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ORIGINAL RESEARCH

No Association Between *ADIPOQ* or *MTHFR* Polymorphisms and Gestational Diabetes Mellitus in South African Women

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Stephanie Dias[1,2]
Sumaiya Adam[2]
Paul Rheeder[3]
Carmen Pheiffer[1,4]

[1]Biomedical Research and Innovation Platform (BRIP), South African Medical Research Council, Cape Town, 7505, South Africa; [2]Department of Obstetrics and Gynecology, University of Pretoria, Pretoria, 0001, South Africa; [3]Department of Internal Medicine, Faculty of Health Sciences, University of Pretoria, Pretoria, 0001, South Africa; [4]Division of Medical Physiology, Faculty of Health Sciences, Stellenbosch University, Cape Town, 7505, South Africa

Purpose: Gestational diabetes mellitus (GDM) is a growing public health concern. GDM affects approximately 14% of pregnancies globally, and without effective treatment, is associated with short- and long-term complications in mother and child. Lower serum adiponectin *(ADIPOQ)* concentrations and aberrant DNA methylation have been reported during GDM. The aim of this study was to investigate the association between the *ADIPOQ* −11377C>G and −11391G>A, and methylenetetrahydrofolate reductase *(MTHFR)* 677C>T polymorphisms and GDM in a population of black South African women.

Materials and Methods: DNA was isolated from the peripheral blood of 447 pregnant women with (n=116) or without (n=331) GDM, where after *ADIPOQ* (rs266729 and rs17300539) and *MTHFR* (rs1801133) polymorphisms were genotyped using TaqMan Quantitative Real-Time PCR analysis.

Results: Women with GDM had a higher body mass index (p=0.012), were more insulin resistant (p<0.001) and had lower adiponectin levels (p=0.013) compared to pregnant women with normoglycemia. Genotypic, dominant and recessive genetic models showed no association between *ADIPOQ* rs266729 and rs17300539 and *MTHFR* rs1801133 polymorphisms and GDM. Intriguingly, the risk G allele of *ADIPOQ* rs266729 was associated with higher fasting glucose and insulin concentrations, while the T allele in *MTHFR* rs1801133 was associated with higher fasting insulin concentrations only.

Conclusion: *ADIPOQ* rs266729 and rs17300539 and *MTHFR* rs1801133 polymorphisms are not associated with GDM in a population of black South African women. These findings suggest that these single nucleotide polymorphisms (SNPs) do not individually increase GDM risk in the African population. However, the role of these SNPs in possible gene-gene or gene-environment interactions remain to be established.

Keywords: SNP genotyping, molecular biomarkers, adiponectin, *ADIPOQ*, methylenetetrahydrofolate reductase, *MTHFR*, gestational diabetes mellitus, GDM

Introduction

Gestational diabetes mellitus (GDM), is defined as glucose intolerance that develops during pregnancy and usually returns to normoglycemia after birth.[1] Globally, it is estimated that approximately 14% of pregnancies are complicated by GDM,[2] although the prevalence varies between <1% and 28% according to the population studied and the diagnostic criteria employed.[3] GDM is associated with adverse perinatal outcomes such as pre-eclampsia, caesarean section, fetal macrosomia, shoulder dystocia, hyperinsulinemia, hypoglycemia, hyperbilirubinemia and

Correspondence: Carmen Pheiffer
Biomedical Research and Innovation Platform (BRIP), South African Medical Research Council, Tygerberg, 7505, South Africa
Tel +27 21 938 0292
Email carmen.pheiffer@mrc.ac.za

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respiratory distress syndrome,[4–8] and an increased risk of developing future metabolic disease such as obesity, type 2 diabetes (T2D), and cardiovascular disease in both mother and child in later life.[9–13] Although the etiology of GDM is not yet fully elucidated, it is widely accepted that factors such as age, high body mass index (BMI), excessive gestational weight gain, family history of diabetes mellitus, previous pregnancies complicated by GDM and genetic polymorphisms increase susceptibility to GDM.[14,15]

