

The Variants in *ADIPOQ* are Associated with Maternal Circulating Adipokine Profile in Gestational Diabetes Mellitus

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Background: Gestational diabetes mellitus (GDM) is the most common association with hyperglycemia and glucose intolerance during pregnancy. The adipokines play an important role to control insulin secretion and glucose. This study aimed to investigate the association between maternal circulating adipokine levels and *ADIPOQ* gene polymorphism among pregnant women subjects with GDM and normal glucose tolerance (NGT).

Methods: Participants including 229 normal pregnant women and 197 GDM pregnant women were enrolled from 2015 to 2018 at Siriraj hospital. Serum adipokine levels including adiponectin, adiponectin, adiponectin, NGAL/Lipocalin-2, total PAI-1, and resistin were measured by immunoassay. *ADIPOQ* variations were investigated including -11377C/G (rs266729), +45T/G (rs2241766), and +276G/T (rs1501299).

Results: Serum adiponectin concentration was also significantly decreased among the GDM who had aged less than 35 years old whereas adiponectin levels were significantly lower among the GDM who had aged more than 35 years old. Also, adiponectin and total PAI-1 levels were significantly lower among the GDM who had a BMI of less than 30 kg/m². The G allele frequency of *ADIPOQ* +45T/G was significantly different between GDM and controls ($p = 0.03$). *ADIPOQ* +45T/G was associated with an increased risk of GDM (odds ratio [OR]: 1.554; 95% confidence interval [CI]: 1.010–2.390; $p = 0.045$). The -11377C/G was affected by the level of adiponectin ($p = 0.04$). The C allele of -11377C/G SNP declined serum adiponectin levels and may be a risk factor for GDM.

Conclusion: This study revealed that genetics play important roles in circulating adipokines among pregnant women. *ADIPOQ* polymorphisms had significant associations with adiponectin levels in GDM patients.

Keywords: adiponectin, genetics, adipokine, gestational diabetes mellitus

Introduction

Diabetes is examined as the main cause of death in most countries and the high percentage of prevalence was 8.8% in 2015.¹ Gestational diabetes mellitus (GDM) can be diagnosed using blood glucose levels during pregnancy and is known as hyperglycemia and the incidence rate of type 2 diabetes in mothers is about 1–14% in different populations.² GDM is defined by early-onset of glucose intolerance during pregnancy and is related to T2D development.³ In a normal pregnancy, it is classified by increasing insulin resistance and insulin secretion by pancreatic β -cells.⁴ GDM women are an imbalance between insulin resistance and insulin secretion capacity, leading to excess glucose circulates.⁵ In the past decades, mothers with GDM related the increasing incidence and deleterious results for offspring.⁶

The pathogenesis of GDM remains unclear but abnormal adipokines may play a role in GDM development.⁷ It is recognized that the key of the endocrine organ is adipose tissue which plays a crucial role in metabolic regulation and is involved with metabolic syndrome, obesity-related chronic low-grade inflammation, and insulin resistance.⁸ More

specifically, adipokines may promote insulin resistance and metabolic diseases. Kampmann et al reported that GDM was constantly elevated with insulin resistance, adipokines, and glucose tolerance deterioration.⁹

Adipokines such as adiponectin, resistin, adipsin, plasminogen activator inhibitor-1 (PAI-1), and neutrophil gelatinase-associated lipocalin (NGAL) are secreted by white adipose tissue, which is now recognized to be an active participant in glucose homeostasis.¹⁰ Recently, this evidence has become robust suggesting that obesity and inflammation are major components of insulin resistance. One of the mechanisms described in patients with a metabolic syndrome characterized by excess visceral adipose tissue is that long-term exposure to higher adipokine levels leads to a chronic sub-inflammatory state that is involved in the development of insulin resistance.¹¹

Adiponectin is the most abundant adipokine with a role in insulin sensitivity.¹² Previous studies have documented that lower adiponectin levels are commonly observed in patients with diabetes and pregnancy.¹³ Apart from the *ADIPOQ* genes are encoded on chromosome 3q27. The SNPs of the *ADIPOQ* gene are associated with metabolic syndrome and diabetes.¹⁴ Gestational diabetes is associated with the polymorphism of the *ADIPOQ* promoter rs266729.¹⁵ The TG and GG genotype of *ADIPOQ* +45T/G (rs2241766) has been reported to be associated with low adiponectin levels in GDM patients.¹⁶ Therefore, we were interested to estimate circulating adipokines and molecular genetics of adiponectin may play an important role in the pathogenesis of GDM.

Based on these findings, we aimed to examine maternal serum adipokines during pregnancy both in women with or without GDM, and their associations were assessed between adiponectin, adipsin/factor D, NGAL/Lipocalin-2, total PAI-1, and resistin with maternal pre-pregnancy weight and BMI, compared with healthy pregnant controls. Using the same population, we also investigate 3 SNPs in *ADIPOQ* genes (−11377C/G, rs266729 in the promoter; +45T/G, rs2241766 in exon 2 and +276G/T, rs1501299 in intron 2 region) between GDM patients and controls.

