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ORIGINAL ARTICLE

Insertion and deletion polymorphism in the alpha-2B adrenoceptor gene in pregnant women ripens gestational diabetes mellitus

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Abstract There are no earlier studies that reported the association of the *12Glu9* polymorphism in the alpha-2B adrenoceptor (*ADRA2B*) gene with gestational diabetes mellitus (GDM). We examined the potential association between the *ADRA2B* gene insertion/deletion (I/D) polymorphism in the Saudi population with GDM. Pregnant women with GDM have been reported to exhibit the same susceptibility as that observed in type 2 diabetes mellitus (T2DM). We have selected I/D polymorphism of the *ADRA2B* gene located in chromosome 2q11.1 that has been extensively related to T2DM and cardiovascular diseases. This case–control study was conducted with 200 GDM and 300 non-GDM pregnant women. Genotyping of I/D polymorphism was performed by conventional PCR method. Biochemical analyses were found to be significantly different between GDM and non-GDM subjects ($p < 0.05$). Genotype (ID + DD vs II, $p = 0.0002$) and allele (D vs I, $p = 0.0002$) frequencies of the *12Glu9* polymorphism were found to be statistically significant. However, a significant difference was found between allele and genotypes of I/D polymorphism of the *ADRA2B* gene or the clinical characteristics of the subjects. Our results obtained in this study indicate the *ADRA2B* gene in the Saudi women was associated with the development of GDM.

1. Introduction

Gestational diabetes mellitus (GDM) remains one of the most common clinical issues that obstetrician's face (Hiraoka et al., 2011). The clinical characteristics of pregnant women that are associated with a high risk for GDM include obesity, increased Body Mass Index (BMI), family history of type 2 diabetes

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mellitus (T2DM)/GDM, advanced maternal age, and glycosuria (Chon et al., 2013). GDM is a common pregnancy complication and a strong predictor for T2DM (Hedderson et al., 2013). Epidemiological studies have confirmed that GDM is associated with increased feto-maternal morbidity and long-term complications in both the mothers and offspring. The pathogenesis of GDM is largely unknown. Women with a history of GDM are at an increased risk of developing T2DM later in their lives, and women with a family history of diabetes may be predisposed to an increased risk of GDM (Wang et al., 2012). GDM and T2DM share common pathophysiological features, including β-cell dysfunction and insulin resistance. The prevalence of GDM and T2DM continue to increase in many racial/ethnic populations. In the Kingdom of Saudi Arabia, the overall prevalence of GDM and T2DM is 22% (Wahabi et al., 2013) and T2DM has 23.1% respectively (Al-Daghri et al., 2012).

A common nonsynonymous variant (*12Glu9*) of the human α_{2B}-adrenergic gene (*ADRA2B*) encodes a receptor protein leading to the insertion/deletion (I/D) of three consecutive glutamate residues at amino acid positions 301–303, and it has been associated with hypertension/T2DM (Vasudevan et al., 2008) and acute coronary events (Heinonen et al., 2002; Snapir et al., 2001). Vasudevan and coworkers (2008) found that the *ADAR2B* I/D polymorphism was associated with T2DM in Malaysian subjects. However, the exon 1 region of the *ADAR2B* I/D polymorphism has not been reported in GDM. Therefore, the goal of the present study was to determine whether the *ADAR2B* I/D polymorphism plays an important role in Saudi pregnant women who develop GDM.

2. Materials and methods

2.1. Study design

In this case–control study, we selected 500 pregnant women from the Department of Obstetrics and Gynecology, King Khalid University Hospital (KKUH), King Saud University, Riyadh, Saudi Arabia. The study included 200 GDM women who developed diabetes during pregnancy and 300 non-GDM women. We excluded 132 antenatal patients who were prediagnosed with type 1 or type 2 diabetes. Non-GDM women had normal glucose levels and demonstrated normal glucose tolerance (NGT). Samples of 5 mL of venous blood were collected from all pregnant women; 3 mL of each serum sample was used for biochemical analysis to confirm the disease, and 2 mL of each ethylenediaminetetraacetic acid (EDTA) sample was used for molecular analysis. The study protocol was approved by the Institutional Review Board at the Faculty of Medicine, King Saud University, and all pregnant women who participated in the study provided written informed consent. GDM and non-GDM samples were obtained by senior physician at KKUH.

