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Regular paper

The genetic polymorphism in the STK11 does not affect gestational diabetes

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Gestational diabetes mellitus (GDM) is defined as carbohydrate intolerance of variable severity that develops during pregnancy. Recent studies indicate that GDM onset is rapid, and that women with GDM will develop other metabolic disorders such as obesity, type 2 diabetes, and cardiovascular disease in their future. Serine/threonine kinase 11 (STK11) is engaged in the insulin signaling pathway and encoded protein is an important activator of adenosine monophosphate activated protein kinase. Based on the previously reported association between the STK11 gene and diabetes, we aimed to investigate whether the rs8111699 polymorphism in *STK11* has any role in gestation diabetes in Saudi women. In this case-control study, we recruited pregnant Saudi women based on biochemical analysis of their blood samples. Genomic DNA was obtained from confirmed subjects (200 GDM cases and 300 non-GDM). PCR-RFLP analysis was performed to detect the C528G polymorphism in the STK11 gene. The anthropometric and clinical data were similar between the GDM and non-GDM subjects ($p>0.05$), whereas the biochemical analysis was significantly different between the cases and controls ($p<0.05$). The genotype and allele frequencies between of the *STK11* gene were not statistically significant difference between the GDM and non-GDM groups (OR=0.82; 95% CI:=0.6–1.0; p=0.12). Our study suggests that the rs8111699 polymorphism has no role in the development of GDM in pregnant Saudi women.

Key words: gestational diabetes, STK11, rs8111699 polymorphism, Saudi women

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INTRODUCTION

Gestational diabetes mellitus (GDM) is a common metabolic disorder appearing in pregnant women that is characterized by carbohydrate intolerance (Han *et al*., 2015). In Saudi Arabia, the prevalence of GDM reaches 22%. The genetic background of type 2 diabetes mellitus (T2DM) may also be a factor in GDM because ample evidence has demonstrated the presence of T2DM in women with GDM. Additionally, the prevalence of T2DM is relatively higher in mothers who developed GDM in pregnancy (Alharbi *et al*., 2014). GDM shares multiple, risk factors and a similar pathophysiology with T2DM (Saucedo *et al*., 2014). Pregnant women diagnosed with GDM are instructed either to modify their diet or start insulin therapy, based on their glucose values. There are many genes associated with diabetes diseases, identified through genetic approaches such as genome-wide association studies (GWAS), linkage scans and candidate gene studies. These studies have shown an involvement of T2DM-associated genes in GDM, consistent with the notion that both types of diabetes share common pathophysiology (Khan *et al*., 2014). Serine-threonine kinase 11 (STK11), also known as LKB1, is an important activator of adenosine monophosphate activated protein kinase (AMPK), phosphorylating it in the activation loop of the catalytic α-subunit (Tkac *et al*., 2015). The AMPK pathway is a master regulator of glucose and lipid metabolism with pleiotropic actions in the liver, skeletal muscle, pancreas, and brain (Bassols *et al*., 2013). A deletion of STK11 in the liver of adult mice resulted in hyperglycemia with increased gluconeogenic and lipogenic gene expression, and *STK11* gene presence was required for metformin efficacy (Legro *et al*., 2008). Diverse polymorphisms in the *STK11* gene have been associated with different metabolic disorders such as T2DM, GDM, polycystic ovarian syndrome, breast, colorectal and other cancers (Tkac *et al*., 2015; Keshavarz *et al*., 2008; Bassols *et al*., 2013; Goldenberg *et al*., 2008; Pierce *et al*., 2011; Penegar *et al*., 2007). Based on these prior reports, the present study aimed to investigate the rs8111699 polymorphism in the STK11 gene and GDM women in the Saudi population.

MATERIALS AND METHODS

Pregnant women. The present case-control study was carried out in King Khalid University Hospitals (KKUH) in Riyadh, Saudi Arabia. The study was granted an ethics clearance from KKUH. Pregnant Saudi women (n=500) were selected based on their willingness to participate, documented in a signed informed consent. The study population comprised 200 GDM cases and 300 non-GDM women/controls as described in our prior reports (Al-Hakeem *et al*., 2014; Alharbi *et al*., 2014). The inclusion and exclusion criteria and the anthropometric and clinical details have been described (Alharbi *et al*., 2014).