Methods

Study Population

This cross-sectional study was approved by the Faculty of Medicine Siriraj hospital ethics review board, Mahidol University, Thailand (Si 577/2015) complies with the Declaration of Helsinki. Written informed consent was obtained from all subjects. This study involved 426 subjects (229 normal pregnant women and 197 GDM pregnant women) with no history of the other type of diabetes; type 1 or type 2; were included. All pregnant women subjects had regular follow-up visits and were diagnosed by Siriraj hospital physicians from the outpatient Department of Obstetrics and Gynecology, Faculty of Medicine Siriraj Hospital, Mahidol University. The screening test for GDM was according to the glucose challenge test (GCT) (measurement of plasma glucose concentration 1 hour after 50 g of oral glucose load). If the screening test was positive (GCT \geq 140 mg/dl), GDM was diagnosed according to 100g 3-hour OGTT with Carpenter/Coustan criteria. GDM was defined if at least 2 values were equal or exceeded the threshold of 95, 180, 155, and 140 mg/dl for fasting plasma glucose, 1-hour plasma glucose, 2 hours plasma glucose, and 3 hours plasma glucose values respectively.

Blood Sample Collection and Laboratory Measurements

Blood samples were collected from every pregnant woman who had follow-up visits at the outpatient department of obstetrics and gynecology, faculty of medicine Siriraj hospital, Mahidol university. Blood samples for adipokine testing were centrifuged at 1500 rev/min for 15 min at room temperature. The serum was separated and determinations were performed within 1 hour at room temperature. The serum/plasma was stored at -80°C until assayed. Also, the buffy coat was kept at 4°C until assayed.

Adipokine levels were investigated in serum by using MILLIPLEX® MAP human adipokine magnetic bead panel 1, in a 96-well plate (Merck Millipore Corporation, MA, USA). The assay sensitivity was 21 pg/mL for adiponectin, 10 pg/mL for adipsin, 3.5 pg/mL for Lipocalin-2, 5.8 pg/mL for PAI-1, and 4.4 pg/mL for resistin. A total of 5 biomarkers (adiponectin, adipsin/factor D, NGAL/Lipocalin-2, total PAI-1, and resistin) were quantified using Luminex® assays (Luminex Corp., Madison, WI).

Genotype Analysis

SNPs -11377C/G (rs266729), +45T/G (rs2241766), and +276G/T (rs1501299) of the *ADIPOQ* gene were genotyped with DNA samples consisting of 197 GDM subjects and 229 controls. Genomic DNA was extracted from the buffy coat by Flexigene® DNA (Qiagen, Valencia, CA, USA). Genomic DNA was amplified with primer-specific genes. PCR primer sequence and annealing temperature are listed in Table 1. Primers were obtained from Integrated DNA Technologies. DNA (125 ng) PCR product was performed by a PCR-restriction fragment length polymorphism (PCR-RFLP) method. The PCR products were digested with *HinPII* (-11377C/G) and *BspHI* (+45T/G) restriction endonuclease (1U, Fermentas, Thermo Scientific, EU) overnight at 37°C, and *BsmI* (+276G/T) restriction endonuclease (1U, Fermentas, Thermo Scientific, EU) at 65°C. The digested products were separated by 12% polyacrylamide gel electrophoresis and visualized by silver staining.

Statistical Analyses

Statistical analyses were performed using SPSS software for window version 18. Continuous variables were given as mean \pm standard deviation (SD). Adipokine concentrations and biochemistry variables between GDM and NGT were evaluated for normality using the Kolmogorov–Smirnov test. Clinical characteristics and adipokine levels were compared and tested for significant differences between GDM and control using Student's *t*-test (normally distributed data) and Mann–Whitney *U*-test (non-parametrically distributed data). All haplotype frequency analyses were determined using the SNPStats tool (<http://bioinfo.iconcologia.net/SNPstats>). Analyses to determine associations between SNPs and GDM were performed under the additive, dominant, and recessive models using logistic regression analysis, with (P^b)/without (P^a) adjustment for age, gestational age at blood collection (weeks), BMI before pregnancy (kg/m^2), BMI during pregnancy (kg/m^2), systolic blood pressure (mmHg), as covariates. The level of statistical significance for all tests was set at $p < 0.05$.

Results

Characteristics of Subjects

A total of 426 pregnant participants were analyzed 197 of the participants had GDM, and 229 were NGT as controls. As shown in Table 2, there was a significant difference between the GDM group and the NGT group in terms of age ($p < 0.0001$) and gestational age ($p < 0.0001$). Pre-pregnancy BMI and BMI at gestation were greater in the women with GDM than in those NGT ($p = 0.004$ and $p = 0.002$, respectively). Systolic and diastolic blood pressure in GDM was significantly elevated when compared with controls ($p = 0.048$ and $p = 0.014$, respectively). Also, glucose concentrations of the 50-g GCT, fasting blood sugar, and for each time of the 100-g, OGTT were significantly greater in the GDM than in the non-GDM group ($p < 0.0001$ for all). Additionally, measurements of serum adipokine levels showed that adiponin and NGAL levels tend to decrease in GDM compared with controls ($p = 0.34$ and $p = 0.23$, respectively). In contrast, serum PAI-1 and resistin levels trend to increase in GDM compared with controls ($p = 0.35$ and $p = 0.28$, respectively). Among adiponectin levels in the GDM group were significantly lower than in the controls ($p < 0.0001$).