2.2. Glucose test

Pregnant women without a previous diagnosis of glucose intolerance were routinely screened for GDM by two methods between 24 and 28 weeks of gestation. Initially, a 50 g glucose challenge test (GCT) was used as a preliminary screen. The GCT was considered positive if the plasma glucose values surpassed 7.8 mmol/L. Pregnant women with positive GCTs were evaluated with the second method, i.e., a 100 g oral glucose tolerance test (OGTT). Diagnosis of GDM was based on criteria set by the American Diabetes Association (Swan et al., 2007). After an overnight fast that followed for three days of unrestricted diet, fasting plasma samples were drawn after one, two, and three hours of glucose administration. The glucose threshold values are shown in Table 1. A diagnosis of GDM was made if two or more of the glucose values met or exceeded the threshold value. NGT was diagnosed when all plasma glucose values were below the threshold values. Based on the above criteria, 200 subjects with GDM and 300 with NGT were recruited into the study. The NGT patients were considered as controls, or non-GDM, for this study.

2.3. Anthropometric measurement

Anthropometric measurements were obtained by trained personnel at the health care centers. Height and body weight were measured to the nearest 0.5 cm and 0.1 kg, respectively. Body Mass Index (BMI) was calculated as weight/height^2 (kg/m^2). Subjects with BMI > 30 kg/m^2 were categorized as obese. Waist circumference was measured to the nearest 0.5 cm at the level of the midpoints of the lowest rib, and the hip circumference was measured to the nearest 0.5 cm at the maximum extension of the buttocks.

2.4. Biochemical analysis

Fasting and postprandial blood and lipid profile biochemical parameters were assessed. High-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), total cholesterol (TC), and plasma glucose were measured with an automated clinical chemistry analyzer (Kit provided by KoneLab, Espoo, Finland) using commercially available kits. Dyslipidemia (low levels of HDL-C) was defined as HDL-C levels < 1.03 mmol/L for men and < 1.29 mmol/L for women (Almeida et al., 2011).

2.5. Genetic analysis

DNA was extracted from peripheral leukocytes using the standard AccuVis Bio DNA extraction kit (AccuVis Bio, UAE). DNA samples were store at −80 °C. The concentration of genomic DNA was quantitatively determined by optical density measurements using a NanoDrop 2000 (Thermo Fisher Scientific, MA, and USA). The purity was determined by calculating the ratio of absorbance at 260 nm to absorbance at 280 nm (A_{260}/A_{280}). Non-annealed DNA should have an A_{260}/A_{280} ratio of 1.7–1.9. For the *12Glu9* I > D polymorphism, the primers 5′-AGGGTGTTTGTGGGGCATCT-3′

Table 1 Diagnosis of GDM with a 100 g oral glucose tolerance test.

	mmol/L*	mg/dL**
Fasting	5.3	95
First hour	10.0	180
Second hour	8.6	155
Third hour	7.8	140

** mg/dL – milligram/deciliter.
* mmol/L – milli molar/liter.

(sense) and 5′-CAAGCTGAGGCCGGAGACACT-3′ (antisense) were used to amplify the *ADAR2B* gene D/D, I/D, and I/I genotypes of the 112 bp fragment. Polymerase chain reactions (PCRs) were performed in a thermal cycler (Applied Biosystems, USA) with initial denaturation at 95 °C for 5 min followed by 35 cycles, with each cycle containing denaturation at 95 °C for 30 s, annealing at 57 °C for 30 s, extension at 72 °C for 45 min, and final extension at 72 °C for 5 min. Amplification of the I/D polymorphism was performed in a total volume of 20 μL reaction mixture composed of 2 μL (10 pmol) of each primer, 6 μL of sterile water, and 10 μL of a 2× master mix that included $MgCl_2$, 10× Taq buffer, 10 unit of Taq DNA polymerase (Norgen Biotek corp, Canada), and 2 μL template DNA. Primers were synthesized by Bioserve Biotechnologies (Hyderabad, India). The amplified products were analyzed by electrophoresis on a 12% polyacrylamide gel. The larger allele of *12Glu9* contained 3 tandem, 9 bp repeats and it was designated the insertion, and the smaller allele of *12Glu9* contained no tandem repeats, being designated as the deletion. The sizes of the PCR products were 112 bp and 103 bp for the insertion and deletion alleles, respectively.