Biochemical analyses. Three milliliters of serum from fasting and non-fasting blood was collected from all the subjects. The pregnant women were instructed to fast overnight following three days of an unrestricted diet. Fasting plasma samples were drawn to initiate the

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Abbreviations: GDM, gestational diabetes mellitus; T2DM, type 2 diabetes mellitus

Glucose Challenge Test (GCT) and Oral Glucose Tolerance Test (OGTT). Serum samples were collected for other specific tests, including fasting blood sugar (FBS), post-prandial blood glucose (PPBG), and a blood lipid profile.

Molecular analysis. Blood samples were collected in EDTA anticoagulant tubes and subjected to molecular analysis. AccuVis extraction kit (AccuVis Bio, UAE) was used to extract genomic DNA from 2 mL of blood. DNA was quantified with Nanodrop. Allelic discrimination was performed with TaqMan SNP genotyping assay (rs8111699 and C_26672045_10) on an Applied Biosystems Prism 7300 Real-Time PCR system using the default cycling conditions (Alharbi *et al*., 2014).

Statistical analysis. Statistical Package for the Social Sciences (SPSS) version 21.0 for Windows (SPSS Inc., Chicago, IL, USA) was used to implement the statistical analyses. Results of continuous variables were reported as mean ± standard deviation (S.D.), percentage. Hardy-Weinberg equilibrium (HWE) was tested using the χ^2 test for goodness of fit, and a p value<0.05 was considered a statistically significant disequilibrium. Allele and genotype frequencies were compared between patients and controls by the χ^2 test or two-sided Fisher's exact test.

RESULTS AND DISCUSSION

Characteristics of pregnant women

The clinical and biochemical characteristics of pregnant women (GDM and non-GDM subjects) are exhibited in Table 1. Anthropometric measurements such as age and body mass index (BMI; height and weight) were similar in both the groups. GDM women (90%) were on a restricted diet to maintain normal glucose values. The remaining 10% of GDM women were using 48 units of insulin due to diet failure. Biochemical values such as FBS, PPBG, GCT, OGTT and blood lipid profiles were obtained. Total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) values were outside of the normal range significantly more frequently in the GDM women compared to the control group (p<0.05). A family history of T2DM and GDM was higher in GDM than in control women (p<0.05).

Genotype analysis

The genotype and allele frequencies of the rs8111699 polymorphism in the STK11 gene in the GDM and non-GDM groups are presented in Table 2. The variants in both GDM and non-GDM subjects were found to be in Hardy-Weinberg equilibrium during pregnancy. The GDM group consisted of 25.5% CC, 59.5% CG, and 15% GG genotypes, while the non-GDM comprised 26.3% CC, 48% CG, and 25.7% GG. The frequency of C and G alleles in the GDM groups was 55% and 45%, respectively, and in the non-GDM groups, 50.3% and 49.7%, respectively. The genotype and allele frequencies of the C528G polymorphism in the *STK11* gene were not significantly different between GDM and non-GDM subjects. For GG *vs.* CC genotypes, p=0.07, and the odds ratio=0.60 (95% CI, 0.3–1.0). For G *vs.* C allele, p=0.12, and the odds ratio=0.82 (95% CI, 0.6, 1.0). Using the dominant model for pregnant women, we could not find any evidence for an association between rs8111699 and risk of GDM (for GG+CG *vs.* CC), p=0.83, and the odds ratio=1.01 (95% CI=0.6–1.5).

This case-control study in pregnant women was carried out in the native Saudi population, follow-

Table 1. Clinical and biomedical characteristics of 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 (1st hour)	10.7±1.8	8.0±1.7	p<0.0001
11	OGTT (2nd hour)	9.2±1.8	6.7±1.6	p<0.0001
12	OGTT (3rd 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 cases.

