The rs10885122 polymorphism of the adrenoceptor alpha 2A (*ADRA2A*) gene in Euro-Brazilians with type 2 *diabetes mellitus*

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ABSTRACT

Objective: To investigate the association of the rs10885122G>T polymorphism in the *ADRA2A* gene in a Euro-Brazilian sample of healthy (controls) and type 2 diabetic (T2D) subjects. **Subjects and methods:** We used fluorescent probes (TaqMan) to genotype 241 subjects, that is, 121 healthy and 120 T2D subjects, who were classified based on the Brazilian Diabetes Association (2013) and American Diabetes Association (2014) criteria. **Results:** The genotype and allele frequencies showed no significant (P > 0.05) difference between the two studied groups. The minor allele (T) frequencies (95%CI) for rs10885122 were 19% (14-24%) and 20% (15-26%) for healthy and T2D groups, respectively. Carriers of the T allele (genotypes GT+TT) were significantly associated (P = 0.016) with approximately a 7-kg body weight reduction compared with the genotype GG, which was only found in the T2D group. **Conclusion:** The rs10885122G>T polymorphism of the *ADRA2A* gene was not associated with T2D in Euro-Brazilians, and carriers of the T allele had lower body weight in the presence of T2D. Arch Endocrinol Metab. 2015;59(1):29-33

Keywords
Type 2 *diabetes mellitus*; *ADRA2A* polymorphism; case-controlled study; SNP

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INTRODUCTION

Type 2 *diabetes mellitus* (T2D) accounts for 90-95% of those with *diabetes mellitus* (DM, diabetes) and is characterized by insulin resistance and relative insulin deficiency (1,2). T2D is a polygenic disease, and several single nucleotide polymorphisms (SNPs) have been associated with risk or protection regarding this disease and its complications (3-5).

The effects of norepinephrine on pancreatic β-cells are primarily mediated by adrenoceptor alpha 2A, which is encoded by the *ADRA2A* gene (HGNC: 281; OMIM: 104210) (6). Since norepinephrine inhibits both of the major pathways by which glucose induces biphasic insulin secretion and adrenoceptor alpha 2A is the responsible for these effects, genetic variations of the *ADRA2A* gene may be candidates for T2D susceptibility (7-12).

Several genetic variations in the human *ADRA2A* gene or in its flanking region are related to decreased insulin secretion and increased T2D risk (13-15). Rosengren and cols. (15), using a tagging SNP approach, identified several polymorphisms in the coding or flanking regions of the *ADRA2A* gene. In particular, a SNP, rs10885122, which is located 0.2 Mb from the *ADRA2A* gene in a non-coding DNA region, was associated with different regulatory expression of nearby or distant genes (16-18). The rs10885122 polymorphism was identified as associated with fasting glucose level and reduced glucose-stimulated insulin release, but not with T2D (14,17,19).

In this study, we investigated the association of the rs10885122 polymorphism of the *ADRA2A* gene in a sample of Euro-Brazilians with and without T2D.

SUBJECTS AND METHODS

Subjects

A total of 241 unrelated Euro-Brazilian subjects, matched by sex, were investigated. Healthy control (n

= 121) and T2D (n = 120) subjects were classified according to the criteria of the American Diabetes Association 2014 (ADA) and Brazilian Diabetes Association 2013 (SBD) (1,2). The control group was recruited among the patients of the Clinical Hospital of Federal University of Parana (HC-UFPR) blood bank in Curitiba City, Parana State, Brazil. T2D patients were recruited from HC-UFPR. Subjects with overt kidney disease or other severe diabetic complications were excluded from this study.

The Federal University of Parana Ethics Committee approved this research (CAAE 05335612.4.0000.0102).

Laboratory data and genotyping

Biochemical parameters were determined by routine laboratory methods (Abbott Diagnostics) in an automated system with reagents, calibrators, and controls provided by the manufacturer (Architect Ci8200, Abbott Diagnostics). The 1,5-anhydroglucitol level was measured enzymatically (GlycoMark, Inc). Glycated hemoglobin was measured by immunoturbidimetry (Architect, Abbott Diagnostics).