Accumulating studies report that polymorphisms in genes involved in metabolic adaptation during pregnancy may increase the risk for developing GDM. At least 34 single nucleotide polymorphisms (SNPs) were reported to be associated with GDM in at least two populations, with variants in the adiponectin (*ADIPOQ*) gene widely investigated during GDM.[16] The expression of *ADIPOQ*, an adipocyte-derived hormone, is decreased during pregnancy and is associated with the development of insulin resistance and glucose intolerance.[17–19] Accordingly, *ADIPOQ* is intensely researched as a biomarker to predict the risk of developing GDM.[20] SNPs within the *ADIPOQ* gene may regulate adiponectin expression during pregnancy and increase the risk for GDM.[21] To date, three *ADIPOQ* SNPs have been investigated during GDM. The rs266729 (−11377C>G) variant in the promoter region of *ADIPOQ* has been associated with an increased risk of GDM in Polish, Bulgarian and Asian populations, and a decreased risk of developing GDM in American populations.[22–25] In a recent meta-analysis conducted in 12 studies, the rs2241766 (45T>G) variant in exon 2 of *ADIPOQ* were reported to be associated with an increased risk of GDM in Iranian, Malaysian, Brazilian and Asian populations,[26] while variant rs1501299 (276G>T) in intron 2 of *ADIPOQ* was not associated with GDM in Polish, Bulgarian and Asian populations.[23,24,26]

Aberrant DNA methylation is associated with the development of GDM.[27–31] DNA methylation is the most widely studied and best characterized epigenetic mechanism, that occurs due to the addition of a methyl group to the fifth carbon position of a cytosine nucleotide, generally leading to transcriptional repression.[32,33] Methylenetetrahydrofolate reductase *(MTHFR)* is an enzyme in the transmethylation pathway and plays a critical role in regulating DNA methylation in response to environmental cues.[34,35] Two *MTHFR* polymorphisms, 677C>T (rs1801133) and 1298A>C (rs1801131), has been shown to impair enzyme function and consequently dysregulate DNA methylation.[36] These polymorphisms are widely studied during metabolic disease,[37–39] and has been associated with insulin resistance,[40] and an increased risk of developing cardiovascular disease,[41,42] T2D[43] and major depressive disorder[44] in South African populations. Recently, the *MTHFR* rs1801133 polymorphism has been linked to higher folate concentrations in early pregnancy and an increased risk of developing GDM in Chinese women,[45] and has been associated with GDM-related pregnancy complications such as gestational hypertension, pre-eclampsia and intrauterine fetal growth restriction.[46,47]

This study aimed to investigate the relationship between the *ADIPOQ* −11377C>G and −11391G>A, and *MTHFR* 677C>T polymorphisms and GDM in a population of South African women. We hypothesized that polymorphisms in these genes may underlie the differences in adiponectin expression[48] and DNA methylation[31] previously reported in this population. To our knowledge this is the first study to investigate the association between *ADIPOQ* and *MTHFR* polymorphisms and GDM in an African population.

Materials and Methods

Study Participants

Ethical approval for this study was granted by the University of Pretoria Health Sciences Ethics Committee (180/2012). The study was conducted according to the Declaration of Helsinki and all women gave written informed voluntary consent after the procedures had been fully explained in the language of their choice. This case-control study is nested within a prospective cohort study where 1000 pregnant women were recruited at a primary care clinic in Johannesburg, South Africa.[49] Black African women with singleton pregnancies, who did not have pre-existing diabetes (type 1 (T1D) and T2D) were enrolled in the study. Random glucose and glycated hemoglobin (HbA1c) concentrations were measured in all participants. Women with random glucose and HbA1c concentrations >11.1 mmol/L and >6.5%, respectively, were excluded. Women who were included were asked to return in a fasted state for GDM testing and blood collection within 2 weeks.

