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RESEARCH

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Association of gene polymorphisms of *ACE*, *AGT*, and *ARNT*-like protein 1 with susceptibility to gestational diabetes

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Abstract

Background and aims: Gestational diabetes mellitus is well-defined as glucose intolerance first documented during pregnancy. In this study, we examined the possible associations between I/D polymorphism of the angiotensin-converting enzyme gene, the M235T variant of angiotensinogen gene, and the rs7950226 polymorphism of the *ARNT*-like protein-1 (*BMAL1*) gene and the risk for diabetes in Egyptian pregnant women.

Subjects and methods: This study recruited 160 gestational diabetes cases and 165 controls. Genomic DNA was derived from peripheral blood leukocytes and *ACE* gene (I/D) genotyping was performed using the method of polymerase chain reaction and the polymerase chain reaction-based restriction fragment length polymorphism was used for identifying the M235T variant of *AGT* gene and the rs7950226 polymorphism of the *BMAL1*.

Results: The II, ID, and DD genotypes of the *ACE* gene have significant differences in cases compared to controls ($P = 0.000$ and $X^2 = 81.77$). The M235T polymorphism of the *AGT* gene was increased with gestational diabetes risk. Furthermore, the AA genotype of the *BMAL1* rs7950226 gene was significantly related to the gestational diabetes risk ($P = 0.000$ and $X^2 = 52.82$). Furthermore, the allele frequencies of the three variants have significant variances between cases and control.

Conclusion: This study suggested significant associations between *ACE* (DD), *AGT* (TT), and *BMAL1* rs7950226 (AA) gene polymorphisms with gestational diabetes susceptibility and there was a possibility to identify that II + MM + GG as protective haplotypes and DD + TT + AA as risk haplotypes for gestational diabetes.

Keywords: Angiotensin-converting enzyme (*ACE*), Angiotensinogen (*AGT* (M235T), *ARNT*-like protein- 1 (*BMAL1*) genes, Gestational diabetes mellitus (GDM)

Introduction

Gestational diabetes mellitus (GDM) is considered an important adverse side effect in pregnancy, in which women no known to have diabetes develop hyperglycemia during pregnancy [1]. In most cases, chronic insulin resistance and pancreatic β-cell dysfunction result from impaired glucose tolerance and hyperglycemia. GDM risk factors include advanced maternal age, overweight and obesity, and diabetic family history [2]. GDM pathophysiological changes are similar to those in type 2 diabetes mellitus (T2DM) [2]. Accordingly, an interaction between GDM and T2DM is recognized, in which multiple genetic polymorphisms previously reported as risk factors for T2DM are found to increase GDM risk. Those gene polymorphisms are involved in glucose metabolism, insulin secretion, and resistance. Women who suffered from GDM before are at increased risk by seven folds to have T2DM postpartum compared with those who did not have GDM [3].

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In normal pregnancy, cardiovascular system changes observed are due to appropriate adaptation to the renin–angiotensin system (RAS) [4]. Angiotensinogen (*AGT*) is converted to angiotensin I by renin and consequently to angiotensin II, which is more potent, by angiotensin-converting enzyme (*ACE*). *ACE* inactivates bradykinin, which has a major effect on the inflammatory process [5]. Moreover, the *ACE* gene is an important gene and T2DM is one of its abnormalities [6]. Other complications of *ACE* gene abnormality are end-stage renal disease [7], diabetic nephropathy [8], and gestational diabetes [9, 10]. *ACE* polymorphism strongly influences *ACE* activity, as about of total phenotypic plasma variant, 47% of them account for I/D (Insertion/Deletion) allele polymorphism of *ACE*. There is two times higher *ACE* activity in subjects with DD (Deletion/Deletion) allele than those with an II (Insertion/Insertion) allele [11]. Angiotensinogen is one of the most important components of the RAS, a single base pair substitution of thymine (T) with cytosine (C) leading to methionine with threonine substitution at position 235 is the gene mutation of M235T angiotensinogen [10]. *AGT* polymorphism was previously studied in the etiology of T2DM [12].

Recently, a relationship between circadian clock function and the T2DM development has been displayed. BMAL1 is a key component of the mammalian molecular clock. In humans, genetic variants in the *ARNT* like protein-1 (*BMAL1*) gene have been recognized to be related to T2DM susceptibility [13]. Despite previous studies, insufficient understood of the genetic history and the predisposition of GDM and given the fact that GDM shares many features with T2DM, so we studied the relationship of *ACE* (I/D), *AGT* (M235T), and the *ARNT*-like 1 (*BMAL1*, G/A) genetic variations or contributed haplotypes with the possibility of GDM development, in Egyptian pregnant women.

