The *GCKR* Gene Polymorphism rs780094 is a Risk Factor for Gestational Diabetes in a Brazilian Population

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Background: The glucokinase regulatory protein (GCKR) regulates the activity of the glucokinase (GCK), which plays a key role in glucose homeostasis. Genetic variants in *GCK* have been associated with diabetes and gestational diabetes (GDM). Due to the relationship between GCKRP and GCK, polymorphisms in *GCKR* are also candidates for genetic association with GDM. The aim of this study was to evaluate the association between the *GCKR* rs780094 polymorphism and GDM in a Brazilian population. *Methods:* 252 unrelated Euro-Brazilian pregnant women were classified as control (healthy pregnant women, $n = 125$) and GDM (pregnant women with GDM, $n = 127$) age-matched groups. Clinical and anthropometric data were obtained from all subjects. The *GCKR* rs780094 polymorphism was genotyped using fluorescent probes (TaqMan®, code C_2862873_10). *Results:* Both groups were in Hardy–Weinberg equilibrium. The *GCKR* rs780094 polymorphism was associated with GDM in codominant and dominant models ($P = 0.022$ and $P = 0.010$, respectively). The minor allele (T) frequency for the control group in the study was 38.4% (95% CI: 32–44%), similar to frequencies reported for other Caucasian populations. *Conclusion:* Carriers of the C allele of rs780094 were 1.41 (odds ratio, 95% CI, 0.97–2.03) times more likely to develop GDM. J. Clin. Lab. Anal. 31:e22035, 2017. © 2016 Wiley Periodicals, Inc.

Key words: genetic association; genetic polymorphism; gestational diabetes mellitus; glucokinase regulatory protein; glucose intolerance; pregnancy

INTRODUCTION

Gestational diabetes (GDM) is defined as glucose intolerance diagnosed in the second or third trimester of pregnancy where there was clearly no previous type 1 or type 2 diabetes (T2DM). GDM increases the risk of adverse outcomes for pregnant mothers, fetuses, and neonates (1). GDM and T2DM share pathophysiological and genetic characteristics. Like T2DM, GDM is a polygenic syndrome and several single nucleotide polymorphisms (SNPs) are associated with susceptibility or protection for both conditions (2–4).

Glucokinase (GCK, HK-IV, HK-D, or ATP:D-hexose 6-phosphotransferase) is the key regulatory enzyme in glucose metabolism and has dual functions: (a) it catalyzes the phosphorylation of glucose in pancreatic beta cells and mammalian hepatocytes and (b) it acts as a "sensor" of glucose, to regulate insulin release by the pancreas (5–7). In hepatocytes, the catalytic activity of GCK is regulated by a 68 kDa protein, the *Gluc*o*k*inase *R*egulatory *P*rotein (GCKRP) or Glucokinase (hexokinase 4) Regulator (8). At basal glucose concentrations, GCK binds to its inhibitory protein, GCKRP, in the nuclei of hepatocytes. Elevated concentrations of glucose cause the dissociation of the GCK-GCKR complex in the liver promoting translocation of GCK to the cytoplasm with consequent increased hepatic glucose phosphorylation

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insulin release from the beta cells and glycogen synthesis (9). After performing its function, GCK reverts to the inactive GCKRP-bound form (8, 10).

GCKRP is primarily expressed in hepatocytes and is encoded by the *GCKR*, which maps to chromosome 2p23 (11). Some variants in the *GCK* gene affect the expression of *GCKR* and may contribute to abnormal glucose concentrations (12, 13). Due to the involvement of GCK in diabetes, and the relationship between GCKRP and GCK, polymorphisms in *GCKR* are also candidates for genetic association with diabetes (14).

The rs780094 polymorphism in *GCKR* is strongly associated with elevated triglyceride concentrations (15, 16) and metabolic syndrome (17). In addition, the T allele of rs780094 is associated with a decreased risk of susceptibility to T2DM in certain populations (15, 18, 19). These data reinforce the hypothesis that this polymorphism increases the activity of GCK, leading to a reduction in glucose and increase in triglycerides by stimulation of lipogenic genes of the glycolytic pathway (20). Further, Japanese carriers of the rs780094 TT genotype have lower glycated hemoglobin (HbA1c) levels than those with other genotypes (21).

