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Impact of gene polymorphism of glutathione S-transferase and ghrelin as a risk factor in Egyptian women with gestational diabetes mellitus

Mai M. Madkour[1*], Afaf M. El-Said[2], Abd El-Aziz A. El-Refaey[3], Abd El-Aziz F. Abd El-Aziz[1] and Fardous F. El-Senduny[1]

Abstract

Background: Gestational diabetes mellitus is the most common metabolic dysfunction that arises during pregnancy. GDM can lead to serious health complications for both the mother during pregnancy and after the delivery of the baby. Additionally, mother–offspring suffers from abnormalities in metabolism. The study aimed to investigate *glutathione S-transferase P1* and *ghrelin* genetic variants in pregnant women diagnosed with gestational diabetes using a tetra-primer amplification refractory mutation system.

Results: This study demonstrated that the frequencies of genotypes in women with GDM were GSTP1-AG (87.1%) and GHRL-GG (100%). The study revealed no significant differences in the frequency of either genotype or allele of both *GSTP1* and *ghrelin* between GDM and healthy pregnant women.

Conclusions: This study may be the first study designed to demonstrate that there is no association between the genotype and allele frequencies of *GSTP1* (rs1695) and *ghrelin* (rs696217) in the development of gestational diabetes mellitus in Egyptian women.

Keywords: Gestational diabetes, Glutathione S-transferase P1 gene, Ghrelin gene, Genetic polymorphism, Lipid profile

Background

Gestational diabetes mellitus (GDM) is the most common metabolic dysfunction of pregnant women detected in the second or third trimester of pregnancy. In recent decades, GDM has been identified in 35% of pregnant women throughout the world [1]. GDM can cause hyperglycemia, macrosomia, high blood pressure, preeclampsia, premature birth, and stillbirth at the end of pregnancy [2]. It is not caused by a lack of insulin but by partially blocking the effect of insulin (known as insulin resistance) by various other hormones produced by the placenta, including steroid hormones (progesterone, estradiol, and cortisol) and peptide placental hormone (human chorionic somatomammotropin (HCS) [3, 4]. Insulin resistance leads to metabolic disorders causing dyslipidemia such as high levels of total cholesterol, triglycerides, LDL-cholesterol, and low levels of HDL-cholesterol. However, hyperlipidemia is a hallmark of the second half of pregnancy to improve fetal growth [5].

Glutathione S-transferase P1 (*GSTP1*) gene maps on the long arm of chromosome 11, which is composed of seven exons. In humans, *GSTP1* exists as a dimer of identical subunits where each subunit contains 210

*Correspondence: maimadkour211@mans.edu.eg
[1] Biochemistry Division, Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt
Full list of author information is available at the end of the article

amino acid residues and two binding sites [6]. *GSTP1* polymorphism (rs1695) is distinguished by an adenine to guanine substitution leading to isoleucine (Ile) to valine (Val) exchange at position 105 of the coding region [7]. The glutathione S-transferases (GSTs) are isoenzyme superfamilies that detoxify toxic substances and protect macromolecules from reactive electrophiles, reactive oxygen species, chemotherapeutic agents, and environmental carcinogens [8].

The short arm of chromosome 3 (3p25-26) contains the sequence for the *ghrelin (GHRL*) gene. *GHRL* is composed of four exons encoding a precursor of preproghrelin (117-aa) [9]. *Ghrelin* polymorphism (rs696217) is distinguished by guanine to thymine substitution leading to leucine (Leu) to methionine (Met) exchange at position 72 in exon 2 [10]. Ghrelin is secreted by enteroendocrine cells in the stomach and binds to its growth hormone secretagogue receptor (GHSR) [11]. Ghrelin regulates the growth hormone secretion from primary pituitary cells via modulating intracellular calcium levels. Additionally, ghrelin plays a role in glucose metabolism. Thus, ghrelin is a key regulator of energy homeostasis [12].

The study aimed to evaluate the single nucleotide polymorphisms (SNPs) in *glutathione S-transferase P1* (A/G rs1695) and *ghrelin* (G/T rs696217) and correlate its genotyping to gestational diabetes mellitus. This may be the first study designed to reveal that there is no association between the genotype and allele frequencies of both *GSTP1* (rs1695) and *ghrelin* (rs696217) in the development of gestational diabetes mellitus in Egyptian women.