2.6. Statistical analysis

Clinical characteristics of all the subjects were expressed as the mean ± SD. Alleles and genotype frequency differences between GDM patients and non-GDM subjects were tested using a chi-square test. Odds ratios (ORs) and 95% confidence intervals are calculated by binomial logistic regression for the allele, genotype, and haplotype frequencies, and the chi-square test was used to identify departures from the Hardy–Weinberg equilibrium. Statistical analyses were performed with SPSS version 19.0 software. A p-value of <0.05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

Clinical, biochemical, and anthropometric data are shown in Table 2 for GDM and non-GDM subjects. The results show that GDM subjects were significantly older than controls, but anthropometric measurements, including weight, height, BMI and LDL-C were not significantly different ($p > 0.05$). The biochemical profile, consisting of FBS, PPBG, GCT, OGTT, TC, TG, and HDL-C was significantly higher in GDM patients compared with non-GDM subjects ($p < 0.05$). Family histories of T2DM and GDM patients were significantly different between the groups ($p < 0.05$). Women diagnosed with GDM were given dietary instructions by a dietician as soon as possible after diagnosis. Ninety percent of the GDM women were on diet, and 10% of them were using insulin because they were unable to maintain normal blood glucose levels by the diet.

3.2. Genotype and allele distribution

The genotype and allele distribution of *12Glu9* variants are summarized in Table 3. Distribution of genotypes and allele frequencies of the *12Glu9* polymorphism in GDM and non-GDM women satisfy the Hardy–Weinberg equilibrium. The genotype and allele frequencies for the *12Glu9* polymorphism were significantly different between GDM and non-GDM subjects. The results from this study show a high prevalence of the DD genotype in GDM women (6.5%) compared to non-GDM (2.3%). The frequency of the D allele was 15.2% in the GDM group and 7% in the non-GDM group. Notably, a significant association was observed between I/D polymorphism and genotypes/allele frequencies, i.e., between the ID + DD genotype and the D allele [OR-2.3 (1.48, 3.8), $p = 0.0002$; OR-2.3 (1.57, 3.6), $p = 0.0002$].

3.3. Association of the ADRA2B gene variants with clinical and biochemical parameters

The prevalence of different components of the *ADRA2B* gene was analyzed based on the *12Glu9* polymorphisms and genotypes. Results showed that the DD and ID genotypes were significantly associated only with TC ($p = 0.03$), whereas the II genotype was found to have high values of weight, height, BMI, FBS, PPBG, GCT, and HDL-C ($p > 0.05$). Family history of T2DM and GDM was found to be high among individuals with the II genotype. More than 88% of the pregnant women were on a diet, and 11.8% of the pregnant women were on insulin (Table 4).

4. Discussion

There are many factors, such as obesity, family history of T2DM, and other complications, that have been reported to influence the pathogenesis of GDM, thought to be a multifactorial disease similar to essential hypertension, T2DM, and chronic heart disease (CHD). The association of gene polymorphisms with GDM has been a major focus of recent research. Our team genotyped a target SNP, rs4426564, located at the 5′-end of exon 1 of the *ADRA2B* gene. We studied I/D (*12Glu9*) polymorphism in Saudi women, grouped according to those who do and do not develop GDM.