In contrast, the C allele of *GCKR* rs780094 polymorphism is associated with an increased risk of T2DM (22, 23). Moreover, the risk allele (C) of rs780094 is associated with GDM in Caucasian populations, reinforcing the premise that T2DM and GDM share genetic similarities (24, 25).

Based on the above evidence, the aim of this study was to evaluate the association between the rs780094 polymorphism in *GCKR* and GDM in a Brazilian population.

MATERIALS AND METHODS

Samples

A total of 252 unrelated Euro-Brazilian pregnant women were included in the study. Healthy Euro-Brazilian pregnant women were classified as controls ($n = 125$) and pregnant women diagnosed with GDM, according to 2015 criteria of the American (26) and Brazilian (27) Diabetes Associations, as the GDM group ($n = 127$). Subjects were recruited from a Public Hospital in southern Brazil after written informed consent and the groups were matched by age. The Ethics Committee of the Federal University of Parana approved the study and the work was carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Clinical and laboratory data

Clinical and anthropometric data were obtained from all subjects. Biochemical parameters were determined by routine laboratory methods (Abbott Diagnostics, Santa Clara, CA) in an automated system with reagents, calibrators, and controls provided by the manufacturer (Architect Ci8200; Abbott Diagnostics). Concentrations of 1,5-anhydroglucitol were measured enzymatically (GlycoMark, Inc., New York, NY). Glycated hemoglobin was measured by immunoturbidimetry (Architect; Abbott Diagnostics).

Genotyping

DNA was extracted from whole blood using a modified "salting out" method (28) and sample concentrations were normalized to 20 ng/μl for subsequent assays. Only samples with 280/260 nm absorbance ratios between 1.8 and 2.0 (NanoDrop; ThermoScientific, Waltham, MA) were used in this study. The rs780094 polymorphism was genotyped using real-time PCR with fluorescent probes (TaqMan®, code C_2862873_10; Life Technologies/Applied Biosystems, Foster City, CA). Genotyping experiments were carried out using a 7500 Fast™ Real-Time PCR System (Life Technologies/Applied Biosystems). Reagents (Master Mix®; and Genotyping Assay® SNPs) and other real-time PCR materials were provided by the manufacturer (Applied Biosystems). The reaction mixture (6 μl final volume) contained 3.0 μl of Master Mix (DNA polymerase, Mg^{2+}, buffer, additives), 0.1 μl of SNP Genotyping Assay (40X), 1.9 μl ultrapure water, and 1.0 μl of genomic DNA (20 ng/μl). The PCR conditions were as follows: one cycle of 1 min at 60°C (pre-PCR), one cycle of 10 min at 95°C, 45 cycles of 15 s at 95°C, followed by 60°C for 2 min, and one final cycle of 30 s at 60°C (final extension). Genotyping quality was ≥98%.

Statistical analyses

Normality was tested with the Kolmogorov–Smirnov test. Comparisons of normally distributed parameters were performed using the Student's *t*-test for independent samples and the Mann–Whitney *U* test was used for non-normally distributed variables. Categorical variables were compared using the Fisher's exact test (two tailed) or the chi-square test, as appropriate. Allele frequencies and Hardy–Weinberg (HW) equilibrium were evaluated by Chi-square test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Data analysis was performed using Statistica for Windows 10.0 software (StatSoft Inc., Tulsa, OK), and

probabilities less than 5% ($P < 0.05$) were considered significant for all analyses.

RESULTS

Anthropometric and laboratory data are presented in Table 1. The control and GDM groups were matched by age. GDM patients were significantly heavier (higher body mass index), and more hypertensive than healthy pregnant women. HbA1c in GDM group (median 5.6%) indicated good glycemic control for these patients. No information was available for healthy controls since this biomarker is not in our routine for non-diabetics pregnant women.

The frequency of hypertension in GDM subjects was significantly higher than that of the control group (14.9% vs. 4.8%, respectively; $P = 0.007$).

The rs780094 polymorphism was in Hardy–Weinberg equilibrium in both control and GDM groups ($P > 0.05$). The genotype and allele frequencies of the polymorphism are shown in Table 2.

