inheritance models

SNP	Comparison	OR	95% CI	p-value
rs3755777	CT vs CC	0.23	0.09–0.55	0.0006
	TT vs CC	0.20	0.07–0.58	0.0019
	Dominant (CT+TT vs CC)	0.22	0.09–0.51	0.0002
	Recessive (TT vs CC+CT)	0.52	0.22–1.19	0.1167
	T vs C (Allelic)	0.43	0.27–0.70	0.0007
rs572169	AG vs AA	0.63	0.31–1.31	0.2183
	GG vs AA	0.27	0.08–0.89	0.0247
	Dominant (AG+GG vs AA)	0.55	0.27–1.09	0.0858
	Recessive (GG vs AA+AG)	0.34	0.11–1.05	0.0523
	G vs A (Allelic)	0.58	0.35–0.96	0.0318
rs495225	CT vs CC	0.60	0.25–1.45	0.2522
	TT vs CC	0.20	0.07–0.53	0.0010
	Dominant (CT+TT vs CC)	0.42	0.18–0.95	0.0352
	Recessive (TT vs CC+CT)	0.27	0.12–0.61	0.0011
	T vs C (Allelic)	0.45	0.28–0.73	0.0011

The dominant model showed a nominally significant association (OR = 0.42, 95% CI: 0.18–0.95, p = 0.0352), but did not meet the Bonferroni-adjusted significance threshold ($p < 0.0167$). The GG genotype was nominally associated with this disease compared to AA, whereas the dominant and recessive models did not show statistically significant associations. The evidence was not consistent across the models and the variant was not independently associated in the multivariable model, so no strong conclusion about its role in susceptibility to T2DM can be made. Previous genetic association studies have found both positive (Selçuk et al., 2022) and negative (Oğuz et al., 2022) associations between ghrelin-related polymorphisms and T2DM in Turkish populations. The differences suggest that genetic influences of the ghrelin pathway might differ depending on population background and other environmental and genetic differences.

Association between genotypes and biochemical parameters

The association of the studied genotypes with biochemical parameters in T2DM patients is presented in Table 6. Only diabetic subjects were compared in this analysis to avoid confounding that would arise from directly comparing patients with healthy controls. For rs3755777, none of the genotype-associated differences remained statistically significant after application of the Bonferroni threshold of $p < 0.0024$.

Although some nominal differences were observed, these should therefore be regarded as exploratory and not as definitive genotype–biochemical associations. Similarly, no statistically significant genotype-associated differences were observed for rs572169 after the multiple-comparison threshold was applied. This finding is consistent with the absence of a strong independent association of rs572169 with T2DM risk. For rs495225, HDL-C differed among genotype groups at the conventional significance level ($p = 0.007$), but this did not meet the Bonferroni-adjusted threshold of $p < 0.0024$. Therefore, the observed HDL-C difference should be considered exploratory rather than a confirmed genotype-associated metabolic effect.

Overall, the updated analysis does not support the earlier interpretation that rs3755777 or rs495225 carriers have definitively more favorable glycemic and lipid profiles. Instead, the findings indicate that the principal evidence in this study concerns T2DM susceptibility, particularly the independent association of rs495225, whereas genotype–biochemical associations require further investigation.

Multivariable logistic regression analysis

Independent associations of clinical and genetic factors with T2DM risk are shown in Table 7. Age was significantly associated with T2DM risk (adjusted OR = 1.078, 95% CI: 1.028–1.131, $p = 0.0021$). Sex was not independently associated with T2DM. The rs495225 T allele remained independently associated with lower odds of T2DM (adjusted OR = 0.469, 95% CI: 0.267–0.824, $p = 0.0085$). In contrast, rs3755777 showed a protective association in the multivariable model (adjusted OR = 0.482, 95% CI: 0.279–0.833, $p = 0.0090$), while rs572169 was not independently associated with T2DM (adjusted OR = 0.690, 95% CI: 0.386–1.233, $p = 0.210$). The Hosmer–Lemeshow test indicated adequate calibration of the primary model ($p = 0.999$), and the Nagelkerke R^2 was 0.259, indicating moderate explanatory performance. Because there was a significant difference between BMI of patients and controls (Table 8), a BMI adjusted sensitivity analysis was performed with the Firth penalized logistic regression. The adjusted OR for BMI was large and significant (6.491; 95% CI: 2.706–NE; $p < 0.001$) and the 95% CIs for the SNP estimates were large, with none of the three SNPs demonstrating significance. This attenuation means that the genetic association can only be interpreted in the context of a significant difference in BMI between study groups that existed in this study.