Subject and method

Study subjects

This case–control study was performed at the antenatal care clinic of the Medical Research Center of Excellence (MRCE), Reproductive Health and Family Planning Department, National Research Centre, Egypt. The study involved 325 midtrimester pregnant women: 160 women with GDM and 165 normal pregnant women with normal glucose levels. Selection, screening, and management of diabetes were done according to the criteria of the NICE guidelines [14].

Exclusion criteria were pregnancy with type 1 diabetes mellitus (T1DM), type 2 diabetes mellitus (T2DM), hyper- or hypo-thyroidism, Cushing syndrome, cardiovascular disease, or cancer from the study.

All participants have signed written informed consent and the study had approval from the Ethical Committee of Medical Research at the National Research Centre (NRC).

Sample collection and DNA extraction

Fasting venous blood samples (5 ml) were collected into ethylenediaminetetraacetic acid (EDTA) tubes when conducting routine medical procedures via hospitalization during 24–28 weeks of gestation.

Clinical data and biochemical measurements

Clinical data and biochemical measurements of all subjects were obtained. For each subject we recorded age, weight, height, gestational duration, blood pressure, and family history of T2DM patients. Body mass index (BMI) was calculated as body weight (kg) divided by height square (m^2).

The fasting plasma glucose (FPG) and glycosylated hemoglobin A1c (HbA1c) levels were measured using Stanbio Laboratory, USA. Serum human insulin levels were determined by enzyme-linked immunosorbent assays (ELISA) using kits from Immunospec. Corporation (USA) manufacturers' instructions. Homeostatic Model assessment of insulin resistance (HOMA-IR) was calculated according to the formula: fasting insulin (μ U/L) x fasting glucose (mg/L)/22.5, using fasting values [15].

Isolation of genomic DNA

Genomic DNA was extracted from leukocytes of whole blood by QIA amplification extraction kit, QIAamp® DNA Blood Mini Kit Cat. No. 51104 according to the manufacturer's instructions; the isolated DNA was quantified via until use.

Genotyping of *ACE* (I/D) gene polymorphism

The *ACE* gene I/D polymorphism was identified using polymerase chain reaction (PCR) and suggested primer by [16]. The template DNA (0.5 μL) was amplified by following primers: forward 5-CTGGAGACCACTCCC ATCCTTTCT-3 and reverse 5-GATGTCGCCATCACA TTCGTCAGAT-3. The PCR amplifications were initial denaturation for 5 min at 95 °C; then 35 cycles of denaturation for 30 s at 94 °C, 30 s at 50 °C and extension for 1 min at 72 °C followed by a final extension cycle of 72 °C for 7 min (T-Gradient Thermal Cycler, Biometra, Germany. PCR products (190 bp for the deletion or 490 bp with insertion) were distinguished by electrophoresis on 3% agarose gel containing Fast green. An ultraviolet

transilluminator was used to detect 490 bp and 190 bp fragments.

Genotyping of AGT (M235T) gene polymorphism

The *AGT* (M235T) gene polymorphisms were genotyped using a polymerase chain reaction/restriction fragment length polymorphism (PCR–RFLP) [17] via amplification with a forward primer 5-GATGCGCAC AAGGTCCTG-3 and a reverse primer 5- CAGGGT GCTGTCCACACTGGCTCGC-3. The PCR conditions were 35 cycles of 1 min at 94 °C, 1 min at 61 °C, and 1 min at 72 °C, followed by a final extension at 72 °C for 7 min (T -Gradient Thermal Cycler, Biometra, Germany), and the products were digested by the restriction enzyme SfaNI (New England Biolabs); then, the restriction products was isolated on a 3% agarose gel. The appeared fragments of 266-bp and 303 bp were detected by ultraviolet transilluminator.

Genotyping of *ARNT*-like protein-1 (*BMAL1*) gene polymorphism

The rs7950226 polymorphism of the *BMAL1* gene was investigated by using the PCR–RFLP method through amplifications with the forward primer 5-CATGCTGTG CTTGAATACTCCT-3 and the reverse primer 5- CTA TGAAACCAAGGCTGAAACA-3 [13]. The 310-bp PCR product was digested by RsaI (New England Biolabs, Ipswich, MA) into two DNA fragments of 151 and 159 bp. The DNA fragments were isolated on a 3.5% agarose gel and detected by an ultraviolet.

transilluminator.