Anthropometric, Biochemical and Clinical Data on Study Participants

At recruitment, age, gestational age (weeks), height (cm) and weight (kg) were obtained using standard procedures, and BMI was calculated as weight (kg)/height squared (m^2). GDM was diagnosed using the 75-g 2-hr oral glucose

tolerance test (OGTT) at 24–28 weeks of pregnancy according to the International Association of Diabetes in Pregnancy Study Group (IADPSG) criteria, and diagnosed if at least one glucose value was met (fasting plasma glucose ≥ 5.1mmol/L, 1 hr OGTT ≥ 10 mmol/L or 2 hr OGTT ≥ 8.5 mmol/L).[50] Serum and whole blood samples were collected from participants in serum separator tubes (SST) and ethylenediaminetetraacetic acid (EDTA) tubes and stored at −80 °C until further analysis. To assess the relationship between GDM and inflammation in these women, C-reactive protein (CRP) levels were measured. CRP and insulin concentrations were measured using the turbidimetric and microparticle enzyme immunoassays (AxSYM, Abbott), respectively, in an accredited laboratory (Vermaak and Partners/Pathcare laboratories, South Africa), while adiponectin concentrations were measured using the human adiponectin enzyme-linked immunosorbent assay (ELISA) (Merck, Darmstadt, Germany). The homeostatic model assessment (HOMA), a measure of insulin resistance, was calculated using the equation: (fasting plasma glucose x fasting serum insulin)/22.5.

DNA Extraction and Genotyping

Genomic DNA was extracted from 2 mL of whole blood, using the QIAamp DNA Blood Midi Kit (Qiagen, Hilden, Germany) as previously described.[51] DNA concentration was measured using the Qubit Fluorometer and the Quanti-iT dsDNA Broad Range assay kit (ThermoFisher, Massachusetts, USA). Genotyping was conducted using quantitative Real-Time PCR (qRT-PCR) with TaqMan genotyping assays[52] (Table 1) on the QuantStudio™ 7 Flex Real-Time PCR System (Applied Biosystems, California, USA). The Quantstudio 7 Real-Time PCR software v1.3 analysis tool was used for base-calling and visualization of the genotyping data (Supplementary Figure S1). Briefly, qRT-PCR was performed using 9.5 ng of DNA, 5 µL of TaqPath ProAmp Master Mix and 0.25 µL of 40X TaqMan SNP Genotyping Assay in a total volume of 10 µL, according to the manufacturer's instructions (Applied Biosystems). The following PCR conditions were used: 10 min at 95 °C (initial denaturation/enzyme activation), 15 sec at 95 °C (denaturation) and 60 sec at 60 °C (annealing/extension) for 40 cycles. For quality control, 20% of samples were randomly selected and genotyped in duplicate. Positive and negative controls were included on all plates. Nine samples were randomly selected, and genotyping validated by DNA sequencing (Central Analytical Facilities, Cape Town, South Africa). Details of sequencing primers are shown in Supplementary Table S1. Primers for sequencing were designed on NCBI using Primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast). The minor allele frequency (MAF) for all SNPs were obtained from Ensemble 1000 genomes project (http://www.ensembl.org/Homo_sapiens/Info/Index). The African (AFR) MAF was determined using published data from seven sub-populations, including Yoruba in Ibadan, Nigeria, Luhya in Webuye, Kenya, Gambian in Western Divisions in the Gambia, Mende in Sierra Leone, Esan in Nigeria, Americans of African Ancestry in SW USA and African Caribbean in Barbados.

Statistical Analysis

Participant characteristics are expressed as the median and interquartile range (25th and 75th percentiles) since data were skewed. Testing for normality was conducted using the Shapiro–Wilk test in STATA 14 (StataCorp, College Station, USA). Univariable and multivariable logistic regression were performed to assess the association between genotype and participant characteristics. Data are expressed as the odds ratio (OR) and 95% confidence interval (CI), and were adjusted for confounding factors age, BMI and gestational age. The *ADIPOQ* rs266729 and

Table 1 Details of *ADIPOQ* rs266729 and rs17300539 and *MTHFR* rs1801133 Single Nucleotide Polymorphisms Assays