It is understood that the etiology of GDM is similar to T2DM, where genetic and environmental factors affect disease onset and progression during pregnancy. The hyperglycemia associated with GDM is detected at one point in a women's life. If glucose levels are already in the diabetic range, GDM could represent glucose intolerance that is limited to pregnancy, where it is chronic but stable, or the detected GDM could indicate progression to diabetes (Buchanan and Xiang, 2005). The *12Glu9* or I/D polymorphism is linked to the autonomic dysfunction and increased sympathetic nervous system activity (Papanas et al., 2007). These characteristics have been associated with adverse metabolic and vascular effects, including reduced basal metabolic rate (Heinonen et al., 1999), obesity (Siitonen et al., 2004), and earlier onset of diabetes (Papazoglou et al., 2006), acute coronary ischemia (Snapir et al., 2001), T2DM with neuropathy (Papanas et al., 2007), sudden cardiac death (Laukkanen et al., 2009), spontaneous abortions (Galazios et al., 2011), migraine (Ni et al., 2010), glucose 6 phosphate dehydrogenase (Alharbi et al., 2013), suicide (Fukutake et al., 2008), and hypertension with and without T2DM (Vasudevan et al., 2008). Several studies of subjects from different ethnic backgrounds have reported a positive or negative association between the *ADRA2B* gene

Table 2 Clinical details.

S. No	Aspects	GDM cases (n = 200)	Controls (n = 300)	Statistical significance
1	Age (Years)	32.43 ± 5.79	31.36 ± 6.02	p = 0.55
2	Weight (kg)	77.1 ± 13.34	74.85 ± 12.09	p = 0.12
3	Height (m^2)	158.51 ± 5.92	157.81 ± 5.31	p = 0.08
4	BMI (kg/m^2)	34.43 ± 4.68	33.36 ± 4.28	p = 0.16
5	Hypertension (%)	76 (38%)	14 (4.6%)	p < 0.0001
6	Mean gestational age	30.27 ± 5.77	NA	NA
7	FBS (mmol/L)	5.0 ± 0.93	4.5 ± 0.87	p < 0.0001
8	PPBG (mmol/L)	6.8 ± 2.0	4.9 ± 1.8	p = 0.0001
9	GCT (mmol/L)	9.5 ± 1.8	6.3 ± 1.5	p < 0.0001
10	OGTT (Fasting hour)	5.2 ± 1.18	4.5 ± 0.87	p < 0.0001
11	OGTT (1st hour)	10.7 ± 1.8	8.0 ± 1.7	p < 0.0001
12	OGTT (2nd hour)	9.2 ± 1.8	6.7 ± 1.6	p < 0.0001
13	OGTT (3rd hour)	5.6 ± 1.7	4.5 ± 1.3	p < 0.0001
14	TG (mmol/L)	2.3 ± 1.8	1.7 ± 0.98	p < 0.0001
15	TC (mmol/L)	5.7 ± 1.2	5.2 ± 1.0	p < 0.0001
16	HDL-C (mmol/L)	0.92 ± 0.38	0.64 ± 0.24	p < 0.0001
17	LDL-C (mmol/L)	3.7 ± 0.93	3.7 ± 1.0	p = 0.82
18	Family History of T2DM (n%)	120 (60%)	55 (18.3%)	p < 0.0001
19	Family History of GDM (n%)	46 (23%)	13 (4.3%)	p < 0.0001
20	R_x (Diet/Insulin)	180 (90%)/20 (10%)	NA	NA

NA = not applicable/not analyzed.

Table 3 Genotype distribution of *12Glu9* polymorphism in the individuals enrolled in the study.

Genotypes	Non-GDM N (%)	GDM N (%)	Odds ratio (95% CI)	p Value
N	300	200		
II	265 (88.4)	152 (76)	Reference	–
ID	28 (9.3)	35 (17.5)	2.1 (1.27, 3.74)	0.003
DD	7 (2.3)	13 (6.5)	1.4 (0.52, 4.2)	0.45
ID + DD	35 (11.6)	48 (24)	2.3 (1.48, 3.8)	0.0002*
I	558 (93)	339 (84.8)	Reference	
D	42 (7)	61 (15.2)	2.3 (1.57, 3.6)	0.0002

* After continuity correction.

Table 4 Anthropometric and metabolic parameters according to genotype of *12Glu9* I > D polymorphism in GDM women.