Methods

This cross-sectional comparative study evaluated women with GDM according to the ethical standards of the Institutional Research Board, Faculty of Medicine, Mansoura University. Informed consent was taken from each participant. One hundred forty pregnant women (seventy women with GDM and seventy healthy women) with a singleton pregnancy were recruited from the Obstetrics and Gynecology Department, Faculty of Medicine, Mansoura University in the period from July 2017 to March 2019.

All participants were at least 18 years of age and were diagnosed with gestational diabetes at > 24-weeks gestation. Pregnant women with type 1 diabetes mellitus, early macrosomia (baby with a birth weight more than 4000 g), polycystic ovary syndrome, or other associated serious medical disorders (hypertension, renal disease, moderate to severe anemia, thyroid disorder, etc.) interfering with maternal and perinatal outcomes were excluded.

Table 1 Primers used in T-ARMS-PCR of *glutathione S-transferase P1* gene (A/G rs1695)

Primer	Sequence (5′–3′)	Fragment size
FOP	CAGGTGTCA GGTGAGCTCTGAGCA CC	A allele 233 bp (FIP + ROP)
ROP	ATAAGGGTG CAGGTTGTGTCTTGTCCC	G allele 290 bp (FOP + RIP)
FIP	CGTGGAGGACCTCCGCTGCAAAT**C**CA	Two outer primers 467
RIP	GCTCACATAGTTGGTGTAGATGAGGGA **T**AC	

FOR forward outer primer, *ROP* reverse outer primer, *FIR* forward inner primer, *RIP* reverse inner primer

[a] The mismatches of the allele-specific primers are emphasized in bold and underlined

Table 2 Primers used in T-ARMS-PCR of *ghrelin* gene (G/T rs696217)

Primer	Sequence (5′–3′)	Fragment size
FOP	GGGGATTTTTTTTTTTATGGGTTGGTGG	G allele 197 bp (FIP + ROP)
ROP	GGAGGACATTGAGGCAGTAGAGCA GTTG	T allele 266 bp (FOP + RIP)
FIP	GTGGGTTGGTGGTGAGATGTTTACCAA **T**AG	Two outer primer 407
RIP	GTGGACATGAGGGACAAAGTACC**C**CA	

FOR forward outer primer, *ROP* reverse outer primer, *FIR* forward inner primer, *RIP* reverse inner primer

[a] The mismatches of the allele-specific primers are emphasized in bold and underlined

Sample collection and DNA extraction

Venous blood samples were taken from each patient and dispensed in an EDTA-containing tube. The blood sample was separated into two portions (3 ml and 2 ml). The first portion phase was used to collect plasma after centrifugation at > 2000 χ *g* for 10 min. The separated plasma was used in the investigation of the biochemical analysis. The second portion was used for DNA extraction. DNA was separated using a DNA extraction kit (ABIOpure™ Genomic DNA, Cat. No. M501DP). All samples showed bands, which represent the genomic DNA when gel electrophoresis was applied. The DNA quantity and quality were measured by reading the absorbance at $\lambda_{230\ \mathrm{nm}}$ and $\lambda_{260\ \mathrm{nm}}$ by Thermo Scientific™ NanoDrop.

Tetra-primer amplification refractory mutation system (T-ARMS-PCR) analysis

PCR analysis for glutathione S-transferase P1 gene

The primers used in this study (FOP, FIP, ROP, and RIP) are provided in Tables 1 and 2. *GSTP1* gene (A/G rs1695) primers were designed by Primer3 software, while *ghrelin*

gene (G/T rs696217) primers were designed by using http://www.primer1.soton.ac.uk/primer1.html. Polymerase chain reaction (PCR) was performed in a 20 µL volume involving 200 ng of genomic DNA, 3 µL of G allele primers (FOP and RIP) or A allele primers (FOP and RIP) (Table 1), and 10 µL 2 × Taq Master Mix (EmeraldAmp® GT PCR Master Mix–Code No. RR310A). The PCR protocol was as follows: 94 °C for 5 min, followed by 35 cycles at 94 °C for 2 min, annealing at 59 °C for 1 min, and extension at 72 °C for 1 min with a final extension at 72 °C for 10 min. The agarose gel electrophoresis (2%) was performed at 125 V and a 100 bp MaestroGen DNA ladder (Cat. No.02001_500) was used to investigate and confirm the quality of the PCR products (Cleaver Scientific Ltd, UK).