Gene Symbol	Assay ID	Sequence (5′-3′)	Global MAF	AFR MAF
ADIPOQ	rs266729	TTGCAAGAACCGGCTCAGATCCTGC**[C/G]** CTTCAAAAACAAAACATGAGCGTGC	C=0.77 G=0.23	C=0.90 G=0.09
ADIPOQ	rs17300539	TCAGAATGTGTGGCTTGCAAGAACC**[G/A]** GCTCAGATCCTGCCCTTCAAAAACA	G=0.97 A=0.03	G=0.99 A=0.01
MTHFR	rs1801133	GAAAAGCTGCGTGATGATGAAATCG**[C/T]** CTCCCGCAGACACCTTCTCCTTCAA	C=0.75 T=0.25	C=0.91 T=0.09

Note: Bold letters indicate the SNP of interest in each sequence. **Abbreviations:** *ADIPOQ*, adiponectin; *MTHFR*, methylenetetrahydrofolate reductase; MAF, minor allele frequency; AFR, African.

rs17300539, and *MTHFR* rs1801133 genotype and allele frequencies, and the dominant and recessive models of inheritance were compared in GDM and non-GDM groups, using the Chi-squared (X^2) test or Fisher's exact test (frequency < 5). A $p \leq 0.05$ was considered statistically significant. The Pearson's X^2 test was performed to determine whether the genotype frequencies at *ADIPOQ* rs266729 and rs17300539 and *MTHFR* rs1801133 were in Hardy-Weinberg equilibrium (HWE) ($p > 0.05$).

Results

Clinical and Biochemical Data

The clinical and biochemical data of the study participants are shown in Table 2. BMI (p=0.012), random (p<0.001) and fasting (p<0.001) glucose concentrations, 1 hr (p<0.001) and 2 hr (p<0.001) OGTT values, fasting insulin (p=0.03) and HbA1c (p=0.005) concentrations, and HOMA (p<0.001) were higher in women with GDM compared to women with normoglycemia, while gestational age (p=0.007) and serum adiponectin concentrations (p=0.013) were lower in women with GDM. CRP levels did not differ between women with GDM compared to women with normoglycemia.

ADIPOQ Genotype Distribution and Association with GDM

The genotype and allele frequency distribution for *ADIPOQ* rs266729 and rs17300539 did not differ in women with GDM nor in women with normoglycemia (Table 3). The genotype frequency distribution of the *ADIPOQ* rs17300539 polymorphism was in accordance with HWE (Chi squared = 0.009; p=0.924), while the *ADIPOQ* rs266729 polymorphisms deviated from HWE (Chi squared = 24.518; p<0.001). Both dominant and recessive genetic models showed that the rs266729 and rs17300539 polymorphisms were not associated with GDM (Table 4). The number of women with the GG genotype (rs266729) and the AA genotype (rs17300539) were low or not observed at all, therefore the heterozygous and homozygous genotypes, CG+GG and GA+AA for rs266729 and rs17300539, respectively, were combined for further analysis. Regression analysis showed that the rs266729 CG+GG genotypes were associated with higher fasting glucose concentrations in women with normoglycemia (p=0.04) and higher fasting insulin concentrations in all women (p=0.004) and in women with GDM (p=0.009), while no association was observed for rs17300539 (Table 5). The association between rs266729 and fasting glucose and insulin concentrations remained significant after adjusting for age, BMI and gestational age. The rs17300539 GA+AA genotypes were not observed in women with GDM, therefore logistic regression could not be conducted.

MTHFR Genotype Distribution and Association with GDM

The genotype and allele frequency for *MTHFR* rs1801133 did not differ in women with GDM nor in women with normoglycemia (Table 6). The genotype frequency