STK11 (rs8111699)	GDM	Non-GDM	Odds ratio[a] (95% CI)	*p* value
N	200	300		
CC	51 (25.5)	79 (26.3)	Reference	
CG	119 (59.5)	144 (48)	1.3 (0.8, 1.9)	0.25*
GG	30 (15)	77 (25.7)	0.6 (0.3, 1.0)	0.07*
CG+GG	149 (74.5)	221 (73.7)	1.0 (0.6, 1.5)	0.83*
CC+GG	81 (40.5)	156 (52)	0.6 (0.4, 0.9)	0.01*
C	221 (0.55)	302 (0.503)	Reference	
G	179 (0.45)	298 (0.497)	0.82 (0.6, 1.0)	0.12

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

ing signed informed consent by participants. All the pregnant women (n=500) were recruited from the obstetrics and gynecology department, King Khalid University Hospitals. Based on the GCT and OGTT results, we separated the GDM (n=200) and non-GDM women (n=300). GDM cases showed high values of HDL-C comparing with the non-GDM subjects. The TaqMan genotyping was performed to detect the rs8111699 polymorphism to explore its association with GDM. The OGTT values were positively associated with the rs8111699 polymorphism in the GDM subjects, compared with non-GDM groups ($p<0.05$). Allele and genotype frequencies were not found to be positively associated with GDM. There are many studies that have shown positive, negative, and nominal associations of genetic polymorphism with various human diseases. The importance of gene polymorphism studies for human diseases is to identify single nucleotide polymorphisms (SNPs) in the coding region of genes. Almost 90% of sequence variants in humans are in DNA sequences that code for proteins. SNPs are common forms of genetic variations that can be used to search for and isolate disease-causing genes. The importance of polymorphism in genetic studies are that (i) SNPs can be used to reconstruct the genome histories (ii) SNPs can be directly responsible for genetic diseases since they may alter the genetic sequence of gene or of a regulatory region, (iii) and SNPs may be utilized as markers to build high-density genetic maps for association studies, and to identify genes of functional importance. However, polymorphisms are not absolute indicators for the development of disease (e.g.: Alzheimer's disease and the ApoE gene) (Collins *et al*., 1998; Sripichai *et al*., 2007).

STK11, originally identified in 1997 as a causative mutation, appears on the 19p13.3 chromosome region, and encodes a protein involved in the AMPK mediated signal pathway. The AMPK signaling pathway controls glucose homeostasis in the liver and mediates therapeutic effects of insulin-sensitizing antidiabetic agents and early metabolic changes in peripheral tissues. It plays critical roles in many metabolic processes, including fatty acid synthesis and gluconeogenesis, and initiates development of insulin resistance, obesity, and T2DM. The Components of the AMPK signaling pathway, including various AMPK subunits, LKB1 and TORC2, are intriguing candidates that might explain the inherited basis of T2DM (Sung *et al*., 2009; Keshavarz *et al*., 2008; Kahn BB *et al*., 2005). This gene (STK11) was targeted in our current study based on a prior report (Bassols *et al*., 2013). GDM is a risk factor that leads to certain adverse pregnancy outcomes and to obesity in offspring of GDM mothers. It is unknown how the maternal glucose status during pregnancy and after delivery in GDM mothers affects the overweight status of their children (Zhang *et al*., 2014). GDM in pregnancy is recognized only by testing of serum samples, because of its asymptomatic nature and because many GDM patients show no classic risk factors (Al-Hakeem *et al*., 2014).

To the best of our knowledge, this is the first study carried out in a population of Saudi women. Our results revealed that the variant G allele in rs8111699 of the STK11 gene is not associated with GDM. Our studies are in agreement with Bassols and coworkers (2013) in the Spanish population. The limitations of our present study included the lack of additional statistics apart from common genotype and odds ratios. Performing a single SNP analysis was another limitation. The final limitation of our study was that patients who were on treatment with metformin were not included in our study.

In conclusion, our study suggests that the rs8111699 polymorphism has no role in GDM in pregnant Saudi women. For future studies, the inclusion of different ethnic populations with larger sample sizes and metformin treated subjects are recommended.

Authors' contribution

AKK and KIA design the work. KIA performed the lab work and interpreted the Data. EMH helped in the lab work. AMM and AZ recruited patients.

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Conflict of Interest

All the authors declare that there is no conflict of Interest related to this article.

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