DNA from blood samples was extracted using a salting out technique (20), and the concentrations were normalized to 20 ng/µL for the assays (NanoDrop, ThermoScientific). Only DNA samples with absorbance ratios (280/260) between 1.8 to 2.0 were used in this study. The rs10885122 polymorphism was genotyped using fluorescent probes (TaqMan®, Life Technologies; code C_175459_10) in the real-time PCR StepOnePlus™ System (Life Technologies) with all reagents supplied by Life Technologies. The reaction mixture (6 µL final volume) contained 3.0 µL of Master Mix (DNA polymerase, Mg^{2+}, buffer, additives), 0.3 µL of SNP Genotyping Assay (40X), 1.7 µL ultrapure water, and 1.0 µL of genomic DNA (20 ng/µL). The cycle sequencing conditions were: 1 cycle of 30 s at 60°C (pre-PCR), 1 cycle for 10 min at 95°C, and 55 cycles of 15 s at 95°C, followed by 60°C for 60 s, and 1 final cycle of 30 s at 60°C (final extension). All genotypes were analyzed by the StepOnePlus software (TaqMan® Genotyper Software version 1.0, Life Technologies) and using a minimum quality threshold of 95% in all analyses.

Statistical analysis

Normality was tested with the Kolmogorov-Smirnov test. The comparison of parameters with normal distribution was performed by the Student's *t*-test for independent samples or Mann-Whitney U test for data with a non-normal distribution. Categorical variables were compared by chi-square test. Allele frequencies and Hardy-Weinberg equilibrium (HW) were evaluated by chi-square test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

Statistical analyses were performed with the software Statistica for windows version 8.0 (StatSoft Inc, Tulsa, OK, USA). A probability lower than 5% ($P < 0.05$) was considered significant.

RESULTS

The anthropometric and clinical characteristics of the studied subjects are shown in table 1. The T2D showed high frequency of family history for diabetes (61.7%) and hypertension (66%). The T2D subjects were older, heavier, and more hypertensive than the control subjects. The mean values for HbA1C (7.9%) and median value for 1,5-anhydroglucitol (8.5 µg/mL) demonstrated that the T2D group showed poor glycemic control. The lipid profile for the T2D group showed serum concentrations similar to triglycerides and total cholesterol, as well as HDL-cholesterol and LDL-cholesterol levels that were significantly lower than those in the healthy subjects. This pattern was expected since antilipemic drugs are commonly used, particularly in high doses, in T2D patients. Kidney function, which was monitored by creatinine and urea, did not show any indication of overt kidney disease, since the included subjects are in the reference range for these parameters. However, albumin was significantly reduced in the T2D group but without clinical significance.

Both studied groups were in Hardy-Weinberg equilibrium ($P > 0.05$), and comparisons of genotype ($P = 0.891$) and allele ($P = 0.698$) frequencies showed no significant differences between the groups (Table 2). A positive and significant correlation between weight and the genotypes of rs10885122 was observed only in the T2D group ($r = 0.277$; $P = 0.006$). The association of the rs10885122 polymorphism of the *ADRA2A* genotypes in the dominant model (GG *vs.* GT+TT) with weight is shown in figure 1. The genotypes with the T allele (GT+TT) were significantly associated ($P = 0.016$) with weight reduction by approximately 7 kg compared with the genotype GG, which was only found in the T2D group.