Table 1 PCR Primer Sequence and Annealing Temperature

| Primer Name | | Sequence (5'→ 3') | Annealing Temperature (°C) | Product Size (bp) |
|----------------------|---|----------------------|----------------------------|-------------------|
| ADIPOQ SNP -11377C/G | F | ACTTGCCCTGCCTCTGTCTG | 60 | 250 |
| | R | GCCTGGAGAACTGGAAGCTG | | |
| ADIPOQ SNP +45T/G | F | TGTGTGTGGGGTCTGTCTCT | 56 | 265 |
| | R | CCTTTCTCACCCTTCTCACC | | |
| ADIPOQ SNP +276 G/T | F | GTGATGGCAGAGATGGCAC | 56 | 201 |
| | R | CCAACCCCAATCACTTCAG | | |

Table 2 Participant Demographic, Anthropometric, and Clinical Characteristics

| Characteristics | Normal Glucose Tolerance Subjects | Gestational Diabetes Mellitus Subjects | P-value |
|--|-----------------------------------|--|----------|
| n (%) | 229 (53.76%) | 197 (46.24%) | |
| Age (years) | 31.28±5.69 | 33.97±4.67 | <0.0001* |
| <30 | 24.10±3.52 | 25.57±2.90 | 0.08 |
| 30–35 | 32.57±1.74 | 32.76±1.47 | 0.44 |
| >35 | 38.21±2.47 | 38.15±2.04 | 0.69 |
| Gestational age at recruitment (weeks) | 13.12±7.50 | 18.01±10.10 | <0.0001* |
| <12 | 7.51±1.86 | 7.66±1.84 | 0.36 |
| 12–24 | 17.42±3.96 | 17.19±4.29 | 0.58 |
| >24 | 28.48±3.59 | 30.71±3.92 | 0.02* |
| Preconception BMI (kg/m ²) | 23.12±4.48 | 24.36±5.01 | 0.004* |
| <25 | 20.70±2.16 | 21.12±2.45 | 0.06 |
| 25–30 | 27.25±1.46 | 27.20±1.51 | 0.95 |
| >30 | 33.24±2.37 | 33.28±2.86 | 0.80 |
| Gestational BMI (kg/m ²) | 24.48±4.62 | 25.88±5.07 | 0.002* |
| <25 | 21.50±2.14 | 21.30±2.44 | 0.71 |
| 25–30 | 27.40±1.36 | 27.52±1.51 | 0.56 |
| >30 | 33.70±2.93 | 33.12±2.52 | 0.39 |
| Systolic blood pressure (mmHg) | 114.7±12.24 | 116.54±13.07 | 0.048* |
| Diastolic blood pressure (mmHg) | 70.56±9.18 | 72.94±9.97 | 0.014* |
| 50g GCT (mg/dL) | 134.39±26.77 | 192.73±38.66 | <0.0001* |
| Fasting blood glucose (mg/dL) | 77.86±7.43 | 85.03±10.83 | <0.0001* |
| 1-h OGTT | 145.92±25.29 | 197.67±20.30 | <0.0001* |
| 2-h OGTT | 116.88±22.47 | 170.47±26.12 | <0.0001* |
| 3-h OGTT | 103.98±22.27 | 140.18±24.20 | <0.0001* |
| Adiponectin (fg/mL) | 78.07±71.90 | 54.04±45.02 | <0.0001* |
| Adipsin/factor D (pg/mL) | 4627.48±1239.37 | 4556.03±1394.23 | 0.34 |
| NGAL/Lipocalin-2 (pg/mL) | 1105.99±616.60 | 1073.59±664.80 | 0.23 |
| Total PAI-1 (pg/mL) | 202.66±65.67 | 203.34±74.04 | 0.35 |
| Resistin (pg/mL) | 161.43±81.89 | 164.29±101.17 | 0.28 |

Notes: Data presented as number and percentage or mean ± standard deviation. *A p-value<0.05 indicates statistical significance.

Abbreviations: BMI, body mass index; OGTT, oral glucose tolerance test.

Comparison of Adipokine Concentrations and Biochemistry Variables by Age, Gestational Age, and at Blood Collection BMI in GDM and NGT Subjects

To explore potential adipokine level changing resulting from variables in GDM, we measured serum adipokine concentrations of GDM patients and controls. The levels of adiponectin were significantly lower in GDM ages less than 30 years ($p = 0.005$) and age range 30–35 years ($p = 0.001$) when compared with controls. Also, we found adipsin/factor D levels were significantly lower in GDM over 35 years of age ($p = 0.001$). During the 12th–24th week of gestational age found that serum adiponectin levels were remarkably lower in GDM patients ($p = 0.02$). Serum NGAL/lipocalin-2 concentration was significantly lower in GDM by over 24th weeks of gestational age ($p = 0.02$). While we measured adipokine levels in pregnancy subgroups of BMI, we found that total PAI-1 was significantly reduced in lean women pregnancies when compared to BMI-matched controls ($p = 0.04$). Serum NGAL/lipocalin-2 concentration was significantly lower in overweight pregnant women with GDM when compared to BMI-matched controls ($p = 0.03$). We further found that adiponectin in GDM normal weight (25–30 kg/m²) was remarkably lower than BMI-matched controls ($p = 0.001$) (Table 3).