PCR analysis for ghrelin gene

The polymerase chain reaction (PCR) was performed in a 25 µL volume involving 200 ng of genomic DNA, 1 µL of T allele primers (FOP and RIP) or G allele primers (FIP and ROP) (Table 2), 12.5 µL 2 × Taq Master Mix (EmeraldAmp® GT PCR Master Mix, Cat. No. RR310A), and 4.5 µL H_2O. The PCR protocol was as follows: 95 °C for 3 min, followed by 35 cycles at 95 °C for 30 s, annealing at 60 °C for 30 s, and extension at 72 °C for 1 min, and 30 s with a final extension at 72 °C for 10 min. The agarose gel electrophoresis (2%) was performed at 125 V and a 100 bp MaestroGen DNA ladder (Cat. No. 02001_500) was used to investigate and confirm the quality of the PCR products (Cleaver Scientific Ltd, UK).

Biochemical measurements

The plasma lipid profile was assayed following the kit's instructions by Biodiagnostic (Cairo, Egypt). Plasma levels of total lipids, total cholesterol, triglycerides, and HDL cholesterol can be determined according to the methods of Zollner and Kirsch [13], Richmond [14], Fassati and Prencipe [15], and Burstein et al. [16] respectively. Plasma levels of LDL-cholesterol and VLDL-cholesterol were calculated by the Friedewald equation where VLDL equals triglycerides divided by 5 [17]:

$$\text{LDL Cholesterol} = \text{Total Cholesterol} - (\text{HDL Cholesterol} + \text{VLDL Cholesterol})$$

Statistical analysis

Statistical analysis was done using the software package, SPSS version 22, and Excel Software. The data were expressed as mean ± SD. One-way ANOVA was used for determining the significant difference between women with gestational diabetes and healthy pregnant women. *P* values < 0.05 were statistically significant. The frequencies of either genotype or allele of *GSTP1* polymorphism between two groups were analyzed by the Fisher exact test and Hardy–Weinberg equilibrium.

Results

Biochemical investigation of studied groups

Table 3 showed the demographic characteristics of pregnant women between the two groups. The data found that there was no significant difference in the mean age and body mass index (BMI) among women with gestational diabetes and healthy pregnant women.

The level of insulin resistance was higher in the gestational diabetes group with a significant difference compared with the control group. Total lipids, total cholesterol, triglycerides, LDL-cholesterol, and VLDL-cholesterol levels were significantly higher, whereas HDL-cholesterol levels were significantly lower in women with GDM than healthy pregnant women (Table 4).

Genetic polymorphism and genotype frequencies

Genotype analysis of GSTP1 gene

The genetic polymorphisms in the *GSTP1* gene were investigated and the genotypes were shown in Fig. 1. The frequencies of the genotype of the *GSTP1* gene (rs1695) between women with gestational diabetes and healthy pregnant women were listed in Table 5. The distribution of genotypes of *GSTP1* for both groups was in alignment with the Hardy_Weinberg equilibrium, which was analyzed by Fisher's exact test (Table 6).

The major risk of gestational diabetes mellitus was evaluated by the codominant, dominant, recessive, and overdominant models as shown in Table 7. In the codominant model (AA vs AG vs GG), there was no significant risk of GDM (OR 2.40, 95% CI 0.38–14.88, $P = 0.342$) with the A/G and G/G genotypes compared with the AA genotype. The dominant model did not show any significant

Table 3 Demographic characteristics of the involved subjects in this study

Parameters	GDM group ($n = 70$)	Control group ($n = 70$)	OR (95% CI)	*P* value
Age (years) Mean ± SD	28.30 ± 4.68	27.73 ± 3.34	1.0 (0.944–1.06)	0.407
BMI (kg/m^2) Mean ± SD	26.30 ± 2.12	25.73 ± 2.07	1.14 (0.89–1.28)	0.109

The data was presented as a mean and standard deviation

OR odds ratio, *95% CI* 95% confidence interval for the difference between the means for both groups, *GDM* gestational diabetes mellitus. *P* is significant when ˂ 0.05

Table 4 Comparison of insulin resistance and lipid profile of gestational diabetes mellitus in pregnant women with healthy pregnant women