Table 2 Participant Characteristics According to GDM Status

Participant Characteristics	Non-GDM	GDM	*p*-value
Number	331	116	
Age (years)	27.0 (23.0–31.0)	29.0 (24.0–32.0)	0.083
BMI (kg/m^2)	25.6 (22.7–29.8)	27.1 (23.6–31.2)	0.012
Gestational age (weeks)	26.0 (23.0–28.0)	25.0 (21.0–27.0)	0.007
Random glucose (mmol/L)	4.4 (4.0–4.8)	4.7 (4.3–5.1)	<0.001
Fasting glucose (mmol/L)	4.4 (4.0–4.6)	5.5 (5.3–6.0)	<0.001
OGTT 1 hr (mmol/L)	5.5 (4.7–6.4)	6.3 (5.4–7.5)	<0.001
OGTT 2 hr (mmol/L)	5.2 (4.5–5.8)	6.0 (5.1–7.2)	<0.001
HbA1c (%)	5.2 (4.9–5.4)	5.3 (5.1–5.5)	0.005
Fasting insulin (mIU/L)	5.2 (3.3–7.5)	5.9 (3.9–8.8)	0.030
HOMA	1.0 (0.7–1.5)	1.5 (0.9–2.2)	<0.001
C-reactive protein (mg/L)	5.7 (3.1–8.8)	7.0 (3.7–10.5)	0.125
Adiponectin (µg/mL)	10.4 (8.0–16.5)	9.1 (5.6–15.1)	0.013

Notes: Data are expressed as the median and interquartile range (25th–75th percentiles). p<0.05, p<0.01, p<0.001.
Abbreviations: BMI, body mass index; OGTT, oral glucose tolerance test; HbA1c, glycated hemoglobin; HOMA, homeostatic model assessment; non-GDM, normoglycemia.

Table 3 Genotype and Allele Frequency of *ADIPOQ* rs266729 and rs17300539 Polymorphisms in GDM and Non-GDM Groups

ADIPOQ	Genotype and Allele Frequency (n (%))				
Variant	**Genotype/Allele**	**Non-GDM (n=331)**	**GDM (n=116)**	**HWE (p-value)**	**p-value**
rs266729	CC CG GG	258 (77.9) 60 (18.1) 13 (4.0)	90 (77.6) 19 (16.4) 7 (6.0)	<0.001	0.606
	C G	576 (87.0) 86 (13.0)	199 (85.8) 33 (14.2)		0.634
rs17300539	GG GA AA	327 (98.8) 4 (1.2) 0 (0)	116 (100.0) 0 (0.0) 0 (0)	0.924	0.577
	G A	654 (99.4) 4 (0.6)	232 (100.0) 0 (0.0)		0.234

Abbreviations: *ADIPOQ*, adiponectin gene; GDM, gestational diabetes mellitus; HWE, Hardy-Weinberg equilibrium; non-GDM, normoglycemia.

Table 4 Association Between *ADIPOQ* rs266729 and rs17300539 Polymorphisms and GDM in Dominant and Recessive Genetic Models

ADIPOQ	Dominant Model (Frequency: n (%))				Recessive Model (Frequency: n (%))			
Variant	**Genotype**	**Non-GDM**	**GDM**	**p-value**	**Genotype**	**Non-GDM**	**GDM**	**p-value**
rs266729	CC CG+GG	258 (77.9) 73 (22.1)	90 (77.6) 26 (22.4)	0.936	CG+CC GG	318 (96.1) 13 (3.9)	109 (94.0) 7 (6.0)	0.345
rs17300539	GG GA+AA	327 (98.8) 4 (1.2)	116 (100) 0 (0.0)	0.577	GA+GG AA	331 (100) 0 (0.0)	116 (100) 0 (0.0)	N/A

Abbreviations: *ADIPOQ*, adiponectin gene; GDM, gestational diabetes mellitus; N/A, analysis not applicable (only one genotype present); non-GDM, normoglycemia.

distribution of *MTHFR* rs1801133 polymorphism deviated from HWE (Chi-squared = 71.934; p<0.001). Both dominant and recessive genetic models showed that the rs1801133 polymorphism was not associated with GDM (Table 7). The number of women with the rs1801133 TT genotype was low, thus, heterozygous and homozygous genotypes, CT+TT were combined for further analysis. Regression analysis showed that the rs1801133 CT+TT genotypes were associated with higher fasting insulin concentrations in all women (p=0.04) and in women with

Table 5 Participant Characteristics According to *ADIPOQ* rs266729 and rs17300539 Genotype Carriers