Table 3 Serum Adipokine Concentrations and Biochemistry Variables by Age, Gestational Age and at Blood Collection BMI in NGT and GDM Subjects

| Characteristic Age (Years) | NGT | GDM | P-value | NGT | GDM | P-value | NGT | GDM | P-value |
|--|-----------------|-----------------|---------|-----------------|-----------------|---------|-----------------|-----------------|---------|
| | <30 (n=67) | <30 (n=28) | | 30–35 (n=114) | 30–35 (n=87) | | >35 (n=48) | >35 (n=82) | |
| Adiponectin (fg/mL) | 63.41±47.38 | 43.98±53.46 | 0.005* | 87.96±86.52 | 54.46±45.19 | 0.001* | 74.05±56.48 | 56.67±42.13 | 0.07 |
| Adipsin/factor D (pg/mL) | 4458.03±1 | 4908.44±1251.36 | 0.12 | 4525.47±1129.32 | 4559.10±1414.89 | 0.93 | 5102.77±1321.91 | 4438.34±1413.38 | 0.001* |
| NGAL/Lipocalin-2 (pg/mL) | 1112.18±541.72 | 1101.78±570.75 | 0.93 | 1068.94±601.84 | 1157.49±790.95 | 0.96 | 1186.89±741.89 | 975.29±525.80 | 0.12 |
| Total PAI-I (pg/mL) | 207.10±72.60 | 221.83±85.66 | 0.91 | 199.93±63.94 | 199.95±75.45 | 0.50 | 202.92±60.37 | 201.00±68.50 | 0.59 |
| Resistin (pg/mL) | 172.77±102.20 | 169.00±111.12 | 0.45 | 156.34±70.31 | 174.17±113.22 | 0.998 | 157.89±76.07 | 152.24±82.62 | 0.34 |
| Gestational age (weeks) | <12 (n=125) | <12 (n=71) | P-value | 12–24 (n=81) | 12–24 (n=64) | P-value | >24 (n=23) | >24 (n=62) | P-value |
| Adiponectin (fg/mL) | 78.96±69.68 | 65.38±54.57 | 0.10 | 83.02±81.19 | 52.92±39.24 | 0.02* | 56.58±42.78 | 41.68±34.06 | 0.13 |
| Adipsin/factor D (pg/mL) | 5112.46±1248.57 | 5100.70±1452.45 | 0.55 | 4028.01±930.62 | 4175.88±1151.89 | 0.50 | 4040.83±964.69 | 4299.72±1369.20 | 0.49 |
| NGAL/Lipocalin-2 (pg/mL) | 1109.64±661.09 | 1234.18±660.48 | 0.13 | 1057.32±511.55 | 1053.86±776.12 | 0.13 | 1256.19±905.73 | 905.73±488.18 | 0.02* |
| Total PAI-I (pg/mL) | 202.18±62.02 | 194.04±73.09 | 0.09 | 199.70±70.37 | 197.78±62.50 | 0.76 | 215.69±69.28 | 219.98±83.98 | 0.80 |
| Resistin (pg/mL) | 160.17±79.17 | 172.38±94.47 | 0.61 | 159.06±80.21 | 156.34±102.56 | 0.21 | 176.21±102.15 | 162.86±108.30 | 0.11 |
| At blood collection BMI (kg/m ²) | <25 (n=140) | <25 (n=84) | P-value | 25–30 (n=64) | 25–30 (n=66) | P-value | >30 (n=25) | >30 (n=38) | P-value |
| Adiponectin (fg/mL) | 92.55±82.39 | 74.08±51.56 | 0.22 | 61.06±44.56 | 39.52±29.96 | 0.001* | 38.76±27.82 | 28.83±19.85 | 0.17 |
| Adipsin/factor D (pg/mL) | 4505.56±1194.11 | 4282.49±1263.61 | 0.13 | 4687.55±1254.98 | 4525.64±1347.08 | 0.34 | 5153.12±1348.74 | 5086.70±1580.74 | 0.92 |
| NGAL/Lipocalin-2 (pg/mL) | 1003.95±524.19 | 1123.50±749.54 | 0.78 | 1245.84±752.45 | 1053.16±650.45 | 0.08 | 1311.64±615.77 | 979.03±514.07 | 0.03* |
| Total PAI-I (pg/mL) | 193.91±65.04 | 180.34±58.45 | 0.04* | 213.84±60.64 | 211.55±76.31 | 0.48 | 222.99±74.89 | 229.78±79.20 | 0.98 |
| Resistin (pg/mL) | 160.72±8.19 | 165.91±103.56 | 0.79 | 163.69±64.17 | 173.29±115.83 | 0.32 | 159.45±59.27 | 146.95±73.01 | 0.25 |

Notes: *A p-value<0.05 indicates statistical significance. Age was stratified into the three following subgroups: <30, 30–35, and >35 years. Gestational age was stratified into the three following subgroups: <12, 12–24, and >24 weeks. BMI was divided in the three following subgroups: <25, 25–30, and >30 kg/m².

Abbreviations: BMI, body mass index; NGT, normal glucose tolerance; GDM, gestational diabetes mellitus.

Genotype Frequencies of –11377C/G (rs266729), +45T/G (rs2241766), and +276G/T (rs1501299) and Haplotype Analysis in GDM and NGT Subjects

The genotypes were investigated by PCR-RFLP. Our results showed significant differences in the major alleles of +45T/G (rs2241766) between GDM and NGT when compared to the minor alleles ($p = 0.03$) (Table 4). The minor alleles of +45T/G (rs2241766) significantly increased the risk of GDM in pregnant women without adjustment for age, gestational age at blood collection, BMI before pregnancy, BMI during pregnancy, systolic blood pressure, diastolic blood pressure (odds ratio [OR]: 1.359; 95% confidence interval [CI]: 1.010–1.828; $p^a = 0.043$) (Table 4). Subsequently, haplotype analysis was performed to assess the combined effect of 3 SNPs in the *ADIPOQ* gene. The haplotype of three loci in the *ADIPOQ* gene, –11377C/G (rs266729), +45T/G (rs2241766), and +276G/T (rs1501299) showed different frequencies between cases and controls (Table 5). The global haplotype association p -value was 0.28. The frequencies of the CGT haplotype as a rare haplotype were significantly higher in GDM subjects compared to NGT ($p < 0.0001$), while the CGG, CTG, CTT, CTG, GTT, and GGG haplotypes were no significant difference between case and controls. However, the CGT haplotype had been found lower frequencies in all participants.