Table 1. Anthropometric and laboratory characteristics of the studied groups

Characteristics	Control n = 121	T2D n = 120	P
Age, years	39.5 ± 14.4	61.9 ± 9.8	< 0.001
Man/Woman, n	61/60	60/60	0.949 **
Body mass index, kg/m^2	23.1 ± 3.7	29.6 ± 5.5	< 0.001
Hypertension, %	16.5	66.0	< 0.001**
Smoking, %	-	70.0	-
Coronary artery disease, %	-	34.0	-
Family history for diabetes, %	-	61.7	-
Family history for obesity, %	-	35.0	-
Fasting glucose, mg/dL	92 (88-97)	136.5 (110-183)	< 0.001*
HbA1C, %	-	7.9 ± 2.3	-
1,5 anhydroglucitol, ug/mL	21.3 (15.5-27.1)	8.5 (3.5–16.4)	< 0.001*
Creatinine, mg/dL	0.8 (0.71-0.85)	0.9 (0.72-1.12)	< 0.001*
Urea, mg/dL	30.5 (25.0-36.0)	37.0 (28.0-48.0)	< 0.001*
Total cholesterol, mg/dL	189.1 ± 39.9	173.8 ± 40.3	0.003
HDL-cholesterol, mg/dL	47.2 ± 12.2	42.8 ± 13.1	0.008
LDL-cholesterol, mg/dL	114.5 ± 33.6	98.9 ± 31.2	< 0.001
Triglycerides, mg/dL	130.0 (92.0–191.0)	146.0 (101.0–201.0)	0.267*
Total Protein, g/dL	7.3 (6.9–7.7)	7.2 (6.8–7.5)	0.187*
Albumin, g/dL	4.2 (3.9–4.4)	3.9 (3.7–4.0)	< 0.001*

Values are mean ± SD, median (interquartile range), or %.

p-values, Student's *t*-test (independent variables), * Mann-Whitney U test or **Chi-square test.

Table 2. Genotype and allele frequencies of the *ADRA2A* rs10885122 polymorphism and a literature review

***ADRA2A* rs10885122**			**Genotype (%)**			**Allele (%)**	**References**
Ethnic group	**Characteristics**	**n**	**GG**	**GT**	**TT**	**T**	
Euro Brazilian	**T2D**	**120**	**65.0**	**29.2**	**5.8**	**20.0**	**This work**
	Healthy (control)	**121**	**67.8**	**26.4**	**5.8**	**19.0**	
UK Asian Diabetes Study	T2D	857	-	-	-	22.0	(30)
Diabetes Genetics in Pakistan	T2D	821	-	-	-	23.0	
Whitehall II Study	T2D	365	78.1	21.1	0.8	11.0	(19)
	Healthy	4,788	77.4	21.2	1.3	12.0	
British Women's Health and Heart Study	T2D	327	83.0	15.0	2.0	9.0	
	Healthy	2,969	76.0	23.0	2.0	13.0	
English Longitudinal Study of Aging	T2D	432	73.2	26.4	0.5	14.0	
	Healthy	5,021	76.8	21.7	1.5	12.0	
Northwick Park Heart Study II	T2D	155	73.6	24.5	1.9	14.0	
	Healthy	2,502	77.0	21.5	1.5	12.0	
White	Participants at high risk for diabetes	1,617	-	-	-	12.0	(29)
African-American	Participants at high risk for diabetes	592	-	-	-	64.4	
Hispanic	Participants at high risk for diabetes	475	-	-	-	15.7	
Asian	Participants at high risk for diabetes	125	-	-	-	13.2	
American Indian	Participants at high risk for diabetes	81	-	-	-	11.1	
Chinese	T2D	3,410	-	-	-	7.6	(31)
	Healthy	3,412	-	-	-	8.0	
Meta-analysis	Nondiabetic	46,186	-	-	-	13.0	(26)
Swedish	Middle-aged adults	4,059	-	-	-	11.0	(27)
Chinese Han	T2D	3,210	-	-	-	8.0	(32)

Bold text indicates data obtained in this study, - indicates no data.

Minor frequency of the T allele [95%CI] in the studied groups: control, 19.0% [14–24]; and T2D, 20.0% [15–26].