Statistical methods

All statistical analyses were achieved using SPSS 20.0. The statistical power and sample size were calculated by PASS 11. Variables were compared by ANOVA test and by the X^2 test for categorical data between studied groups. Compared differences between allele frequencies and genotype distributions in patients with and without GDM were statistically processed using the Hardy–Weinberg equilibrium tests and the chi-square test (X^2) or the Fisher's exact test. The association between the genetic polymorphisms and some risk factors among GDM and controls were assessed using univariate analysis.

Results

Table 1 shows the genotype distribution of *ACE*, *AGT* (M235T), and *ARNT* (*BMAL1*) genes in cases and control group. The results revealed that there were significant change in the *ACE* (I/D), *AGT* M235T, and *ARNT* (G/A) gene polymorphisms among GDM women when compared to the controls, and the frequency of DD, TT, and AA genotypes was significantly higher in GDM ($X^2=81.77$; $P=0.000$, $X^2=109.83$; $P=0.000$ and $X^2=52.82$; $P=0.000$), respectively, when compared to controls.

The relationship between different genotypes of the *ACE* (I/D), *AGT* (M/T), and *ARNT* (G/A) and risk factors between GDM and controls are presented in Tables 2, 3, and 4, respectively. There are significant associations between the above-mentioned genes and some risk factors such as BMI, glucose, HBA1c, insulin, and HOMA-IR (as an index of diabetes) with a *P* value of 0.000. The risk of different variables of GDM depending on the genotypes of the above-mentioned genes is indicated in Table 5.

Gene–gene interactions

The results of gene–gene interactions using the stratification method and haplotype analysis are shown in Table 6. Haplotype analysis indicated the remarkably significant evidence of association with single-nucleotide polymorphism (SNP) combination *ACE* (DD), *AGT* (TT), and *ARNT* (AA) with GDM patients due to the decreased risk of the wild genotypes of *ACE* (II), *AGT* (MM), and *ARNT* (GG) in pregnant women with GDM (0%) as compared to controls (100%) with a *P* value of 0.001, OR 2.4, and 95% CI 1.229–4.688.

Discussion

Gestational diabetes mellitus (GDM) is supposed to have multifactorial causes; one of them may result from gene variations with the influence of some individual effects. GDM is supposed to have multifactorial causes; one of them may result from gene variations with the influence of some individual effects. For prediction, prevention, and management of GDM, genetic risk scores were implemented. These scores were derived from several genes and their single-nucleotide polymorphisms (functional polymorphisms) or haplotypes [18].

This study was conducted on pregnant Egyptian women with GDM; we have investigated the probable associations of *ACE* (I/D), *AGT* (M235T), and *ARNT*-like protein-1 (G/A) genetic variations with the risk of GDM. Though the actual mechanism of GDM development through gestation is still unknown, it could be recognized by increasing insulin resistance caused by maternal adiposity superimposed by the effect of insulin-desensitizing placental products as prolactin, estrogen, and human placental lactogen [19].

In an insertion/deletion (I/D) polymorphism of *ACE* gene with intron 16, 287-base pair *Alu* repetitive sequence is a common occurring variant [20]. This result

Table 1 Genotype distribution of ACE, AGT, and ARNT genes polymorphisms in the studied groups