Analysis of Association Between SNPs *ADIPOQ* Gene and GDM

Three SNPs in 197 GDM patients and 229 controls were genotyped. The genotype distributions of SNPs are shown in Table 4. All three SNPs genotypes including –11377C/G (rs266729), +45T/G (rs2241766), and +276G/T (rs1501299) were in Hardy-Weinberg equilibrium (HWE) in GDM and control group (all $p > 0.05$). Logistic regression analysis was used to examine the association between each SNP and GDM patients in three different models (additive, dominant and recessive model). There was a significant increase in the risk of GDM patients after adjustment for age, gestational age at blood collection, BMI before pregnancy, BMI during pregnancy, systolic blood pressure, diastolic blood pressure (odds ratio [OR]: 1.554; 95% confidence interval [CI]: 1.010–2.390; $p^b = 0.045$). No associations between *ADIPOQ* –11377C/G (rs266729) or +276G/T (rs1501299) and GDM were found in three genetic models.

Clinical and Genotype Frequencies of SNPs *ADIPOQ* Gene in GDM and NGT Subjects

To compare serum adipokine concentrations and biochemistry variables between subcategories of major allele and minor alleles in 3 SNPs *ADIPOQ* gene, the 50g GCT levels were significantly decreased in major alleles than minor alleles ($p = 0.01$) in SNPs at –11377C/G (rs266729) in GDM patients. Furthermore, analysis of glucose concentrations after 1 hr by OGTT, the results showed that glucose levels were significantly elevated in major alleles compared with minor alleles in GDM subjects ($p = 0.02$). Interestingly, the levels of adiponectin were significantly reduced in major alleles (–11377C/G, rs266729) when compared with minor alleles in GDM ($p = 0.04$). Moreover, fasting blood glucose was significantly decreased at SNPs +276G/T (rs1501299) major alleles compared to minor alleles in GDM ($p = 0.02$) (Table 6). Levels of adipisin were significantly elevated in major alleles compared to minor alleles in SNPs +276G/T (rs1501299) in NGT subjects ($p = 0.005$) (Table 7).

Discussion

GDM is a pregnancy-related complication. It shows the risk factors of both poor maternal and poor newborn health.¹⁷ The most symptoms of GDM are premature birth, premature rupture of membranes, gestational hypertension, pre-eclampsia, cesarean section, and macrosomia.¹⁸ It is well known that insulin resistance (IR), which causes a change of increased maternal adipose tissues and anti-insulin in the placenta.¹⁹ Previous studies of adipokines show that they have been associated with insulin resistance related to pregnancy in women with a history of GDM.²⁰ However, no previous evidence has investigated a cohort of these five adipokines and their relationship to glucose level with advancing pregnancy within a single sample of women. To our knowledge, there have been few published reports on simultaneously determining genetic factors, and maternal concentrations of adipokines in pregnancy subsequently developing GDM.⁷ Type 2 diabetes (T2D) after pregnancy has also been linked to GDM as a risk factor.³ The meta-analysis of T2D variants was relevant in various tissue such as adipose subcutaneous tissue, adipose visceral omentum, liver, and pancreas.²¹

Adiponectin is a protein released from adipocytes, which plays a role in the pathogenesis of GDM.²² In this current study, serum adiponectin levels were lower in GDM subjects compared to NGT subjects. Pala et al reported similar

Table 4 Analysis of the Association Between SNPs in *ADIPOQ* and NGT and GDM

| Polymorphism | Group | Allele Frequency, n | | | | | Genotype Frequency, n | | | | | Addictive Model | | Dominant Model | | Recessive Model | |
|-------------------------|-------|---------------------|----------|-------|-------------------------------|-------------------------------|-----------------------|-----|-----|------|------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| | | A Allele | B Allele | P | P ^a OR (95% CI) | P ^b OR (95% CI) | A/A | A/B | B/B | P | P ^{HWE} | P ^a OR (95% CI) | P ^b OR (95% CI) | P ^a OR (95% CI) | P ^b OR (95% CI) | P ^a OR (95% CI) | P ^b OR (95% CI) |
| -11377C/G (rs266729) | GDM | 290 | 104 | 0.29 | 0.414 | 0.876 | 105 | 80 | 12 | 0.40 | 0.48 | 0.246 | 0.833 | 0.698 | 0.698 | 0.234 | 0.728 |
| | NGT | 322 | 136 | | 0.881 (0.651– 1.193) | 1.027 (0.734– 1.438) | 115 | 92 | 22 | | 0.57 | 0.640 (0.301– 1.359) | 0.914 (0.397– 2.104) | 0.926 (0.630– 1.363) | 1.089 (0.708– 1.676) | 1.559 (0.750– 3.239) | 1.154 (0.515– 2.586) |
| +45T/G (rs2241766) | GDM | 259 | 135 | 0.03* | 0.043* | 0.078 | 82 | 95 | 20 | 0.07 | 0.32 | 0.151 | 0.310 | 0.035* | 0.045* | 0.234 | 0.728 |
| | NGT | 333 | 125 | | 1.359 (1.010– 1.828) | 1.342 (0.967– 1.863) | 120 | 93 | 16 | | 0.72 | 1.709 (0.823– 3.549) | 1.524 (0.676– 3.435) | 1.519 (1.030– 2.241) | 1.554 (1.010– 2.390) | 1.559 (0.750– 3.239) | 1.154 (0.515– 2.586) |
| +276G/T (rs1501299) | GDM | 293 | 101 | 0.30 | 0.476 | 0.447 | 109 | 75 | 13 | 0.58 | 0.82 | 0.531 | 0.701 | 0.533 | 0.384 | 0.234 | 0.728 |
| | NGT | 326 | 132 | | 0.895 (0.659– 1.214) | 0.878 (0.627– 1.228) | 116 | 94 | 19 | | 0.99 | 0.786 (0.370– 1.671) | 0.852 (0.376– 1.932) | 0.884 (0.601– 1.301) | 0.827 (0.538– 1.270) | 1.559 (0.750– 3.239) | 1.154 (0.515– 2.586) |

