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The C Allele of the rs4929949 STK33 gene Polymorphism is Associated with Gestational Diabetes Mellitus

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Gestational diabetes mellitus (GDM) is a growing health concern that typically appears during the latter half of pregnancy. It is defined as any form of diabetes or glucose intolerance with the onset of or first recognition during pregnancy. Pregnant women with GDM are at risk for certain neonatal complications. Our aim was to investigate the association between the serine/threonine kinase 33 (*STK33*) rs4929949 polymorphism and the risk of diabetes developed during pregnancy in Saudi women. We genotyped the rs4929949 polymorphism in a hospital-based case-control study, comparing pregnant 200 GDM women with 300 non-GDM women. Genotyping was performed using the TaqMan assay method. All the glucose values and lipid profiles examined, excluding LDL-C, were associated with an increased risk of GDM ($p < 0.05$). A significant variation with respect to the genotypic and allele distribution in the disease group was observed when compared to the non-GDM women (OR = 1.83 [95% CI = 1.0, 3.1], $p = 0.02$) and (OR = 1.33 [95% CI = 1.0, 1.7], $p = 0.02$). The present study revealed a significant association of the *STK33* gene polymorphism with GDM women in a Saudi population.

Key words: Gestational diabetes, *STK33*, rs4929949 and Saudi population.

Gestational diabetes mellitus (GDM) is a common pregnancy-related condition characterized by glucose intolerance without a previous diagnosis of diabetes (Cavicchia *et al*., 2014). GDM presents itself primarily in the second half of gestation and results from insulin resistance that is thought to be induced by excessive placental hormones. GDM mothers and their offspring are at increased risk of developing type 2 diabetes mellitus (T2DM) in the future (Xu *et al*., 2014). The T2DM genetic background may be a risk factor in GDM; ample evidence demonstrates that the prevalence of T2DM is relatively higher in mothers with GDM after pregnancy (Chon *et al*., 2013). The definition of GDM applies whether insulin or diet modification is used for treatment and whether the condition persists after pregnancy. The prevalence of GDM, which affects 2–22% of all pregnancies, varies across populations (e.g., ethnic groups) (Alharbi *et al*., 2014). GDM is associated with obesity in later life (Jamenez *et al*., 2014). The serine/threonine kinase 33 (*STK33*) gene, associated with obesity and body weight, has been identified at the 11p15.5 locus by a genome-wide association study (GWAS) (Rask *et al*., 2013). A single nucleotide polymorphism (SNP), rs4929949, is a high-frequency SNP located within intron 1 of *STK33* and has a reported minor allele frequency of approximately 46%, making it suitable for replication in smaller independent cohorts. Rs4929949 gave the strongest signal in body mass

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association tests, but several other SNPs spanning a 200-kb region, including the entire *STK33* gene and its proximal upstream area, were also strongly associated. *STK33* is located in a gene-rich region on chromosome 11p15.5 (Speliotes *et al*., 2010). GDM is associated with an equivalent risk of obesity; however, there is no data relating *STK33* SNPs and GDM. To the best of our knowledge, the association between the *STK33* gene and the risk of GDM in a Saudi population has not been assessed. Because genetic differences are likely to exist in different communities and GDM is of significant concern to society, we conducted a case-controlled study to investigate the potential association between the rs4929949 *STK33* polymorphism and GDM in Saudi women.

MATERIALS AND METHODS

Participants

This is a case-control study carried out in Riyadh, the capital city of the Kingdom of Saudi Arabia. The study population consisted of 200 women diagnosed with GDM who were admitted to King Khalid University Hospital (KKUH), part of King Saud University, between 2011 and 2013. All the pregnant women were natives of Saudi Arabia by self-identification. The study protocol was approved by the Institutional Review Board of KKUH (Riyadh).

Screening of GDM women

Clinical and laboratory examinations of women were conducted at KKUH. The criteria for inclusion in the study group were (a) Saudi nationality; (b) pregnancy with confirmed GDM or a history of GDM in the second or third trimester of gestation; (c) signed informed consent for the study; (d) age range between 18–40 years; (e) body mass index (BMI) <40 kg/m^2. The control women (n = 300) were selected from pregnant females with similar characteristics as the GDM women, but they had a history of normal glucose tolerance and were termed non-GDM women. The glucose challenge test (GCT) and oral glucose tolerance tests (OGTT) were performed for all the pregnant women who had signed the informed consent form to participate in this study. Before the OGTT was performed, all the pregnant women were told to fast overnight and to consume an unrestricted diet for three days. Fasting plasma samples were drawn for the OGTT test. The confirmation of the GDM women was performed as described previously (Alharbi *et al*., 2014).