The goal of the use of Firth penalized regression was to account for some of the biases and instabilities that may occur in sparse data and near complete separation. The large confidence intervals and the upper bound that cannot be estimated for some predictors is also a reflection of the lack of

Table 6. Association between *GHRL* and *GHSR* genotypes and biochemical parameters among patients with T2DM (n=100)

SNP	Genotype	HbA1c (%)	FBG (mg/dL)	TC (mg/dL)	TG (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	VLDL-C (mg/dL)
rs3755777	CC (n=46)	8.33 ± 0.88	170.28 ± 28.73	229.98 ± 21.58	243.96 ± 59.32	38.74 ± 6.06	140.26 ± 21.02	45.46 ± 13.48
	CT (n=39)	8.62 ± 0.87	175.03 ± 24.17	227.49 ± 32.99	233.15 ± 62.15	38.41 ± 6.70	137.97 ± 24.78	47.76 ± 13.13
	TT (n=15)	8.07 ± 0.87	169.20 ± 25.32	229.00 ± 21.84	238.07 ± 69.81	39.60 ± 6.96	149.67 ± 19.63	47.59 ± 15.21
	p-value	0.092	0.650	0.912	0.726	0.832	0.228	0.712
rs572169	AA (n=50)	8.46 ± 0.95	170.44 ± 24.75	226.18 ± 27.67	234.88 ± 71.80	38.84 ± 6.44	137.40 ± 20.08	45.15 ± 14.00
	AG (n=44)	8.32 ± 0.88	173.09 ± 29.82	231.18 ± 24.86	238.07 ± 48.48	38.75 ± 6.58	143.84 ± 24.24	48.05 ± 13.56
	GG (n=6)	8.55 ± 0.26	176.50 ± 8.64	234.17 ± 29.19	277.83 ± 49.43	37.83 ± 5.49	146.50 ± 27.93	49.35 ± 8.37
	p-value	0.686	0.812	0.583	0.273	0.937	0.315	0.522
rs495225	CC (n=34)	8.46 ± 0.83	172.12 ± 28.62	231.71 ± 29.92	248.59 ± 62.05	38.79 ± 5.88	142.24 ± 19.79	47.88 ± 13.27
	CT (n=52)	8.36 ± 0.98	170.21 ± 23.55	229.23 ± 24.46	235.52 ± 63.30	39.98 ± 6.33	141.23 ± 22.65	47.03 ± 13.54
	TT (n=14)	8.44 ± 0.70	178.14 ± 31.53	220.57 ± 24.53	227.64 ± 54.64	34.00 ± 6.01	135.57 ± 28.49	42.44 ± 14.22
	p-value	0.864	0.612	0.414	0.485	0.007	0.638	0.435

Data are presented as mean ± SD. Differences among genotype groups were evaluated using one-way ANOVA. These comparisons were exploratory; the Bonferroni threshold for the 21 comparisons is $p < 0.0024$.

precision of the BMI-adjusted estimates in this moderate sample size.