Notes: P-values were calculated using logistic regression without (P^a) or with (P^b) adjustment for age, gestational age at blood collection (weeks), BMI before pregnancy (kg/m²), BMI during pregnancy (kg/m²), systolic blood pressure (mmHg). The OR and 95% CI of having the risk allele are shown in allele frequency. The OR and 95% CI of having the risk allele are shown in the additive and dominant model. The OR and 95% CI of having the A/A homo major allele, A/B heterozygote allele are shown in the recessive model. *A p-value<0.05 indicates statistical significance.

Abbreviations: SNPs, single-nucleotide polymorphisms; NGT, normal glucose tolerance; GDM, gestational diabetes mellitus; HWE, Hardy-Weinberg equilibrium, A major allele, B minor allele, A/A homo major allele, A/B heterozygote allele, B/B homo minor allele.

Table 5 Haplotype Frequencies in NGT Controls and GDM Cases

| No. | Haplotype† | Total Frequency | Frequency (NGT) | Frequency (GDM) | Odds Ratio (95% CI) | P |
|-----|------------|-----------------|-----------------|-----------------|---------------------|------|
| 1 | CGG | 0.2780 | 0.2511 | 0.3096 | 1 | – |
| 2 | CTG | 0.2641 | 0.2703 | 0.2566 | 0.76 (0.50–1.15) | 0.19 |
| 3 | CTT | 0.1721 | 0.1817 | 0.1613 | 0.72 (0.46–1.13) | 0.16 |
| 4 | GTG | 0.1614 | 0.1685 | 0.1530 | 0.73 (0.47–1.15) | 0.17 |
| 5 | GTT | 0.0972 | 0.1065 | 0.0865 | 0.66 (0.39–1.15) | 0.14 |
| 6 | GGG | 0.0230 | 0.0219 | 0.0245 | 0.91 (0.25–3.32) | 0.89 |

Notes: †Haplotype (–11377 C/G, +45 T/G and +276 G/T); Global haplotype association $p=0.28$.

Abbreviations: NGT, normal glucose tolerance; GDM, gestational diabetes mellitus; CI, confidence interval.

Table 6 Serum Adipokine Concentrations and Biochemistry Variables by ADIPOQ Single Nucleotide Polymorphism Genotype in GDM Subjects

| Phenotype | –11377C/G | | P | +45T>G | | P | +276G>T | | P |
|---------------------------------|-----------------|-----------------|-------|-----------------|-----------------|------|-----------------|-----------------|-------|
| | C/C | C/G + G/G | | TT | TG + GG | | GG | GT + TT | |
| n | 105 | 80+12 | – | 82 | 95+20 | – | 109 | 75+14 | – |
| Systolic blood pressure (mmHg) | 117.46±11.80 | 115.53±14.32 | 0.27 | 115.73±13.10 | 117.12±13.07 | 0.59 | 116.46±12.84 | 116.63±13.40 | 0.98 |
| Diastolic blood pressure (mmHg) | 73.49±8.74 | 72.34±11.18 | 0.38 | 73.00±10.51 | 72.90±9.61 | 0.80 | 72.72±9.85 | 73.19±10.16 | 0.96 |
| 50g GCT (mg/dL) | 186.74±37.54 | 199.36±39.01 | 0.01* | 195.15±31.13 | 191.03±43.26 | 0.12 | 192.53±39.30 | 192.98±38.12 | 0.87 |
| Fasting blood glucose (mg/dL) | 85.75±10.48 | 83.93±11.39 | 0.35 | 85.14±9.79 | 84.95±11.54 | 0.85 | 83.23±11.63 | 87.18±9.48 | 0.02* |
| 1 h OGTT | 201.35±18.76 | 192.16±21.49 | 0.02* | 199.73±21.16 | 196.36±19.80 | 0.36 | 194.34±22.86 | 201.74±15.97 | 0.06 |
| 2 h OGTT | 171.04±22.86 | 169.61±30.74 | 0.80 | 164.69±26.50 | 174.27±25.38 | 0.14 | 170.31±22.45 | 170.67±30.19 | 0.95 |
| 3 h OGTT | 138.99±25.47 | 142.00±22.33 | 0.43 | 137.54±23.40 | 141.91±24.75 | 0.65 | 138.53±22.94 | 142.16±25.75 | 0.47 |
| Adiponectin (fg/mL) | 48.46±41.69 | 60.18±47.91 | 0.04* | 55.32±47.09 | 53.10±43.64 | 0.73 | 54.63±45.34 | 53.33±44.89 | 0.99 |
| Adipsin/factor D (pg/mL) | 4390.45±1346.60 | 4736.66±1430.21 | 0.09 | 4833.43±1523.38 | 4356.40±1263.30 | 0.09 | 4531.07±1384.84 | 4585.09±1412.76 | 0.79 |
| NGAL/ Lipocalin-2 (pg/mL) | 1076.49±661.40 | 1070.36±672.35 | 0.78 | 1109.44±750.94 | 1047.70±597.26 | 0.93 | 1089.98±631.76 | 1054.12±705.36 | 0.33 |
| Total PAI-1 (pg/mL) | 196.13±68.30 | 211.36±79.58 | 0.30 | 212.66±75.37 | 196.60±72.67 | 0.13 | 196.90±73.12 | 210.98±74.84 | 0.26 |
| Resistin (pg/mL) | 164.97±102.77 | 163.55±99.98 | 0.96 | 164.98±109.07 | 163.79±95.62 | 0.61 | 162.10±95.14 | 166.83±108.29 | 0.84 |