Parameters	GDM group ($n=70$)	Control group ($n=70$)	OR (95% CI)	*P* value
Insulin resistance Mean ± SD	24.20 ± 6.76	7.42 ± 2.66	undefined	˂0.001
Total lipids (mg/dL) Mean ± SD	1145.60 ± 171.20	605.32 ± 155.50	1.02 (1.01–1.03)	˂0.001
Total cholesterol (mg/dL) Mean ± SD	229.11 ± 34.78	150.03 ± 22.95	1.127 (1.09–1.17)	˂0.001
Triglycerides (mg/dL) Mean ± SD	299.25 ± 53.55	140.31 ± 32.26	5.25 (undefined)	˂0.001
LDL-cholesterol (mg/dL) Mean ± SD	142.0 ± 32.69	76.53 ± 18.05	1.16 (1.12–1.23)	˂0.001
VLDL-cholesterol (mg/dL) Mean ± SD	60.0 ± 11.45	29.06 ± 7.05	undefined	˂0.001
HDL-cholesterol (mg/dL) Mean ± SD	25.37 ± 6.09	51.45 ± 6.60	undefined	˂0.001

The data was presented as a mean and standard deviation

OR odds ratio, *95% CI* 95% confidence interval for the difference between the means for both groups, *GDM* gestational diabetes mellitus. *P* is significant when ˂ 0.05

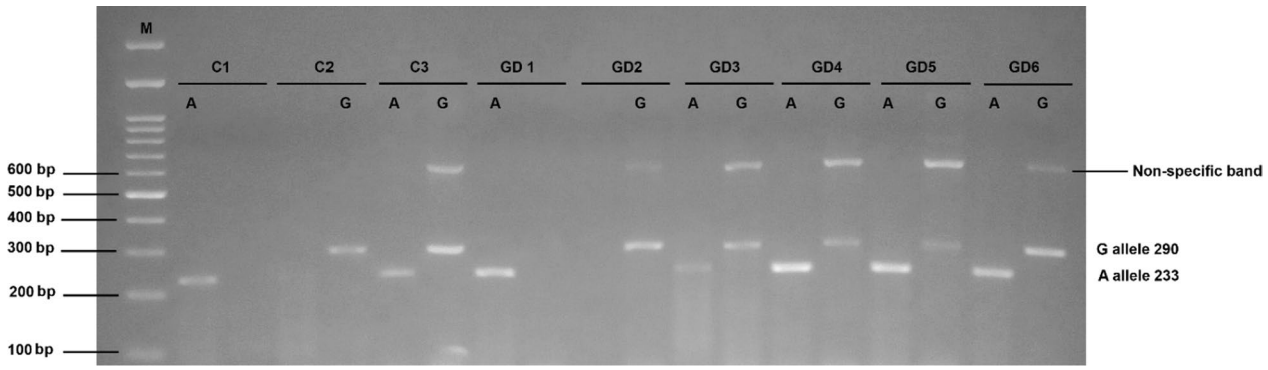

Fig. 1 Agarose gel electrophoresis (2%) of PCR product of tetra-primer ARMS-PCR for analyzing the rs1695 polymorphism in the *glutathione S-transferase P1* gene (*GSTP1*). Lane M showed DNA marker; lane C1 and GD1 showed AA genotype; lane C2 and GD2 showed GG genotype; lanes C3, GD3, GD4, GD4, GD5, and GD6 showed AG genotype (Where *C* control group, *GD* gestational diabetes group)

Table 5 Genotype frequencies of *GSTP1* gene (rs1695) in the current study

Genotype	Amino acid change	All subjects ($n=140$)	GDM group ($n=70$)	Control group ($n=70$)
A/A	Ile/Ile	11 (7.9%)	6 (8.6%)	5 (7.1%)
A/G	Ile/Val	120 (85.7%)	61 (87.1%)	59 (84.3%)
G/G	Val/Val	9 (6.4%)	3 (4.3%)	6 (8.6%)

The data was expressed as frequency and percentage

GDM gestational diabetes mellitus

risk of GDM when compared with the AA genotype (OR 1.22, 95% CI 0.354–4.195, $P=0.753$). The recessive model did not display any significant risk of GDM compared with the A/A-A/G genotype (OR 2.09, 95% CI 0.502–8.73, $P=0.301$). Similarly, the overdominant did not show any significant risk of GDM compared with the A/A-G/G genotype (OR 0.791, 95% CI 0.306–2.05, $P=0.629$).