Genotypes	GDM (n = 160)	Control (n = 165)	X^2 values	*P* values	Risk /OR (95% CI)
ACE (I/D)					
II	8 (5%)	80 (48.5%)			
ID	68 (42.5%)	50 (30.3%)			
DD	84 (52.5%)	35 (21.2%)	81.77	**0.000***	
II	8 (5%)	80 (48.5%)			
ID + DD	152 (95%)	85 (51.5%)	77.8	**0.000***	17.88 (8.25–38.77)
ID + II	76 (47.5%)	130 (78.8%)			
DD	84 (52.5%)	35 (21.2%)	34.3	**0.000***	4.1 (2.53–6.67)
I	84 (26.2%)	210 (63.6%)			
D	236 (73.8%)	120 (36.4%)	91.7	**0.000***	4.9 (3.52–6.87)
AGT (M/T)					
MM	20 (12.5%)	115 (69.7%)			
MT	92 (57.5%)	30 (18.2%)			
TT	48 (30%)	20 (12.1%)	109.83	**0.000***	
MM	20 (12.5%)	115 (69.7%)			
MT + TT	140 (87.5%)	50 (30.3%)	109.4	**0.000***	0.062 (0.035–0.11)
MT + MM	112 (70%)	145 (87.9%)			
TT	48 (30%)	20 (12.1%)	15.7	**0.000***	0.32 (0.18–0.57)
M	132 (41.2%)	260 (78.8%)			
T	188 (58.8%)	70 (21.2%)	95.6	**0.000***	0.19 (0.13–0.27)
ARNT (G/A)					
GG	30 (18.7%)	90 (54.6%)			
GA	68 (42.5%)	55 (33.3%)			
AA	62 (38.8%)	20 (12.1%)	52.82	**0.000***	
GG	30 (18.7%)	90 (54.6%)			
GA + AA	130 (81.3%)	75 (45.4%)	44.69	**0.000***	5.2 (3.15–8.6)
GA + GG	98 (61.2%)	145 (87.9%)			
AA	62 (38.8%)	20 (12.1%)	30.5	**0.000***	4.6 (2.6–8.1)
G	128 (40%)	235 (71.2%)			
A	192 (60%)	95 (28.8%)	64.2	**0.000***	3.7 (2.7–5.2)

Data are expressed as numbers (percentage (X^2: chi-square statistic

95% CI: 95% confidence intervals. OR: odds ratio

Bold values indicate a significant difference between the studied groups (GDM and Control)

*$P < 0.001$ was considered highly significant

indicated that the DD genotype and D allele of ACE was significantly associated with an increased risk of GDM.

Our results are in agreement with the present study by Dmitrenko et al. [21] who suggested a significant association between the DD homozygous type of ACE gene and GDM women with preeclampsia in the general inheritance model. This is, also, reported with a positive association in women of Brno and Indian population [22]. In the same line, a study by Khan et al. [19] in an Asian Indian population of pregnant women revealed the relationship between polymorphism of *ACE* (ID + DD) and the risk for GDM development. Alternatively, the frequency of DD genotype showed no significant difference among GDM patients in a Saudi population, and D allele frequency had a nonsignificant statistical difference between GDM and controls [23]. Moreover, Dostalova et al.[22] had one study in which polymorphism of *ACE* gene had no significant relation with GDM. *ACE* catalyzes the cleavage of the decapeptide angiotensin I to the octapeptide angiotensin II, which improves the vasoconstriction activity of the angiotensin [24]. *ACE* has a significant effect on the angiotensin II production not only in the circulating blood, but also on the synthesis and interaction of renin–angiotensin system (RAS) components, including in the beta cells of the Langerhans islets and the placenta [25]. Local RAS in the pancreas and the placenta are supposed to be involved in physiological and pathophysiological processes in pregnancy [26].

Table 2 Relation between various genotypes of the ACE (I/D) and risk factors among GDM and controls

Parameters	Genotype	ACE (I/D)		P-value	
Age (years) M + SD	Controls	30.69 ± 4.17/29.57 ± 2.16		0.578/0.413	
	GDM	30.32 ± 5.47/30.19 ± 6.04			
Duration (weeks) M + SD	Controls	23.35 ± 3.67/24.86 ± 3.77		0.120/0.431	
	GDM	24.32 ± 4.62/24.24 ± 3.94			
BMI (kg/m^2) M + SD	Controls	27.72 ± 4.69/24.14 ± 2.62		**0.001*/0.001***	
	GDM	32.13 ± 5.48/33.53 ± 8.41			
Family history N (%)	Controls (No, Yes)	95 (73.1), 35 (26.9)	25 (71.4), 10 (28.6)	**0.001***	**0.017***
	GDM (No, Yes)	28 (36.8), 48 (63.2)	40 (47.6), 44 (52.4)		
Abortion history N (%)	Controls (No, Yes)	86 (66.2), 44(33.8)	30 (85.7), 5 (14.3)	**0.050***	**0.042***
	GDM (No, Yes)	60 (78.9), 16 (21.1)	56 (67.5), 27 (32.5)		
Hypertension N (%)	Controls (No, Yes)	130 (100), 0 (0.0)	30 (85.7), 5 (14.3)	**0.001***	0.535
	GDM (No, Yes)	64 (84.2), 12 (15.8)	68 (81.0), 16 (19.0)		
Glucose M + SD	Controls	77.5 ± 7.32/77.88 ± 10.23		**0.0001*/0.0001***	
	GDM	106.5 ± 0/129.76 ± 54.55			
HBA1c M + SD	Controls	5.96 ± 0.23/5.79 ± 0.27		**0.0001*/0.0001***	
	GDM	11.0 ± 1.68/8.0 ± 1.9			
Insulin M + SD	Controls	11.10 ± 1.56/9.15 ± 1.23		**0.0001*/0.0001***	
	GDM	31.10 + 0/31.46 ± 12.04			
HOMA-IR M + SD	Controls	2.12 ± 0.28/1.74 ± 0.16		**0.0001*/0.0001***	
	GDM	3.38 ± 0.55/9.94 ± 5.45			