Participant Characteristics	***ADIPOQ* rs266729 (CC and CG+GG)**			***ADIPOQ* rs17300539 (GG and GA+AA)**		
	All	**Non-GDM**	**GDM**	**All**	**Non-GDM**	**GDM**
	OR (95% CI)	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**	**OR (95% CI)**
BMI (kg/m^2)	0.99 (0.95–1.04)	0.99 (0.94–1.05)	1.01 (0.94–1.09)	0.78 (0.59–1.05)	0.79 (0.59–1.06)	N/A
Fasting glucose (mmol/L)	1.05 (0.85–1.29)	1.96 (1.02–3.76)*	0.88 (0.58–1.34)	0.36 (0.08–1.58)	0.53 (0.07–3.67)	N/A
1 hr OGTT (mmol/L)	0.95 (0.82–1.11)	0.90 (0.74–1.09)	1.05 (0.80–1.37)	1.15 (0.61–2.16)	1.37 (0.67–2.80)	N/A
2 hr OGTT (mmol/L)	0.96 (0.81–1.13)	0.82 (0.64–1.06)	1.08 (0.85–1.37)	0.69 (0.29–1.68)	0.81 (0.31–2.13)	N/A
HbA1c (%)	1.28 (0.71–2.28)	1.24 (0.64–2.42)	1.42 (0.42–4.83)	0.49 (0.05–5.21)	0.59 (0.06–6.41)	N/A
Fasting insulin (mIU/L)	1.08 (1.03–1.14)‡	1.02 (0.90–1.15)	1.10 (1.03–1.19)‡	0.38 (0.06–2.26)	0.39 (0.07–2.24)	N/A
Adiponectin (µg/mL)	1.02 (0.99–1.05)	1.03 (0.99–1.06)	1.01 (0.95–1.07)	1.06 (0.98–1.15)	1.06 (0.97–1.15)	N/A

Notes: Data are expressed as the odds ratio and 95% confidence interval. Significant values are indicated by: *p<0.05; ‡p<0.01.
Abbreviations: BMI, body mass index; OGTT, oral glucose tolerance test; HbA1c, glycated hemoglobin; GDM, gestational diabetes mellitus; non-GDM, normoglycemia; N/A, not applicable (risk allele not present); OR, odds ratio; CI, confidence interval.

Table 6 Genotype and Allele Frequency of *MTHFR* rs1801133 Polymorphisms in GDM and Non-GDM Groups

MTHFR	Genotype Frequency (n (%))				
Variant	**Genotype**	**Non-GDM**	**GDM**	**HWE (p-value)**	**p-value**
rs1801133	CC CT TT	295 (89.1) 27 (8.2) 9 (2.7)	106 (91.4) 6 (5.2) 4 (3.4)	<0.001	0.559
	C T	617 (93.2) 45 (6.8)	218 (93.9) 14 (6.1)		0.687

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase gene; GDM, gestational diabetes mellitus; HWE, Hardy-Weinberg Equilibrium; non-GDM, normoglycemia.

normoglycemia (p=0.04) (Table 8). However, after adjusting for age, BMI and gestational age, only the association between rs1801133 CT+TT genotype and fasting insulin concentrations in women with normoglycemia remained significant. *MTHFR* genotypes were not associated with global DNA methylation levels (Supplementary Table S2).

Discussion

This study investigated the association between *ADIPOQ* and *MTHFR* polymorphisms, and GDM in a population of black South African women. Results of this study showed that women with GDM had a higher body mass index, were more insulin resistant and had lower adiponectin levels compared to pregnant women with normoglycemia. Genotypic, dominant and recessive genetic models showed no association between *ADIPOQ* rs266729 and rs17300539 and *MTHFR* rs1801133 polymorphisms and GDM in this population. Intriguingly, the risk G allele in *ADIPOQ* rs266729 was associated with higher fasting glucose in women with normoglycemia, and higher fasting insulin in all women and in women with GDM, while the T allele in *MTHFR* rs1801133 was associated with higher fasting insulin, in women with normoglycemia.