DISCUSSION

In gestational diabetes, insulin resistance induces a compensatory insulin release by the pancreas, which can increase weight gain. The risk for GDM increases with increase in the BMI of the pregnant woman (29).

TABLE 1. Anthropometric and Laboratory Characteristics of the Study Groups

Characteristics	Controls ($n = 125$)	GDM ($n = 127$)	P
Age, years	30.6 ± 4.7	31.9 ± 6.4	0.070
Body mass index, kg/m^2	26.9 ± 5.0	32.7 ± 6.3	<0.001
Hypertension, %	4.8	14.9	0.007[a]
Family history of diabetes, %	–	70.1	–
Fasting glucose, mg/dl	84.0 (79–88)	88 (81–95)	<0.001[b]
2 h, 75 g glucose, mg/dl	105.0 (93–111)	161.0 (149–176)	<0.001[b]
HbA1c, %	–	5.6 (5.3–5.9)	–
1,5-Anhydroglucitol, µg/ml	11.7 ± 6.9	9.8 ± 5.1	0.060
Total cholesterol, mg/dl	213.9 ± 50.0	224.7 ± 45.6	0.074
HDL-cholesterol, mg/dl	55.4 ± 15.8	56.8 ± 12.5	0.451
LDL-cholesterol, mg/dl	130.9 ± 41.5	123.5 ± 38.9	0.146
Triglycerides, mg/dl	124.0 (96–171)	221.0 (175–270)	<0.001[b]
Total Protein, g/dl	6.9 ± 0.8	6.4 ± 0.5	<0.001
Albumin, g/dl	4.2 ± 0.6	3.4 ± 0.4	<0.001
Creatinine, mg/dl	0.80 (0.7–0.9)	0.70 (0.60–0.72)	<0.001[b]
Urea, mg/dl	20.4 ± 5.3	16.1 ± 4.8	<0.001
Uric acid, mg/dl	3.6 (3.0–3.9)	4.3 (3.7–4.9)	<0.001[b]

Values are presented as mean ± SD, median (interquartile range), or %, –, no information available; Controls, healthy pregnant women; GDM, pregnant women with gestational diabetes; *P*, *P*-values calculated by Student's *t*-test (independent variables)
[a]Chi-square test or [b]Mann–Whitney *U* test.

TABLE 2. Genotype and Allele Frequencies of *GCKR* rs780094 in the Absence (Controls) or Presence of Gestational Diabetes Mellitus (GDM)

Gene/SNP	Model	Genotypes	Controls $n = 125$	GDM $n = 127$	P
GCKR rs780094 (C>T)	Codominant	CC	43 (34.4)	64 (50.4)	**0.022**[a]
		CT	68 (54.4)	48 (37.8)	
		TT	14 (11.2)	15 (11.8)	
	Allele T Frequency [95% CI]		38.4 [32–44]	30.7 [25–36]	0.069
	Dominant	CC/CT+TT	43/82	64/63	**0.010**
	Recessive	TT/CC+CT	14/111	15/112	0.879

Genotypes depicted as number (%).95% CI, 95% confidence interval; *P*, probability determined by chi-square or [a]two-tailed Fisher's exact test.
Significant *p* values ($P < 0.05$) are in bold.

Obese pregnant women are more likely to have larger than expected infants and are also more likely to undergo cesarean section (30). The frequency of hypertension among women with GDM in our study (14.9%) was also higher than that reported in the literature (5–10%) (31).

Subjects in the GDM group also had a high prevalence of diabetes in their family history (approximately 70%). Pregnant women with a family history of DM are at increased risk of developing GDM and of giving birth to macrosomic children (32).

Fasting glucose and HbA1c levels of the GDM group were within the reference range, suggesting that these patients showed good glycemic control. As a marker for post-prandial hyperglycemia, 1,5-Anhydroglucitol (1,5 AG) indicates the occurrence of hyperglycemic excursions. During pregnancy, 1,5 AG levels decrease significantly, probably due to increased glomerular filtration, the glycosuria associated with pregnancy and the dilution effect due to increased plasma volume that occurs in pregnancy (33). In a previous study, our group demonstrated that pregnant women with GDM had lower 1,5 AG levels than healthy pregnant women, with less variation during pregnancy (34). Similarly, the GDM group in the present study showed lower levels of 1,5 AG.