Biological samples

Venous blood samples (3 mL) were collected for biochemical analysis and 2 mL of each anticoaggulated blood sample was collected in an EDTA tube for DNA analysis. The samples were maintained at 4 °C and centrifuged. Separated plasma was stored at "80 °C for further use.

Physical and biochemical examination

The physical examination included height and weight measurements. BMI was calculated as weight/height^2 (kg/m^2). The women with BMI <30 kg/m^2 and >30 kg/m^2 were considered as overweight and obese, respectively. Fasting and postprandial blood lipid profile parameters included triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), GCT, and OGTT were measured using commercial kits as described previously (Alharbi *et al*., 2014).

DNA analysis

Total genomic DNA was extracted from venous blood samples using the AccuVis DNA extraction kit (AccuVis Bio, Abu Dhabi, and UAE). DNA samples were stored at "80 °C until further use. The quantity and quality of the DNA was assessed using a Nanodrop and gel electrophoresis, respectively. Allelic discrimination (rs4929949 and C_11595944_10) was performed using the TaqMan SNP Genotyping Assay and Applied Biosystems Prism 7300 Real-time Polymerase Chain Reaction apparatus using the default cycling conditions (Alharbi *et al*., 2014[b]).

Statistical analysis

Statistical package for the social sciences (SPSS) version 21.0 for windows (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Results of continuous variables are reported as mean ± standard deviation, median for quantitative variables, and percentage of categorical variables. Hardy-Weinberg equilibrium (HWE) was tested using the Ç^2 test for goodness of fit. A p-value of <0.05 was considered as a significant disequilibrium. Allele and genotype frequencies were compared between patients and controls by the Ç^2 test or two-sided Fisher's exact test (p < 0.05 was considered significant). Chi-Square methods were used to

compare genotype frequencies between GDM and non-GDM pregnant women, assuming both additive and recessive models.

RESULTS

Participant characteristics

The clinical and biomedical characteristics of the participating women are displayed in Table 1. No statistically significant differences in maternal age and prepregnancy BMI was found in the study groups. Biochemical serum parameters such as fasting blood sugar, postprandial blood glucose, GCT, OGTT, and lipid profile tests, including TC, TG, HDL-C, and LDL-C values, occurred significantly more frequently in the GDM women compared to the control group ($p < 0.05$). Family history of GDM and T2DM were higher in GDM women ($p < 0.05$). Predictable GDM women (90%) were prescribed a diet to maintain normal glucose values, whereas 10% of GDM women were administered 4–8 units of insulin due to failure of the diet.

Table 1. Clinical and Biomedical characteristics of the pregnant women

S.No	Aspects	GDM Cases (*n*=200)	Non-GDM (*n*=300)	*p* value
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	Mean Gestational Age	30.27±5.77	NA	NA
6	FBS (mmol/L)	5.0±0.93	4.5±0.87	$p < 0.0001$
7	PPBG (mmol/L)	6.8±2.0	4.9±1.8	$p = 0.0001$
8	GCT (mmol/L)	9.5±1.8	6.3±1.5	$p < 0.0001$
9	OGTT (Fasting hour)	5.2±1.18	4.5±0.87	$p < 0.0001$
10	OGTT (1^{st} hour)	10.7±1.8	8.0±1.7	$p < 0.0001$
11	OGTT (2^{nd} hour)	9.2±1.8	6.7±1.6	$p < 0.0001$
12	OGTT (3^{rd} hour)	5.6±1.7	4.5±1.3	$p < 0.0001$
13	TG (mmol/L)	2.3±1.8	1.7±0.98	$p < 0.0001$
14	TC (mmol/L)	5.7±1.2	5.2±1.0	$p < 0.0001$
15	HDL-C (mmol/L)	0.92±0.38	0.64±0.24	$p < 0.0001$
16	LDL-C (mmol/L)	3.7±0.93	3.7±1.0	$p = 0.82$
17	Family History of T2DM (n %)	120 (60%)	55 (18.3%)	$p < 0.0001$
18	Family History of GDM (n %)	46 (23%)	13 (4.3%)	$p < 0.0001$
19	R_x (Diet/Insulin)	180 (90%)/ 20 (10%)	NA	NA