Data are presented as means ± standard deviation (SD)

Bold values indicate a significant difference

*$P \leq 0.05$ was considered significant

AGT gene encodes the precursor of all peptides of angiotensin. This gene is located on chromosome 1 (lq42-43) and has been considered as hypertension gene [27] and preeclampsia [28, 29]. To our knowledge, this study is the first one that evaluates the influence of this common SNP on GDM in Egyptian women. Our findings showed significant associations between the TT genotype and T allele of *AGT* and the risk of GDM. A study done by Ludwi et al. [30] suggested that T allele of *AGT* M235T polymorphism as an independent risk factor for CAD, while Gurkan et al. [31] findings did not support the idea that persons with T allele of *AGT* 235 gene will have high levels of *AGT* and so will yield more Ang. II, which lead to destruction of the tissue, as this genetic variant was more frequent in healthy subjects. Furthermore, we observed a significant correlation between the recessive model of *AGT* (MM + MT/ TT) with BMI, hypertension, and HOMA-IR. An earlier Japanese study was in agreement with our results, in which M235T reported a positive association with visceral obesity in obese women and T2DM morbid obese patients [32, 33]. M235T, also, has been investigated and showed a positive relation with T2DM in the Tunisian population [34]. Association between *AGT* rs699 and hypertension induced by pregnancy was investigated by another meta-analysis in the Chinese population. This meta-analysis found that the dominant genetic model (MT + TT) and the recessive genetic model (MT + MM) have significant associations [35]. Angiotensin II acts as a final hormone affecting the renin–angiotensin system. Its reactive production baseline can be increased by variants of angiotensinogen as M235T. Autoregulatory mechanisms could be provoked by continuous over-stimulation which leads to increase vascular tone and vascular hypertrophy. The sensitivity to angiotensin II then increased and the plasma levels of most angiotensinogen systems are reduced [36].

Strong evidence for the *ARNT*-like protein-I (*BMALI*) gene role has been provided in T2DM pathogenesis [37–39]. Given the genetic and pathophysiological feature's similarities between T2DM and GDM, in this study, we investigated the informative variants of the relationship between the *BMAL1* gene and GDM in Egyptian women.

Table 3 Relation between various genotypes of the AGT (M/T) and risk factors among GDM and controls

Parameters	Genotype	MM + MT/TT			
		M + SD		*P* value	
Age (years)	Controls	30.31 ± 3.96/31.50 ± 2.76		0.233/0.687	
	GDM	29.56 ± 5.70/31.91 ± 5.61			
Duration (weeks)	Controls	23.72 ± 3.83/23.25 ± 3.02		0.655/**0.04***	
	GDM	23.96 ± 4.61/25.02 ± 3.21			
BMI (kg/m2)	Controls	27.04 ± 4.74/26.36 ± 3.07		**0.001*/0.001***	
	GDM	32.81 ± 7.78/32.99 ± 5.58			
Family history	Controls (No, Yes) *n*,%	110 (75.9), 35 (24.1)	10 (50.0), 10 (50.0)	**0.001***	0.936
	GDM (No, Yes) *n*,%	44 (38.9), 69 (61.1)	24 (51.1), 23 (48.9)		
Abortion history	Controls (No, Yes) *n*,%	101 (69.7), 44 (30.3)	15 (75.0), 5 (25.0)	0.139	0.268
	GDM (No, Yes) *n*,%	88 (77.9), 25 (22.1)	28 (60.9), 18 (39.1)		
Hypertension	Controls (No ,Yes) *n*,%	140 (96.6), 5 (3.4)	20 (100), 0 (0.0)	**0.021***	**0.003***
	GDM (No, Yes) *n*,%	101 (89.4), 12 (10.6)	31 (66.0), 16 (34.0)		
Glucose	Controls	75.26 ± 6.53/70.50 ± 0.89		**0.001*/0.001***	
	GDM	115.30 ± 50.27/120.53 ± 76.63			
HBA1c	Controls	7.46 ± 1.65/7.23 ± 1.60		**0.001***/0.534	
	GDM	8.30 ± 1.80/7.91 ± 2.38			
Insulin	Controls	10.40 ± 2.61/10.23 ± 1.58		**0.001*/0.001***	
	GDM	36.36 ± 13.12/27.89 ± 12.33			
HOMA-IR	Controls	1.93 ± 0.53/1.80 ± 0.32		**0.001*/0.001***	
	GDM	9.90 ± 4.55/8.64 ± 7.44			