Studies investigating the association between *ADIPOQ* rs266729 polymorphisms and GDM have reported conflicting results. Consistent with our findings, Gueuvoghlanian-Silva et al, found no association between *ADIPOQ* rs266729 and GDM in a sample of 248 pregnant Brazilian women.[53] However, these authors found that the CC genotype was associated with higher serum adiponectin concentrations, whereas this association was not demonstrated in our study. Contradictory to our study, four studies reported that the *ADIPOQ* rs266729 polymorphism is associated with GDM. The G allele was associated with GDM in studies conducted in 130 Chinese women,[22] 411 Polish women,[24] and 562 Iranian women.[54] In contrast to these findings, Beltcheva et al reported that the C allele was associated with GDM in a Bulgarian population.[23] Discrepancies between these studies may be due to BMI, stage of pregnancy, sample size and ethnicity, which are critical limitations of GDM associated genetic studies.[25] Well-designed, large scale, studies that are conducted in diverse populations are required to further explore the role of this polymorphism in the development of GDM. Moreover, different risk allele frequencies of SNPs could further explain the differences observed between studies. As such, the rs266729 G allele occurs at a lower frequency in Africans (9.0%) compared to Europeans (28.1%), East Asians (27.6%), South Asians (28.4%) and Americans (24%). However, in our sample of 447 black South African women, the G allele occurred at a frequency of 22.1% (99 women), a frequency almost double than previously reported in the African population. These result suggest that the G allele in rs266729 may not be a significant risk factor for GDM in the South African population. GDM is a multifactorial disorder that occurs due to the interplay between genetic and environmental factors, thus, the interaction between diet, physical activity, smoking, alcohol consumption and genetics may influence susceptibility to GDM.[55–57] Although, many studies have investigated the association between *ADIPOQ* rs17300539 polymorphisms and metabolic disease,[58–62] ours is the first to investigate

Table 7 Association Between *MTHFR* rs1801133 Polymorphisms and GDM in Dominant and Recessive Genetic Models

MTHFR	Dominant Model (Frequency: n (%))				Recessive Model (Frequency: n (%))			
Variant	**Genotype**	**Non-GDM**	**GDM**	**p-value**	**Genotype**	**Non-GDM**	**GDM**	**p-value**
rs1801133	CC CT+TT	295 (89.1) 36 (10.9)	106 (91.4) 10 (8.6)	0.491	CC+CT TT	322 (97.3) 9 (2.7)	112 (96.5) 4 (3.5)	0.688

Abbreviations: *MTHFR*, methylenetetrahydrofolate reductase gene; GDM, gestational diabetes mellitus.; non-GDM, normoglycemia

Table 8 Participant Characteristics According to *MTHFR* rs1801133 Genotype Carriers

Participant Characteristics	*MTHFR* rs1801133 (CC and CT+TT)		
	All	Non-GDM	GDM
	OR (95% CI)	OR (95% CI)	OR (95% CI)
BMI (kg/m^2)	0.97 (0.98–1.03)	0.97 (0.92–1.06)	0.92 (0.79–1.06)
Fasting glucose (mmol/L)	0.92 (0.66–1.28)	0.94 (0.43–2.07)	1.02 (0.61–1.69)
1 hr OGTT (mmol/L)	0.96 (0.78–1.19)	1.03 (0.79–1.33)	0.88 (0.57–1.35)
2 hr OGTT (mmol/L)	1.05 (0.85 1.29)	1.02 (0.73–1.41)	1.19 (0.86–1.64)
HbA1c (%)	0.96 (0.44–2.10)	0.88 (0.37–2.09)	1.94 (0.29–13.10)
Fasting insulin (mIU/L)	0.85 (0.73–0.99)*	0.81 (0.65–0.99)*	0.92 (0.76–1.12)
Adiponectin (µg/mL)	1.03 (0.99–1.07)	1.03 (0.98–1.07)	1.04 (0.97–1.12)

Notes: Data are expressed as the odds ratio and 95% confidence interval. Significant values are indicated by: *p<0.05.
Abbreviations: BMI, body mass index; OGTT, oral glucose tolerance test; HbA1c, glycated hemoglobin; GDM, gestational diabetes mellitus; non-GDM, normoglycemia; OR, odds ratio; CI, confidence interval.