During pregnancy, changes in lipid metabolism occur to ensure the supply of nutrients to the growing fetus (35). Both groups had high concentrations of total cholesterol and LDL-cholesterol, with no significant difference between them ($P > 0.05$). Triglycerides concentrations in the GDM group were approximately two-fold those of the control group ($P < 0.001$). GDM induces a state of dyslipidemia, consistent with insulin resistance (36).

None of the subjects had clinical symptoms of kidney disease or serum creatinine levels > 1.4 mg/dL.

TABLE 3. Comparisons of Allele Frequencies of Pregnant Women with those Reported by Other Studies

GCKR rs780094 polymorphism			Genotype (%)			Allele (%)	
Population	Characteristics	*n*	CC	CT	TT	T	Reference
Euro-Brazilians	GDM	127	50.4	37.8	11.8	30.7	Present work
	Controls	125	34.4	54.4	11.2	38.4	
Finnish	GDM	526				37.0	(24)
	Controls	404				32.0	
Danish	T2DM	3878	42	46	12	34.9	(16)
	Controls	4891	45	43	12	33.1	
CaucasianAfrican-American	GDM					42	(25)
	GDM					**14**	
Chinese	T2DM and obese	2894	19.6	51.1	29.3	**45.2**	(18)
Japanese	Dysglycemia	283	25.1	48.1	26.8	**50.9**	(39)
	Controls	1747	21.2	49.4	29.4	**54.1**	

Frequencies are presented as %. The minor allele (T) frequencies that differ significantly from those of the healthy group in this study are highlighted in bold.

Creatinine and urea did not show any indication of overt kidney disease. Total protein and albumin was significantly reduced in the GDM group and uric acid was higher, although this was not clinically significant.

The rs780094 variant of the *GCKR* gene was associated with GDM in the study population ($P = 0.022$ and $P = 0.010$ in codominant and dominant models, respectively). The odds ratio for the risk C allele was 1.41 (95% CI; 0.97–2.03). In a Finnish population, the C allele was associated with a 1.25-fold increase in the risk of developing GDM (24), consistent with our findings.

The minor allele (T) frequency for the control group in the study was 38.4% (95% CI: 32–44%), similar to frequencies in populations with different ethnicities (Table 3); however, African Americans showed significantly lower, and Asians (Chinese and Japanese) significantly higher, T allele frequencies.

The association between the T allele of rs780094 and increased triglyceride levels was strongly demonstrated in a study examining different populations (15); however, a similar association was not observed in the present study (data not shown), which may be due to the sample size or population characteristics.

The frequency of the T allele in a Chinese study of individuals who were obese or had T2DM was associated with high concentrations of triglycerides, but also with lower BMI and lower risk for T2DM (18). Han Chinese C-allele carriers are 1.22 times more likely to develop T2DM than those with the T allele (22).

The frequency of the T allele among women with GDM in this study was similar to that reported for Finnish and Danish women with T2DM, and lower than the frequencies reported in Asians. African Americans with GDM had a lower frequency of the T allele compared to pregnant women with GDM in this study, probably because of the ethnic differences between the populations.

In our study, carriers of the C allele of rs780094 were 1.41 (odds ratio, 95% CI, 0.97–2.03) times more likely to develop GDM. Glucokinase is an enzyme that phosphorylates glucose and determines hepatic glucose clearance. The glucokinase regulatory protein enables adaptive regulation of hepatic glucose disposal (37, 38). However, the effect of rs780094 polymorphism on GCKR expression is not known. Sparso et al. (16) proposed that this intronic polymorphism may be in linkage disequilibrium with other genes responsible for the effect on the concentrations of glucose or triglycerides. In addition, we did not find any associations between rs780094 genotypes and glucose, 2-h, 75 g glucose, HbA1c, or triglyceride concentrations in both groups including in dominant or recessive models (data not shown). The sample size in our study has no power to exclude definitely the association among the genotypes with laboratory markers. Finally, the association of the polymorphism with GDM also needs to be confirmed in a large sample size.

In conclusion, the C allele of the *GCKR* rs780094 polymorphism is a risk factor for GDM in a Brazilian population.

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