Data are presented as means ± standard deviation (SD)

Bold values indicate significant difference

*$P \leq 0.05$ was considered significant

GDM susceptibility knowledge is still limited, concerning genes or epigenetic alterations induced by glucose which occur during gestation, despite the wide spread of active research using genomic technologies [40]. This study provides evidence that the clock gene variants of the *ARNT* like the protein-1 (*BMAL1*) gene may be a predisposing factor to GDM in the Egyptian population. There was a positive association between the mutant genotype AA and A allele and the development of GDM. Circadian clock genes contribute to reproductive processes in mammals. *BMAL1* plays an essential role in female reproduction [41].

Our findings were consistent with the previous studies conducted by Pappa et al.[13] who indicated for the first time that the rs7950226 (G > A) of the *BMAL1* gene can be associated with increased GDM susceptibility. To our knowledge, these polymorphisms were found to be associated with T2DM in current studies by Kelly et al. [38], further increasing the list of sharing genetic parameters and common variants between GDM and T2DM. Also, the results are in agreement with Woon et al. [42] who reported T2DM patients independently, and found that the same polymorphisms in rs7950226 (G > A) were highly associated with T2DM susceptibility. However, our findings differ from Kelly et al. [38], who displayed that the haplotype of rs7950226A/rs11022775T of the *BMAL1* gene has a positive association with T2DM.

The circadian clock distribution differences in gene polymorphism frequencies between populations worldwide were recently highlighted, giving us a powerful genetic component associated with the environment, not as a result of natural selection [43]. Specifically, the significant role of the clock genes, mainly *BMAL1* in circadian self-keeping oscillations involved in glucose metabolism, growth, and pancreatic islets insulin signaling, has been recently underscored. These delayed phase oscillations when applied on mutant mice by conditional β-cell knockout lead to hyperinsulinemia and diabetes [37]. An early stage of dysfunction of β-cell may be reflected as a temporal derangement of the secretion of insulin. This also augments the clock (s) function of dynamic relation links in different tissues and increases the impact of disruption leading to T2DM [13].

As for the assumed GDM pathophysiological mechanisms that involve the clock genes, also regarding common features between both types of diabetes (T2DM and GDM) such as decreased insulin secretion and β-cell dysfunction, and increased insulin resistance during

Table 4 Relation between different genotypes of the ARNT (G/A) and risk factors among GDM and controls