S. No	Aspects	ID + DD (n = 48)	II (n = 152)	p Value
1	Age (Years)	33.58 ± 6.51	32.07 ± 5.46	p = 0.11
2	Weight (kg)	75.96 ± 14.99	77.46 ± 12.80	p = 0.15
3	Height (m^2)	157.42 ± 6.30	158.86 ± 5.81	p = 0.46
4	BMI (kg/m^2)	29.89 ± 4.89	30.48 ± 4.62	p = 0.59
5	Mean gestational age	31.12 ± 6.71	29.93 ± 5.54	p = 0.08
6	FBS (mmol/L)	3.29 ± 2.38	3.55 ± 8.29	p = 0.61
7	PPBG (mmol/L)	3.91 ± 3.31	4.04 ± 3.97	p = 0.14
8	GCT (mmol/L)	1.39 ± 3.45	1.81 ± 3.84	p = 0.39
9	TG (mmol/L)	1.81 ± 1.04	1.74 ± 0.96	p = 0.46
10	TC (mmol/L)	5.41 ± 1.26	5.12 ± 1.00	p = 0.03
11	HDL-C (mmol/L)	0.62 ± 0.25	0.64 ± 0.24	p = 0.69
12	LDL-C (mmol/L)	3.95 ± 1.06	3.60 ± 0.87	p = 0.07
13	Family history of T2DM (n%)	20 (41.6%)	100 (65.8%)	NA
14	Family history of GDM (n%)	11 (22.9%)	35 (23%)	NA
15	R_x (Diet/Insulin)	46 (95.8%)/2 (4.2%)	134 (88.2%)/18 (11.8%)	NA
16	OGTT (Fasting hour)	4.26 ± 2.68	3.70 ± 2.54	p = 0.61
17	OGTT (1st hour)	8.41 ± 4.89	7.66 ± 5.07	p = 0.79
18	OGTT (2nd hour)	7.28 ± 4.20	6.47 ± 4.37	p = 0.77
19	OGTT (3rd hour)	3.76 ± 2.92	3.26 ± 3.14	p = 0.57

NA = not applicable/not analyzed.

Table 5 Association of *12Glu9* polymorphism of *ADRA2B* gene in relation to different diseases and population of ethnicity.

S. No	Disease	Population	*p* Value	Association	References
1	Type 2 diabetes mellitus + Hypertension	Malaysian	$p = 0.51$	No	Vasudevan et al. (2008)
2	Acute coronary events	Finland/Sweden	$p = 0.03$	Yes	Heinonen et al. (1999, 2002)
3	Acute coronary events	Finland	$p = 0.02$	Yes	Snapir et al. (2001, 2003)
4	T2DM + Neuropathy	Greece	$p = 0.0008$	Yes	Papanas et al. (2007)
5	Insulin Secretion + T2DM	Finland	$p = 0.04$	Yes	Siitonen et al. (2004)
6	T2DM	Greece	$p = 0.40$	No	Papazoglou et al. (2006)
7	Sudden cardiac death (SCD)	Finland	$p < 0.05$	Yes	Laukkanen et al. (2009)
8	Spontaneous recurrent abortions	Greece	$p = 0.78$	No	Galazios et al. (2011)
9	Migraine	Chinese	$p = 0.35$	No	Ni et al. (2010)
10	Glucose 6 phosphate dehydrogenase	Saudi	$p = 0.68$	No	Alharbi et al. (2013)
11	Suicide	Japanese	$p = 0.04$	Yes	Fukutake et al. (2008)
12	Artery compliance	Chinese	$p = 0.73$	No	Zhang et al. (2005)
13	Cardiac conduction	Russian	$p < 0.05$	Yes	Chernova et al. (2013)
14	Hypertension	Scandinavian	$p = 0.04$	Yes	Von Wowern et al.(2004)
15	Myocardial ischemia + T2DM	Chinese	$p < 0.05$	Yes	Chen et al. (2010)
16	Obesity	Greece	$p > 0.05$	No	Sykiotis et al. (2003)
17	Myocardial infarction + SCD	Finland	$p = 0.01$	Yes	Snapir et al. (2001, 2003)
18	Gestational diabetes mellitus	Saudi	$p = 0.0002$	Yes	Present study

Table 6 Genotype distribution of *12Glu9* polymorphism of *ADRA2B* gene in controls vs different forms of diabetes in relation to ethnicity.