Table 6 Fisher exact test for Hardy–Weinberg equilibrium in the current study

	GDM group ($n=70$)	Control group ($n=70$)
HWE	$\chi^2=38.96$	$\chi^2=32.95$
P value	$P<0.001$*	$P<0.001$*

GDM gestational diabetes mellitus, χ^2 chi-square test, *HWE* Hardy–Weignberg equilibrium

*Statistically significant if $P<0.05$

Correlation between insulin resistance, lipid profile, and GSTP1 (rs1695) SNP

The association between lipid profile, insulin resistance, and *GSTP1* gene (rs1695) SNP in the population under the study was presented in Table 8. In all these study cases, no significant difference was found between the lipid profile, insulin resistance, and *GSTP1* (rs1695) SNP.

Genotype analysis of GHRL gene

The genetic polymorphisms in the *GHRL* gene were analyzed and the genotypes were shown in Fig. 2. The frequencies of genotype of the *GHRL* (rs696217) between pregnant women diagnosed with gestational diabetes and healthy pregnant women were listed in Table 9. In the distribution of genotypes, there were no significant differences between groups.

Discussion

Gestational diabetes mellitus is the most prevalent disease in pregnant women worldwide. It is a complex metabolic state that is distinguished by insulin resistance [18]. In this study, we assessed a possible association between *glutathione S-transferase P1* Ile105Val SNP (rs1695) as well as *ghrelin* Leu72Met SNP (rs696217) and patient risk of gestational diabetes among Egyptian women.

Our results showed that insulin resistance was related to women with GDM. Previous studies have reported that women with GDM with high insulin resistance had a higher blood glucose level in either an early or a late pregnancy than women with GDM with less insulin resistance [19].

Hyperlipidemia is one of the metabolic disturbances that have been diagnosed in women with GDM. Insulin resistance and estrogen stimulation lead to an increase in plasma lipid levels throughout pregnancy [20]. The levels of total cholesterol, triglycerides, LDL-cholesterol, and

Table 7 Association between genotypes of *GSTP1* with response status in the current study

Model	Genotype	GDM ($n=70$)	Control ($n=70$)	OR (95% CI)	*P*
Codominant	A/A	6 (8.6%)	5 (7.1%)	2.40 (0.38–14.88)	0.342
	A/G	61 (87.1%)	59 (84.3%)	2.07 (0.494–8.65)	0.31
	G/G	3 (4.3%)	6 (8.6%)	1	
Dominant	A/A	6 (8.6%)	5 (7.1%)	1.22 (0.354–4.195)	0.753
	A/G-G/G	64 (91.4%)	5 (92.9%)	–	
Recessive	A/A-A/G	67 (95.7%)	64 (91.4%)	2.09 (0.502–8.73)	0.301
	G/G	3 (4.3%)	6 (8.6%)	–	
Overdominant	A/A-G/G	9 (12.9%)	11 (15.7%)	0.791 (0.306–2.05)	0.629
	A/G	61 (87.1%)	59 (84.3%)	–	

The data was presented as percentage and frequency

GDM gestational diabetes mellitus, *OR* odds ratio, *95% CI* 95% confidence interval for the difference among the means for both groups. *P* is significant when < 0.005

Table 8 Correlation between insulin resistance, lipid profile and *GSTP1* (rs1695) SNP in the current study

Parameters	Genotype AA ($n=11$)	Genotype AG ($n=120$)	Genotype GG ($n=9$)	*P*
Insulin resistance	23.39 (16.19–29.0)	24.08 (17.68–28.89)	30.04 (20.60–35.18)	0.533
Total lipids (mg/dL)	1196.01 ± 229.76	1138.91 ± 170.10	1180.91 ± 16.89	0.697
Total cholesterol (mg/dL)	245.01 ± 25.54	227.59 ± 34.26	228.29 ± 63.70	0.510
Triglycerides (mg/dL)	321.93 ± 70.07	295.51 ± 51.69	329.93 ± 55.77	0.312
HDL-cholesterol (mg/dL)	27.22 ± 2.66	25.24 ± 6.01	24.25 ± 12.83	0.717
LDL-cholesterol (mg/dL)	151.29 ± 27.96	141.39 ± 32.78	135.91 ± 48.77	0.743
VLDL-cholesterol (mg/dL)	63.87 ± 14.37	59.83 ± 11.26	55.79 ± 11.44	0.582