Parameters	Genotype	ARNT (G/A) GG+GA/AA			
		M+SD		*P* value	
Age (years)	Controls	30.69 ± 3.90/28.63 ± 2.97		0.147/0.492	
	GDM	31.58 ± 5.22/27.91 ± 5.96			
Duration (weeks)	Controls	23.48 ± 3.65/25.11 ± 4.12		**0.016***/0.112	
	GDM	24.76 ± 4.38/23.41 ± 3.93			
BMI (kg/m2)	Controls	26.88 ± 4.68/27.60 ± 3.69		**0.001*/0.001***	
	GDM	32.25 ± 7.10/33.94 ± 7.27			
Family history	Controls (No, Yes) n,%	105 (71.9), 41 (28.1)	15 (78.9), 4 (21.1)	**0.001***	**0.027***
	GDM (No, Yes) n,%	39 (38.2), 63 (61.8)	29 (50), 29 (50)		
Abortion history	Controls (No, Yes) n,%	102 (69.9), 44 (30.1)	14 (73.7), 5 (26.3)	0.451	0.802
	GDM (No, Yes) n,%	75 (74.3), 26 (25.7)	41 (70.7), 17 (29.3)		
Hypertension	Controls (No, Yes) n,%	145 (99.3), 1 (0.7)	15 (78.9), 4 (21.1)	**0.001***	0.576
	GDM (No, Yes) n,%	83 (81.4), 19 (18.6)	49 (84.5), 9 (15.5)		
Glucose	Controls	75.22 ± 6.52/70.53 ± 0.91		**0.001*/0.001***	
	GDM	111.50 ± 59.02/126.21 ± 58.37			
HBA1c	Controls	7.37 ± 1.68/7.93 ± 1.28		**0.001***/0.457	
	GDM	8.16 ± 2.00/8.22 ± 1.98			
Insulin	Controls	10.38 ± 2.61/10.34 ± 1.54		**0.001*/0.001***	
	GDM	33.75 ± 14.18/34.10 ± 12.11			
HOMA-IR	Controls	1.93 ± 0.53/1.81 ± 0.32		**0.001*/0.001***	
	GDM	9.06 ± 5.61/10.37 ± 5.42			

Data are presented as means ± standard deviation (SD)

Bold values indicate significant difference

*$P \leq 0.05$ was considered significant

Table 5 The risk of different variables of GDM depending on the different genotypes

Variables	ACE (I/D) DD/ ID + II Risk		AGT (M235T) TT/ MT + MM Risk		ARNT (G/A) AA/GA + GG Risk	
	95% CI	*P*	95% CI	*P*	95% CI	*P*
Age (years)	29.14–30.79/30.30–31.57	0.064	31.6–33.1/28.9–30.2	**.000***	29.5–31.4/29.9–31	.858
Duration (weeks)	24.39–25.44 /23.27–24.18	**0.001***	24.7–25.8/23.1–24	**.000***	24–25.3/23.5–24.1	**.047***
Abortion history	.57–.94/.64–.96	.717	.74–1.1/.54-.84	**.040***	.6–1.1/.62-.9	.517
BMI (kg/m^2)	30.3–32.4/30.7–32.1	.899	32.1–33.9/29.8–31	**.000***	31.8–33.8/30.2–31.6	**.005***
Glucose	116.7–133.7/100.2–111.2	**0.000***	121.7–140.7/98.9–109.6	**.000***	115–134.3/103.5–114.4	**.004***
HBA1c	8.3–8.8/7.5–7.9	**0.000***	7.9–8.5/7.8–8.2	.210	8–8.7/7.7–8.1	**.020***
Insulin	29.8–37.1/24.9–29.2	**0.002***	33.5–41.8/23.5–27.2	**.000***	28.8–34.7/26.3–31	.167
HOMA-IR	18.5–23.7/15.4–18.7	**0.007***	20.8–26.4/14.4–16.6	**.000***	17–21.8/16.5–20	.499

95% CI: 95% confidence intervals

*$P < 0.001$ was considered highly significant

gestation, it is believable that the associated decrease in clock genes allele variants especially *BMAL1* gene, as it was documented in this study, may increase the risk of GDM and glucose intolerance [39]. Besides, decreased susceptibility of the GDM alleles makes complementary β-cell mass increase [39, 40, 44]. The related deficiency of the allele variants of the clock genes and particularly of the *BMAL1* gene, as recognized in our study, might

Table 6 Haplotype association of ACE (I/D), AGT (M/T), and ARNT (G/A) between GDM and control groups