Genotype/allele	Saudi ($n = 500$)		Greece ($n = 190$)		Malaysian ($n = 210$)		
	GDM *N* (%)	Non-GDM *N* (%)	Diabetic Neuropathy *N* (%)	Non-Diabetic Neuropathy *N* (%)	Controls *N* (%)	Hypertension *N* (%)	T2DM N (%)
II	152 (76)	265 (88.4)	72 (55.4)	46 (76.70)	6 (8.57)	7 (10.77)	18 (24)
ID	35 (17.5)	28 (9.3)	46 (35.4)	14 (23.3)	34 (48.57)	28 (43.08)	35 (46.67)
DD	13 (6.5)	7 (2.3)	12 (9.2)	0 (0)	30 (42.86)	30 (46.15)	22 (29.33)
ID + DD	48 (24)	35 (11.6)	60 (44.6)	14 (23.3)	64 (91.43)	58 (89.23)	57 (76)
I	339 (84.8)	558 (93)	190 (73.07)	106 (88.3)	46 (32.86)	42 (32.31)	71 (47.33)
DD	61 (15.2)	42 (7)	70 (26.93)	14 (11.7)	94 (67.14)	88 (67.69)	79 (52.67)
OR	2.3 (1.5,3.6)		2.789 (1.4–5.1)		0.545 (0.3–0.8)		
p-Value	$p = 0.0002$		$p = 0.0008$		$p = 0.012$		

variants in different diseases. The *12Glu9* polymorphism was studied in relation to multiple diseases, and the reports are summarized in Table 5.

In this case–control study, we find significant differences in the distribution of the *12Glu9* or I/D polymorphism among GDM and non-GDM subjects. A significant association was found between the genotypes and the GDM status, obesity, or dyslipidemia, indicating that these polymorphisms may be important risk determinants of cardio or metabolic disease in Saudi women. Furthermore, our data suggest that I/D polymorphism contribute to variation in expression of the *ADRA2B* gene. In addition, no associations were noted between genotype and anthropometric parameters, including age and BMI, indicating that this polymorphism may not independently contribute to obesity and advanced maternal age as stated in the studied population. The results of this study also indicated that the frequency of the heterozygous carriers and homozygous variants of I/D polymorphism of *ADRA2B* was significantly higher in GDM women than in non-GDM women.

Papanas et al. (2007) studied T2DM with and without nephropathy. The results of their study indicate that D allele was significantly higher ($p = 0.001$) in group A (26.9%) as compared to group B (11.7%). Papanas et al. (2007) and co-authors concluded that patients with neuropathy exhibit a significantly higher frequency of the D allele. Essential hypertension in a Malaysian population with and without T2DM was studied earlier in three separate groups, controls, hypertension with T2DM, and hypertension without T2DM. There was a significant difference between the genotype and clinical data ($p < 0.05$), but there is no significant difference between the three genotypes and the clinical characteristics of the study subjects ($p > 0.05$). The results obtained in the Malaysian study indicated that the D allele in the *ADRA2B* gene was associated with essential hypertension with and without T2DM in Malaysian subjects (Vasudevan et al., 2008). In this present study, the genotype distribution of the *12Glu9* polymorphism was strongly associated with GDM. This consistency might be due to differences in genetic background and sample size (Table 6). No clinical effects of this

polymorphism were observed in this prospective study. This is clearly a limitation of the study and indicates that the influence on obesity risk of I/D polymorphism may be of major importance in GDM women. However, the subjects in our study had a mean age greater than 30, which indicates the presence of obesity in both GDM and non-GDM subjects.

In conclusion, the present study shows that *12glu9* polymorphism of the *ADRA2B* gene is associated with the risk of GDM in a Saudi population. Further research is warranted to confirm the causality. The association of the DD genotype and the D allele with GDM should be examined in multiple, well-designed genetic epidemiological studies, and the physiological effects should be identified.

Conflict of interest

We confirm that all the authors have no actual or potential competing interests regarding the submitted article.

Authors' contributions

AMM and AZ have helped to collect the samples and edited the manuscript. AKK was the PI of the project and design the study, helped us to finalize the manuscript. KIA had performed the experiment and written and finalized the manuscript. All the authors read and approved the final manuscript.

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