The data was presented as median and interquartile range or mean and standard deviation

95% CI 95% confidence interval for the difference between the means for both groups. *P* is significant when ˂ 0.05

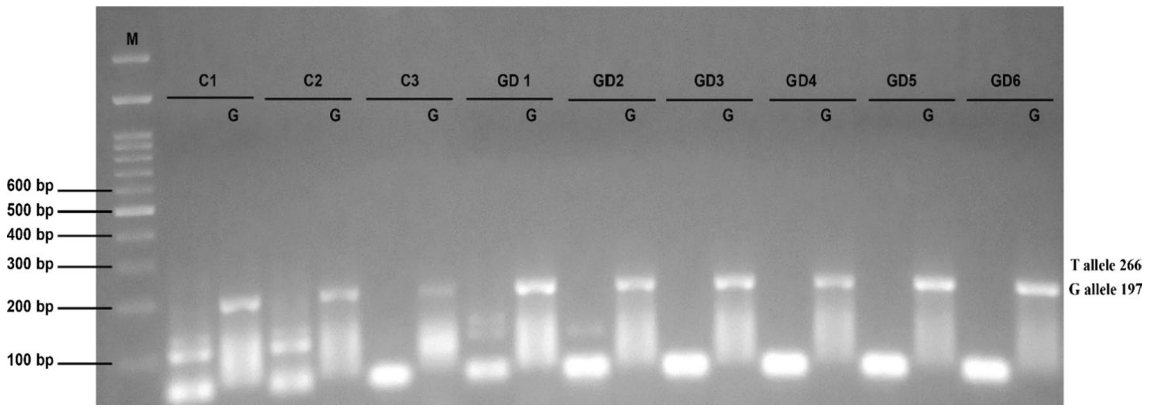

Fig. 2 Agarose gel electrophoresis (2%) of PCR product of tetra-primer ARMS-PCR for analyzing the rs696217 polymorphism in the *GHRL* gene. Lane M showed DNA marker; lane C1, C2, and C3 showed GG genotype; lanes GD1, GD2, GD3, GD4, GD4, GD5, and GD6 showed GG genotype (Where *C* control group, *GD* gestational diabetes group)

Table 9 Genotype frequencies of *GHRL* gene (rs696217) in the current study

Genotype	Amino acid change	All subjects ($n=140$)	GDM group ($n=70$)	Control group ($n=70$)
G/G	Leu/Leu	100 (100%)	70 (100%)	70 (100%)
G/T	Leu/Met	0 (0%)	0 (0%)	0 (0%)
T/T	Met/ Met	0 (0%)	0 (0%)	0 (0%)

The data was expressed as frequency and percentage

GDM gestational diabetes mellitus

VLDL-cholesterol were elevated in GDM due to increasing hyperphagia (an abnormally excessive appetite for the consumption of food-related to hypothalamic damage), progesterone, lipogenesis, and fat storage in the first two-thirds of gestation [21].

Our results showed that total lipids, total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol levels increased and HDL-cholesterol levels decreased in women with GDM. Previous studies revealed that GDM changes cholesterol metabolism where total cholesterol levels were significantly elevated in GDM compared with normal pregnant women. Women with GDM had high levels of LDL cholesterol [22]. Both VLDL cholesterol and triglycerides were significantly elevated in GDM than in normal pregnant women. Shen et al. [23] reported that the levels of lipids increased steadily throughout pregnancy and reached a peak before delivery, but the levels of HDL cholesterol increased from the 1st to 2nd trimester accompanied by a little decrease in the 3rd trimester. However, hyperlipidemia may be a physiological or pathological condition, so it is difficult to determine it. In addition, there are no standards for measuring maternal lipid levels due to the heterogeneity in meta-analysis and the region of the world's population [24].