this polymorphism during GDM. The A allele was associated with T2D in French[58] and German[59] Caucasian populations, however, studies in Pakistani[60] and African American populations,[61] failed to show an association between the rs17300539 polymorphisms and T2D. Interestingly, Olckers et al showed that the G allele was associated with T2D in a black South African population.[62] Although T2D and GDM are both associated with insulin resistance and glucose intolerance, the pathophysiologic mechanisms that underlie these conditions during pregnancy may differ, possibly explaining the differences observed between our study and Olckers et al.[62]

The *MTHFR* rs1801133 polymorphism was not associated with GDM in our study and it is consistent with findings by Khan et al and Franzago et al who similarly failed to see an association between rs1801133 and GDM in a South Indian[63] and Italian population,[64] respectively. Previously, the T allele was shown to be associated with HOMA and serum insulin concentrations in non-pregnant Iranian women at high risk of developing insulin resistance,[40] and with Chinese participants at risk of metabolic syndrome.[39] In our study, the T allele was associated with higher fasting insulin concentrations. Thus, these results suggest that *MTHFR* polymorphism may be associated with pathophysiological mechanisms underlying the development of GDM. However, further work is required to explore the potential mechanism associated with *MTHFR* polymorphisms and serum insulin levels in the South African population.

To the best of our knowledge, this is the first study to investigate the association between the *ADIPOQ* rs266729 and rs17300539, and *MTHFR* rs1801133 polymorphisms and GDM in a South African population. A strength of our study is that GDM was diagnosed using the IADPSG criteria,[50] which is widely advocated to improve diagnosis of GDM. Women were recruited at a primary health care facility, which supports generalizability of our study findings to the community. Furthermore, genotyping results were validated by DNA sequencing, minimizing the possibility of genotyping error. However, a few limitations should be considered when interpreting the study results. Due to limited serum samples and the cross-sectional nature of the study, lipid profiles and gestational weight gain during and after pregnancy,[65,66] known to affect GDM risk, were not assessed in this study, and may have been associated with *ADIPOQ* polymorphisms. Our sample size was moderate, and although it is larger than many previous studies investigating SNPs during GDM, a lower risk allele frequency compared to previous studies may have led to our study being underpowered to detect significant associations between the investigated SNPs and GDM.[67] Replication of this analysis in a larger sample size is required to definitively rule out the association between the investigated polymorphisms and GDM. Furthermore, genotype frequencies of *ADIPOQ* rs266729 and rs1801133 deviated from HWE, suggesting that these SNPs may be under possible selection pressure. We recommend that technologies such as the H3A array, which contains SNPs specific to the African population be conducted to improve the ability to detect genetic susceptibility loci for genetic association studies in the African population. Importantly, gene-gene and gene-environment interactions[68] may also contribute to the risk of GDM and should be accounted for in genetic association studies.[55–57]

Conclusion

ADIPOQ rs266729 and rs17300539 and *MTHFR* rs1801133 polymorphisms are not associated with GDM in a population of black South African women. These findings suggest that these SNPs do not individually increase GDM risk in the African population. However, the role of these SNPs in possible gene-gene or gene-environment interactions remain to be established.

Abbreviations

GDM, gestational diabetes mellitus; *ADIPOQ*, adiponectin gene; *MTHFR*, methylenetetrahydrofolate reductase gene; SNP, single nucleotide polymorphism; SA, South Africa; T2D, type 2 diabetes; T1D, type 1 diabetes; HbA1c, glycated hemoglobin; OGTT, oral glucose tolerance test; IADPSG, International Association of Diabetes in Pregnancy Study Group; CRP, c-reactive protein; HOMA, homeostatic model assessment; ELISA, enzyme-linked immunosorbent assay; EDTA, ethylenediaminetetraacetic acid; HWE, Hardy-Weinberg Equilibrium; BMI, body mass index.

Data Sharing Statement

The data analyzed during the current study are available from the corresponding author on reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare no conflicts of interest in this work.

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