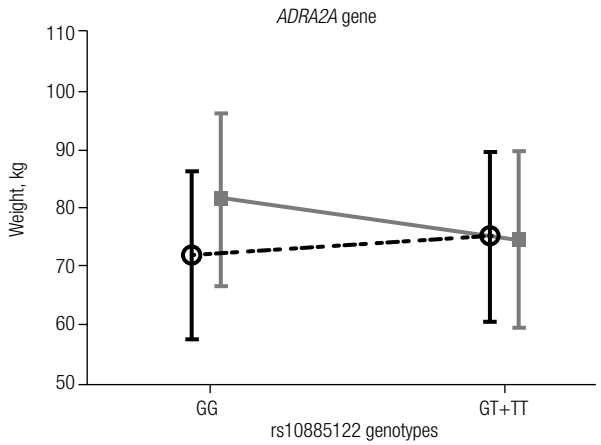

Figure 1. Association of *ADRA2A* rs10885122 genotypes with body weight.

Values indicate mean and standard deviation. Healthy subjects (*black, open circle, dashed line*) and type 2 diabetic patients (*gray, filled square, solid line*). Comparisons (GG *vs.* GT+TT) with Student's *t*-test: healthy subjects (controls), P = 0.247; and type 2 diabetic patients, P = 0.016.

DISCUSSION

T2D is a burdensome epidemic that is highly associated with genetic susceptibility (20). Few studies were available that compared the association of SNPs with T2D in the Brazilian population. In our study, we revealed that the characteristics of the T2D group indicated poor glycemic control, as demonstrated by the concentration of the biomarkers HbA1C (> 7%) and 1,5-anhydroglucitol (< 10 µg/mL) (21,22). In addition, the minor but significant reduction in serum albumin concentration associated with high frequency of hypertension in the T2D group could suggest an increase in albumin loss (microalbuminuria) by the kidney (23,24).

Polymorphisms of the *ADRA2A* gene have been primarily studied by meta-analysis (25). The G allele of the *ADRA2A* rs10885122 polymorphism was described as a risk factor associated with higher fasting glucose and reduced insulin secretion in non-diabetic subjects (14,17,26,27). In contrast, others studies did not find an association of this polymorphism with T2D in European Caucasians (17,19,25), Japanese (28), Chinese (25), and several other ethnicities (29). We showed that the *ADRA2A* rs10885122 SNP in the Euro-Brazilian population was not associated with T2D. The minor allele frequencies (T allele) of rs10885122 observed in this study were similar (close to the 95%CI) to those of different ethnicities that are shown in table 2. Chinese and African Americans showed significantly lower and higher T allele frequencies, respectively (Table 2).

The lack of consistent association of the *ADRA2A* variant with glycemic traits in the present report is similar to the results of others studies, including studies by Talmud and cols. (19) involving 1,307 diabetic subjects and by Florez and cols. (29) involving 1,307 subjects with prediabetes. In addition, the meta-analysis of glucose and insulin-related traits consortium (MAGIC) study (17) and four UK studies (19), comprised of over 118,000 and 17,000 individuals, respectively, also did not find an association of rs10885122 with T2D. Although, one of the limitations of our study was the relatively small sample size, our results were comparable and consistent with these works.

Dupuis and cols. (17) showed an association of the rs10885122 T allele with lower fasting glucose in subjects with normal glucose tolerance. On the other hand, the same authors found an association with higher fasting glucose concentration for the G allele of this polymorphism, and Boesgaard and cols. (14) described a reduction in glucose-stimulated insulin release for this allele. In our study, the T allele was associated with a reduction of weight in the T2D group (Figure 1). We hypothesized that T2D patients that are carriers of the T allele could have better weight control and consequently improved glycemic control, which is consistent with the findings of Dupuis and cols. (17). The mechanisms that explain the effects of the rs10885122 SNP of the *ADRA2A* gene on weight or glucose concentration are currently unknown.

In conclusion, the rs10885122 polymorphism of the *ADRA2A* gene was not associated with T2D in the studied Euro-Brazilian population. T2D patients that are carriers of the T allele of rs10885122 was significantly associated with lower weight compared with those who carried the G allele.

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