NA= Not applicable/ Not analyzed

Table 2. Genotype and allele distribution of the *STK11* (C528G) gene polymorphism for GDM and non-GDM

STK33 (rs4929949)	GDM	Non-GDM	Odds ratio[a] (95 % CI)	*p* value	Odds ratio[b] (95 % CI)	*p* value
N	200	300				
TT	47 (23.5)	98 (32.7)	Reference			
TC	110 (55)	153 (51)	1.49 (0.9, 2.2)	0.06*	1.2 (0.8, 2.7)	0.07
CC	43 (21.5)	49 (16.3)	1.83 (1.0, 3.1)	0.02*		
TC+CC	153 (76.5)	202 (67.3)	1.57 (1.0, 2.3)	0.02*	1.4 (1.0, 2.8)	0.04
T	204 (0.51)	349 (0.58)	Reference			
C	196 (0.49)	251 (0.42)	1.33 (1.0, 1.7)	0.02		

aCrude odds ratio (95% CI); [bOdds] ratio (95%CI) adjusted for age and BMI
*Genotype and allele frequency distribution with GDM and non-GDM subjects.

Table 3. Anthropometric and metabolic parameters according to genotype of rs4929949 polymorphism in GDM women

S.No	Aspects	TC+CC (n = 153)	TT (n = 47)	p-value
1	Age (Years)	32.74±5.84	31.41±5.30	p = 0.44
2	Weight (Kg)	77.51±14.00	75.72±10.84	p = 0.04
3	Height (m^2)	158.48±5.98	158.61±5.90	p = 0.94
4	BMI (kg/m^2)	30.42±4.83	30.04±4.19	p = 0.26
5	Mean Gestational Age	30.6±5.97	28.93±5.30	p = 0.35
6	FBS (mmol/L)	3.31±2.42	3.35±2.32	p = 0.75
7	PPBG (mmol/L)	5.43±3.43	4.61±3.63	p = 0.60
8	GCT (mmol/L)	1.86±3.86	1.27±3.36	p = 0.27
9	TG (mmol/L)	1.79±1.03	1.62±0.78	p = 0.03
10	TC (mmol/L)	5.21±1.11	5.13±0.96	p = 0.25
11	HDL-C (mmol/L)	0.62±0.25	0.61±0.19	p = 0.03
12	LDL-C (mmol/L)	3.74±0.96	3.76±0.83	p = 0.25
13	Family History of T2DM (n %)	92 (60.1%)	28 (59.6%)	<0.0001
14	Family History of GDM (n %)	36 (23.5%)	10 (21.3%)	<0.0001
15	R_x (Diet/Insulin)	136 (75.5%)/17 (8.5%)	44 (93.6%)/03 (6.4%)	<0.0001
16	OGTT (Fasting hour)	3.74±2.55	4.15±2.67	p = 0.66
17	OGTT (1^{st} hour)	7.76±5.10	8.09±4.79	p = 0.63
18	OGTT (2^{nd} hour)	6.5±4.37	7.23±4.22	p = 0.80
19	OGTT (3^{rd} hour)	3.90±3.26	3.58±3.12	p = 0.74

NA= Not applicable/ Not analyze

STK33 rs4929949 genotype

The genotype and allele frequencies of the rs4929949 *STK33* gene polymorphism in the GDM and non-GDM groups are tabulated in Table 2. The *STK33* genotype distribution met the requirements of HWE in both groups of pregnant women. There was a significant difference in the genotype and allele frequencies of the rs4929949 *STK33* polymorphism between GDM and non-GDM women. For CC vs. TT, $p = 0.02$, odds ratio = 1.49 (95% CI, 1.0–3.1). A significant difference was observed in the frequency of C and T alleles in GDM women and non-GDM women (for C vs. T, $p = 0.02$, odds ratio = 1.33 (95% CI, 1.0–1.7). When we performed the dominant model for pregnant women, we found that there was evidence for the association between rs4929949 and the risk of GDM (for CC + TC vs. TT), $p = 0.02$, odds ratio = 1.57 (95% CI = 1.0–2.3).