Gene polymorphisms change the gene expression, structure, and quantity of the products that affect gene function. This is the first study to demonstrate that the frequencies of genotype and the allele of rs1695 in *GSTP1* were not associated with gestational diabetes in Egyptian women. The dispersal of genotypes was in alignment with Hardy–Weinberg equilibrium. Similar to other studies, in a Chinese population, *GSTP1* IIe105Val polymorphisms did not have an impact on the risk of gestational diabetes mellitus [25]. Li et al. [26] found that the *GSTP1* IIe105Val polymorphism was not associated with an elevated risk of gestational diabetes mellitus in a Chinese population. Yalin et al. [27] found that the *GSTP1* polymorphism was suggested to have no effect on the development of diabetes mellitus in Turkish patients. There was no significant association between the *GSTP1* IIe105Val polymorphism and developing type 2 diabetes mellitus in overall studies [28].

Zhang et al. [29] revealed that the *GSTP1* heterozygous genotype is significantly associated with type 2 diabetes mellitus in the north Indian population. There was an

association between the *GSTP1* Ile105Val gene polymorphism in overweight and obese patients for more than 60 years in southern Brazil [30]. Our study showed that there was no significant difference between the lipid profile, insulin resistance, and *GSTP1* (rs1695) SNP in women with gestational diabetes. The current study is in agreement with Amer et al. [31], who demonstrated that there was no significant influence of different genotypes of the *GSTP1* gene on lipid profile in the Egyptian population.

Ghrelin is a key factor in the hypothalamic melanocortin system, which is involved in various bioactivities [32]. The present study revealed that the *GHRL* gene (G/T rs696217) polymorphism was not significantly associated with gestational diabetes in Egyptian women. Rocha et al. revealed that the Gln90Leu polymorphism of the *preproghrelin* gene was not correlated with gestational diabetes in the Euro-Brazilian population [33]. Kim et al. [34] found that the Leu72Met polymorphism of the *preproghrelin* gene is not related to type 2 diabetes mellitus or to its complications. Joatar et al. [35] found that the Leu72Met polymorphism of *GHRL* was not associated with T2DM, IR, or serum ghrelin levels in a Saudi population. No associations were found between genotypes and ghrelin serum levels in a Mexican population [36]. Bai et al. [37] found that the genotype and allele frequencies of *GHRL* gene polymorphisms in participants with obesity showed no significant difference compared to those in nonobese controls in Chinese subjects.

In disagreement with other studies, the Leu72Met polymorphism of the *GHRL* gene had an impact on type 2 diabetes in the Finnish population [38]. In the Caucasian population, there was an association between the Leu72Met polymorphism of the *GHRL* gene and a decreased risk of type 2 diabetes [39]. A *Ghrelin* Arg51Gln polymorphism was detected in the Helsinki population with type 2 diabetes [40]. The Leu72Met polymorphism contributes to the development of obesity in the Swedish population [41].

Conclusions

Worldwide, pregnant women are at high risk of developing gestational diabetes. The study of risk factors will decrease the incidence. Therefore, this study aimed to evaluate the correlation between gene polymorphism in *GSTP1* and *ghrelin* in the development of gestational diabetes. It was found that *glutathione S-transferase P1* IIe105Val (A/G rs1695) and *ghrelin* Leu72Met SNP (G/T rs696217) were not correlated with gestational diabetes mellitus in Egyptian women.

Abbreviations

GDM: Gestational diabetes; GSTP1: Glutathione S-transferase P1; GHRL: Ghrelin; HCS: Human chorionic somatomammotropin; GSTs: Glutathione S-transferases; GHSR: Growth hormone secretagogue receptor; SNPs: Single-nucleotide polymorphisms; T-ARMS-PCR: Tetra-primer amplification refractory mutation system polymerase chain reaction.

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Authors' contributions

MMM contributed to the methodology, analysis, investigation, and writing original manuscript. FE contributed to the methodology, acquisition, analysis, and investigation. AE contributed to the methodology, analysis and investigation. AAE contributed to interpretation of data, supervision, reviewing, and editing of the manuscript. AFA contributed to designing the work, supervision, reviewing, and editing of the manuscript. All authors have read and approved the manuscript for publication.

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Availability of data and materials

The authors can confirm that all relevant data are included in the manuscript.

Declarations

Ethics approval and consent to participate

This study was approved by ethical standards of the Institutional Research Board, Faculty of Medicine, Mansoura University. The patient provided written informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Biochemistry Division, Department of Chemistry, Faculty of Science, Mansoura University, Mansoura, Egypt. [2]Genetic Unit, Department of Pediatrics, Faculty of Medicine, Mansoura University, Mansoura, Egypt. [3]Department of Obstetrics and Gynecology, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

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