Haplotype	Group		Chi-square (X^2)	*P* value	Risk	
	GDM	Control			OR	95% CI
II + MM + GG	0 (0.0%)	50 (100.0%)	32.879	**0.001***	2.400	1.229–4.688
II + MM + AA	4 (100.0%)	0 (0.0%)	2.424	0.119	1.714	1.063–2.765
II + MT + GG	0 (0.0%)	10 (100.0%)	8.556	**0.003***	2.400	1.229–4.688
II + MT + GA	0 (0.0%)	10 (100.0%)	8.556	**0.003***	2.400	1.229–4.688
II + TT + GG	0 (0.0%)	5 (100.0%)	4.958	**0.026***	2.400	1.229–4.688
II + TT + GA	3 (100.0%)	0 (0.0%)	1.875	0.171	1.714	1.063–2.765
II + TT + AA	1 (16.7%)	5 (83.3%)	2.812	0.094	0.143	0.013–1.630
ID + MM + GG	1 (6.2%)	15 (93.8%)	9.115	**0.003***	0.048	0.005–0.488
ID + MM + GA	8 (28.6%)	20 (71.4%)	3.175	0.075	0.286	0.070–1.171
ID + MM + AA	3 (37.5%)	5 (62.5%)	0.833	0.361	0.429	0.068–2.684
ID + MT + GG	7 (58.3%)	5 (41.7%)	0.001	1.000	1.000	0.197–5.068
ID + MT + GA	24 (82.8%)	5 (17.2%)	2.746	0.098	3.429	0.766–15.342
ID + MT + AA	13 (100.0%)	0 (0.0%)	6.771	**0.009***	1.714	1.063–2.765
ID + TT + GG	1 (100.0%)	0 (0.0%)	0.677	0.411	1.714	1.063–2.765
ID + TT + GA	5 (100.0%)	0 (0.0%)	2.951	0.086	1.714	1.063–2.765
ID + TT + AA	6 (100.0%)	0 (0.0%)	3.462	0.063	1.714	1.063–2.765
DD + MM + GA	4 (16.7%)	20 (83.3%)	6.545	**0.011***	0.143	0.030–0.688
DD + MM + AA	0 (0.0%)	5 (100.0%)	4.958	**0.026***	2.400	1.229–4.688
DD + MT + GG	11 (100.0%)	0 (0.0%)	5.856	**0.016***	1.714	1.063–2.765
DD + MT + GA	9 (100.0%)	0 (0.0%)	4.922	**0.027***	1.714	1.063–2.765
DD + MT + AA	28 (100.0%)	0 (0.0%)	13.333	**0.001***	1.714	1.063–2.765
DD + TT + GG	10 (66.7%)	5 (33.3%)	0.199	0.656	1.429	0.297–6.877
DD + TT + GA	15 (100.0%)	0 (0.0%)	7.670	**0.006***	1.714	1.063–2.765
DD + TT + AA	7 (58.3%)	5 (41.7%)	**Reference**			

Data are presented as number (percentage %). X^2: chi-square statistic

95% CI: 95% confidence intervals. OR: odds ratio

Bold values indicate significant difference. **P* value ≤ 0.05 was considered significant

further result in the increased risk of glucose intolerance and GDM.

Conclusion

Our results proposed that the *ACE* (I/D), AGT (M235T), and *BMAL1* rs7950226 (G/A) gene polymorphisms might have an important role in GDM development in Egyptian pregnant women. In this study, it was possible to identify that II + MM + GG were the protective haplotypes for GDM while DD + TT + AA were the risk haplotypes for GDM. In the future, it could allow using this genetic marker as the principle in assessing the individual prediction of GDM development, which will assist to take efficient protective efforts for timely improvement in the pregnancy outcome.

Limitation

The limitation of this research study included the lack of evaluation of the *ACE*, *AGT*, and *ARNT* like protein-1 (*BMAL1*) levels and uses the only one SNP for each one. Studies with larger sample sizes are necessary to investigate the associations between gene polymorphisms and GDM in Egyptian populations.

Abbreviations

ACE: Angiotensin converting enzyme; AGT (M235T): Angiotensinogen; BMAL1: ARNT-like protein-1; BMI: Body mass index; ELISA: Enzyme-linked immunosorbent assays; GDM: Gestational diabetes mellitus; HBA1c: Glycated Hemoglobin; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; OR: Odds ratio; T2DM: Type 2 diabetes mellitus; 95% CI: 95% Confidence Intervenes.

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Authors' contributions

Conceived and designed the study: EA, MA, OMF. Diagnosis and selection of all participants in the study: AO. Contributed reagents/materials/analysis tools: WG, LM and EA. WG, LM, AO, and EA: Contributed to the analysis of the results and the writing of the manuscript. All authors read and approved the final manuscript.

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Availability of data and material
The datasets supporting the results are included within the article. The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available because of privacy or ethical restrictions.

Declarations

Ethics approval and consent to participate
This study was approved by the Human Ethical Committee of the National Research Centre (The patient provided written consent).

Consent for publication
Written informed consent was obtained from all patients and controls.

Competing interests
The authors declare that they have no competing interests.

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