DISCUSSION

GDM is defined as a spectrum of metabolic disorders that are genetically heterogeneous with multiple genes located on different chromosomes contributing to its susceptibility. GDM occurs when the pancreas fails to produce enough insulin to regulate blood sugar efficiently. Hormones produced by the placenta makes certain women essentially resistant to their own insulin. There is ample evidence that GDM and T2DM have a strong genetic basis. In the current case-control study, we reported a common genetic polymorphism in the *STK33* gene, rs4929949, associated with an increased risk of GDM in Saudi women. Screening for GDM is recommended because of its asymptomatic nature and many women fail to present classic risk factors. The exact identification of GDM women during pregnancy is recognized only by plain serum samples like fasting tests, OGTT, and GCT. To date, no procedure was established for identifying GDM by genetic and molecular analyses. To the best of our knowledge, this is the first study conducted on pregnant Saudi women. The results of our study revealed that the C allele variant of the rs4929949 *STK33* polymorphism is significantly associated with GDM. We investigated the positive association of *STK33* gene and GDM in a Saudi population.

Mouse models are complementary to human genetic studies and offer unique

advantages, including the ability to control environmental variance, perform dangerous or invasive procedures, conduct well-defined crosses, functionally evaluate candidate genes in vivo or in vitro, and undertake rigorous mechanistic studies. Quantitative trait locus studies have been successful in identifying chromosomal regions associated with body weight in mice, yet gene identification has remained elusive (Parker *et al*., 2011). Rs4929949 is located 500-kb downstream from the gene encoding tubby protein homolog, which has been linked to body weight and obesity in mouse studies (Parker *et al*., 2013) and in early genetic studies (Shiri-Sverdlov *et al*., 2006; Snieder *et al*., 2008), but it remains unconfirmed in GWAS. To date, no functional data exists linking any of the genes in the proximity of rs4929949, or rs4929949 itself, to GDM.

In the current study, we have analyzed the effect of the rs4929949 polymorphism on anthropometric, clinical, and biochemical parameters, and we analyzed the distribution of these variables in relation to TC + CC and TT genotypes. The analysis revealed that TC + CC genotypes in comparison with TT was significantly associated with increased weight (77.51 ± 14.00 vs. 75.72 ± 10.84 kg, $p = 0.04$), BMI (33.42 ± 4.93 vs. 30.04 ± 3.61 kg/m^2, $p = 0.02$), TG (1.79 ± 1.03 vs. 1.62 ± 0.78 mmol/L, $p = 0.03$), TC (5.21 ± 1.11 vs. 5.13 ± 0.76 mmol/L, $p = 0.03$), HDL-C (0.62 ± 0.25 vs. 0.61 ± 0.19 mmol/L, $p = 0.03$) and LDL-C (3.69 ± 0.69 vs. 3.79 ± 0.89 mmol/L, $p = 0.02$). Family history of both T2DM and GDM was associated ($p < 0.0001$) (Table 3). In contrast, the TC + CC genotype was significantly higher in the R_x (diet/insulin) than with the TT genotype ($p < 0.0001$) (Table 3).

In this case-control study, a significant association was found between the alleles and the GDM status, obesity, and BMI, indicating that these polymorphisms are important in estimating the risk of cardiovascular or metabolic diseases in Saudi women. Furthermore, our data suggest that the rs4929949 polymorphism contributes to variation in the expression of the *STK33* gene.

Our study has several limitations. First, although our sample size was sufficient, we assume that the relative sample size may reduce the statistical power and overestimate the OR value. Second, we only investigated one SNP in *STK33* gene. Although this SNP was associated with obesity in one study, the relationship between STK33 gene polymorphism and BMI, serum lipids, and GDM risk may not be fully elucidated. Finally, there are no prior studies on any metabolic disorder apart from obesity.

To summarize, SNP rs4929949 in intron 1 of the *STK33* gene in a Saudi population might alter its expression and contribute to the genetic background of GDM, a pregnancy-associated disease that emerges from the interactions of multiple genes, variants, and environment factors. Large-scale studies are required to confirm the disease in different ethnicities.

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