Notes: Values are presented as mean ± standard deviation in each category. The Mann–Whitney U was used to compare each parameter among two groups of each ADIPOQ variation. *A p -value<0.05 indicates statistical significance.

Abbreviation: OGTT, oral glucose tolerance test.

Table 7 Serum Adipokine Concentrations and Biochemistry Variables by ADIPOQ Single Nucleotide Polymorphism Genotype in NGT Controls

| Phenotype | –11377C/G | | P | +45T>G | | P | +276G>T | | P |
|---------------------------------|-----------------|-----------------|------|-----------------|-----------------|------|-----------------|-----------------|--------|
| | C/C | C/G + G/G | | TT | TG + GG | | GG | GT + TT | |
| n | 115 | 92+22 | – | 120 | 93+16 | – | 116 | 94+19 | – |
| Systolic blood pressure (mmHg) | 113.83±12.81 | 114.32±11.68 | 0.62 | 114.02±12.18 | 114.13±12.35 | 0.98 | 114.43±13.26 | 113.71±11.13 | 0.54 |
| Diastolic blood pressure (mmHg) | 71.31±9.20 | 69.81±9.13 | 0.20 | 70.55±9.27 | 70.58±9.12 | 0.87 | 70.00±9.49 | 71.14±8.85 | 0.39 |
| 50g GCT (mg/dL) | 135.20±25.39 | 133.58±28.17 | 0.52 | 132.32±26.58 | 136.69±26.90 | 0.18 | 134.84±27.17 | 133.93±26.46 | 0.96 |
| Fasting blood glucose (mg/dL) | 77.47±9.05 | 78.28±5.18 | 0.54 | 78.51±7.60 | 77.30±7.32 | 0.82 | 78.46±7.62 | 77.29±7.29 | 0.95 |
| 1 h OGTT | 143.59±21.96 | 148.47±28.56 | 0.55 | 151.55±28.44 | 141.02±21.31 | 0.06 | 144.88±26.76 | 146.91±24.07 | 0.86 |
| 2 h OGTT | 116.80±24.50 | 116.97±20.31 | 0.87 | 116.40±23.95 | 117.30±21.36 | 0.89 | 117.90±23.21 | 115.91±21.97 | 0.91 |
| 3 h OGTT | 104.77±22.49 | 103.12±22.28 | 0.58 | 104.19±22.175 | 103.80±22.61 | 0.90 | 104.44±21.87 | 103.55±22.91 | 0.91 |
| Adiponectin (fg/mL) | 73.84±65.10 | 82.53±78.49 | 0.62 | 85.15±78.47 | 70.33±63.42 | 0.37 | 77.57±68.54 | 78.60±75.60 | 0.89 |
| Adipsin/factor D (pg/mL) | 4694.31±1210.17 | 4558.84±1270.53 | 0.54 | 4600.16±1293.07 | 4657.64±1182.66 | 0.70 | 4858.95±1239.34 | 4393.94±1200.21 | 0.005* |
| NGAL/ Lipocalin-2 (pg/mL) | 1095.12±664.02 | 1117.26±566.12 | 0.55 | 1144.39±608.78 | 1063.15±625.35 | 0.31 | 1145.83±692.75 | 1064.66±526.23 | 0.70 |
| Total PAI-1 (pg/mL) | 206.94±66.96 | 198.34±64.35 | 0.35 | 200.38±63.11 | 205.17±68.58 | 0.13 | 204.66±65.90 | 200.60±65.66 | 0.46 |
| Resistin (pg/mL) | 164.19±89.11 | 158.52±73.81 | 0.91 | 170.84±91.88 | 151.32±68.57 | 0.12 | 161.46±74.60 | 161.40±89.16 | 0.55 |

Notes: Values are presented as mean ± standard deviation in each category. The Mann–Whitney U was used to compare each parameter among two groups of each ADIPOQ variation. *A p -value<0.05 indicates statistical significance.

Abbreviation: OGTT, oral glucose tolerance test.

findings in their study.²³ Adipsin is secreted in abundance in adipose tissue. It activates glucose transport through an insulin mechanism.²⁴ This finding identified adipsin as a circulating factor linking fat cells to beta-cell function, more specifically, adipsin potentiates insulin secretion.²⁵ Resistin is a hormone secreted from adipose tissue. It belongs to the family of cysteine-rich, c-terminal proteins, and actively opposes insulin action in peripheral tissues.²⁶ Some evidence has approved lower resistin levels in GDM than in NGT with a further decline after childbirth.²⁷ NGAL or lipocalin-2 is a potential mediator involved in the inflammatory marker in insulin resistance, high blood glucose, and obesity.²⁸ PAI-1 is the regulator of the fibrinolytic system. It is produced by the endothelium but is also secreted by adipose tissue, liver, lung, and muscle.²⁹ Increased PAI-1 levels in plasma accompany symptoms of metabolic syndromes, such as glucose intolerance and insulin resistance.³⁰ However, this study did not find significant differences in adipsin, NGAL/lipocalin-2, PAI-1, and resistin levels between GDM and NGT.