Overall interpretation and study significance

The present study confirms the previous study that genetic polymorphisms of ghrelin signaling pathway is associated with the susceptibility to T2DM in Iraqi population. Among the investigated variants, *GHRL* rs3755777 and *GHSR* rs495225 showed significant case–control differences, while rs495225 remained independently associated with T2DM after adjustment for age and sex. These results are compatible with experimental evidence showing that ghrelin and GHSR are involved in the regulation of insulin secretion and glucose metabolism (Tschöp et al., 2000; Broglio et al., 2001; Kojima & Kangawa, 2005; Sun et al., 2007; Tong et al., 2010; Pradhan et al., 2013; Müller et al., 2015). However, the current study does not establish a direct functional mechanism for any of the investigated variants. The genetic effects of the ghrelin/GHSR pathway have also differed among previous populations (Selçuk et al., 2022; Oğuz et al., 2022), supporting the possibility that ethnicity, environmental exposures, lifestyle and gene–gene interactions may influence the observed associations. The present study also examined genotype–biochemical relationships among diabetic patients, providing information regarding possible metabolic heterogeneity after disease onset. However, after correction for multiple comparisons, the biochemical findings were largely non-significant. Thus, these results should be considered hypothesis-generating rather than evidence of established metabolic effects. Several limitations need to be taken into account. The sample size was moderate, particularly after stratification by genotype, and no *a priori* power

Table 7. Multivariable logistic regression analysis of factors associated with risk of T2DM

Predictor	Adjusted OR	95% CI	p-value
Age	1.078	1.028–1.131	0.0021
Sex (Male vs Female)	1.268	0.591–2.723	0.542
rs3755777 (per T allele)	0.482	0.279–0.833	0.0090
rs572169 (per G allele)	0.690	0.386–1.233	0.210
rs495225 (per T allele)	0.469	0.267–0.824	0.0085

Model adjusted for age and sex. Hosmer–Lemeshow goodness-of-fit test: p=0.999, indicating adequate model calibration. Nagelkerke R^2=0.259, indicating moderate explanatory performance of the model.

Table 8. BMI-adjusted sensitivity analysis using Firth penalized logistic regression

Predictor	Adjusted OR	95% CI	p-value
Age (per year)	1.122	0.888–1.750	0.328
Sex (male vs female)	5.407	0.496–NE	0.195
BMI (per kg/m²)	6.491	2.706–NE	<0.001
rs3755777 per T allele	0.485	0.036–2.473	0.394
rs572169 per G allele	1.376	0.178–8.588	0.709
rs495225 per T allele	0.952	0.141–13.111	0.960

Model adjusted for age, sex and BMI; SNPs are modeled per minor allele. Confidence intervals are profile penalized-likelihood intervals and p values are likelihood-ratio tests; NE, upper limit not estimable because of near-complete separation. Hosmer–Lemeshow p=0.954.

calculation was performed. Functional assays were not performed, so the biological mechanisms underlying the observed associations remain speculative. The study had a candidate-gene exploratory design and was conducted in a single Iraqi population, limiting generalizability to other populations. Although quality-control genotyping of ten samples showed 100% concordance, the study remains subject to the limitations inherent in a moderate-sized case–control genetic study. In addition, the substantial difference in BMI between patients and controls affected the precision of the BMI-adjusted sensitivity analysis. Conclusions should therefore be viewed with caution and confirmed in larger independent populations.

Conclusions

The current case–control study suggests that genetic polymorphisms of *GHRL* and *GHSR* may be associated with T2DM susceptibility in an Iraqi population. The *GHRL* rs3755777 and *GHSR* rs495225 variants showed significant differences between patients and controls, while rs495225 remained independently associated with lower odds of T2DM after adjustment for age and sex. In contrast, rs572169 showed only weak allelic evidence and was not independently associated with T2DM. The genotype-biochemical analyses provided limited evidence after correction for multiple comparisons, and the BMI-adjusted

sensitivity analysis demonstrated the importance of adiposity as a potential confounding factor, with the individual SNP associations attenuated after BMI adjustment. These findings support further investigation of ghrelin-pathway genetic variation in Iraqi and other populations, with larger samples, functional validation and multicenter recruitment needed to determine whether these variants have reproducible and biologically meaningful effects.

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Authors' contributions

H. A. Salih:
Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Writing – original draft; Writing – review & editing.

Conflict of interest

Author declare no conflict of interest.

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