Furthermore, few studies investigated the relationships between adiponectin levels determined early in pregnancy and GDM. Low adiponectin levels were associated with an increased risk of GDM.³¹ Contradictory evidence can be partly explained by experimental designs, confounding factors, and the cutoff of impaired glucose regulation in pregnancy.³¹ Adiponectin concentrations in the circulation are associated with triacylglycerol and HDL levels.²³ Besides, we examined the blood glucose and circulating adiponectin as early as 14 weeks of GDM pregnancy, compared with those who avoid GDM recurrence.²⁰

Maternal adipokine profiles and a glucose tolerance assay will be used in early pregnancy to prognosis women at high risk of GDM recurrence, as maternal adiponectin may function for fetal growth and birth weight.³² Based on adipokine functions, further studies on the role of these adipokines are important to understand in the pathogenesis of insulin resistance and GDM and may help to identify biomarkers of GDM prediction or prognosis. The current study showed that adiponectin concentration was significantly decreased in GDM after subcategorized by age, gestational age, and blood collection BMI. Adiponectin concentrations were lower in GDM at age <30, 30–35 years old, 12th–24th week of gestational age, and 25–30 kg/m² of gestational BMI when compared to NGT. It has been similar to the prospective cohort study, adiponectin was negatively correlated with age, glucose, and BMI, and positively correlated with gestational age at delivery.³³

The relationship between *ADIPOQ* polymorphism and GDM has been the subject of many recent studies. The present study revealed that the GDM had a higher distribution of +45T/G (rs2241766) G allele frequency than the NGT group. It was considered that the adiponectin SNP +45T/G (rs2241766) might be associated with GDM, and the G allele might be the ultimate risk factor for GDM in Thai women. Our finding was confirmed with the previous document that Han women with GDM, G allele at SNP +45T/G (rs2241766) might be correlated with declined plasma adiponectin concentrations and inverse clinical outcomes.³⁴ In agreement with our findings, GDM resembles an early stage of type 2 diabetes, which was associated with low adiponectin levels and the *ADIPOQ* gene. SNP –11377C/G (rs266729) demonstrated the prevalence of Korean T2D.³⁵ Our results have shown that adiponectin levels significantly declined in GDM with SNP –11377 C/G (rs266729) C allele. Nomani et al found that an elevated level of adiponectin was associated with the G allele of SNPs –11377 C/G (rs266729) in the Iran population.³⁶ It might indicate the association between SNP –11377 C/G (rs266729) in the promoter region of the adiponectin gene and the regulation of adiponectin expression. However, there was no association between SNP –11377C/G and GDM in Asian, South American, and European populations which may be due to the difference between the ethnic group.³⁷ In this study, fasting blood glucose levels were significantly higher in GDM patients with +276G/T (rs1501299) minor alleles compared to those with major alleles. Obese subjects with the T allele of *ADIPOQ* +276G/T (rs1501299) had higher fasting glucose levels on the Mediterranean hypocaloric diet.³⁸ Moreover, our results demonstrated that maternal age, pre-pregnancy BMI, and increasing weight were not predictive factors for GDM. Our sample size may be small conducted with less power to predict glucose levels in GDM.

In conclusion, our studies illustrated that adiponectin concentrations were remarkably decreased in GDM pregnant women than in NGT. Adiponectin levels were controlled by *ADIPOQ* gene polymorphisms. GDM patients with minor alleles of *ADIPOQ* –11377C/G (rs266729) had higher levels of adiponectin. *ADIPOQ* +45T/G (rs2241766) was associated with an increased risk of GDM in Thai pregnant women (Figure 1). These results were implicated in biomarker risk prediction in early diagnosis and preventing high glucose during gestation.

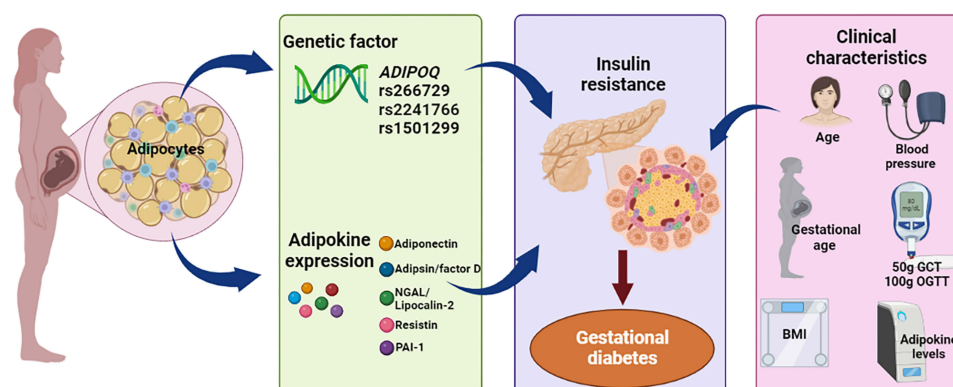


Figure 1 Schematic diagram showing the association between adipokines level, *ADIPOQ* variants (−11377C/G, rs266729; +45T/G, rs2241766 and +276G/T, rs1501299) and pregnant women (Created with BioRender.com).

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Disclosure

The authors declare no conflict of interest.

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