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Association of polymorphisms at the *ADIPOR1* regulatory region with type 2 diabetes and body mass index in a Brazilian population with European or African ancestry

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Association studies between *ADIPOR1* genetic variants and predisposition to type 2 diabetes (DM2) have provided contradictory results. We determined if two single nucleotide polymorphisms (SNP c.-8503G>A and SNP c.10225C>G) in regulatory regions of *ADIPOR1* in 567 Brazilian individuals of European (EA; N = 443) or African (AfA; N = 124) ancestry from rural (quilombo remnants; N = 439) and urban (N = 567) areas. We detected a significant effect of ethnicity on the distribution of the allelic frequencies of both SNPs in these populations (EA: -8503A = 0.27; AfA: -8503A = 0.16; P = 0.001 and EA: 10225G = 0.35; AfA: 10225G = 0.51; P < 0.001). Neither of the polymorphisms were associated with DM2 in the case-control study in EA (SNP c.-8503G>A: DM2 group -8503A = 0.26; control group -8503A = 0.30; P = 0.14/SNP 10225C>G: DM2 group 10225G = 0.37; control group 10225G = 0.32; P = 0.40) and AfA populations (SNP c.-8503G>A: DM2 group -8503A = 0.16; control group -8503A = 0.15; P = 0.34/SNP 10225C>G: DM2 group 10225G = 0.51; control group 10225G = 0.52; P = 0.50). Similarly, none of the polymorphisms were associated with metabolic/anthropometric risk factors for DM2 in any of the three populations, except for HDL cholesterol, which was significantly higher in AfA heterozygotes (GC = 53.75 ± 17.26 mg/dL) than in homozygotes. We conclude that *ADIPOR1* polymorphisms are unlikely to be major risk factors for DM2 or for metabolic/anthropometric measurements that represent risk factors for DM2 in populations of European and African ancestries.

Key words: Association study; Adiponectin receptors; Type 2 diabetes; Polymorphism for ancestry-admixture mapping; HDL cholesterol; Association of *ADIPOR1* with DM2

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Adiponectin is a circulating peptide secreted from adipose tissue that acts as an antidiabetic adipokine (1). In 2003, two adiponectin receptors encoding genes were described: adiponectin receptor 1 (*ADIPOR1*) and 2 (*ADIPOR2*) (2). Several case-control studies on single nucleotide polymorphisms (SNPs) in the *ADIPOR1* gene and type 2 diabetes mellitus (DM2) have been conducted in different populations but with contradictory results. Although association has been found between polymorphisms at *ADIPOR1* and DM2 in the Amish (3), all other studies have been negative, including American (4), Japanese (5), French (6), and UK populations (7). Interestingly, associations between *ADIPOR1* SNPs and liver fat content or insulin sensitivity (8), and with anthropometric measurements, such as height, weight, body mass index (BMI) (9), have been suggested.

In order to determine the association of *ADIPOR1* with susceptibility to DM2 and with anthropometric measurements, we investigated if variants in regulatory regions of *ADIPOR1* (SNPs c.-8503G>A [rs6666089] in the promoter and c.10225C>G [rs7539542] in the 3' UTR region) are associated with DM2 or with anthropometric measurements and also with metabolic traits in DM2 Brazilian patients with European (EA) and African (AfA) ancestries from both rural (quilombo remnants) and urban areas. We also evaluated if the frequency of these SNPs differs between individuals of EA and AfA.

The total sample consisted of 567 individuals: 313 from a cohort being followed at the Federal University of Rio Grande do Sul (UFRGS) (10) and 254 at the Hospital das Clínicas of the Universidade de São Paulo (HC-USP). Among them, 443 were EA (Portuguese, Spanish, Italian, and German) descendants and 124 AfA (West Africa, Angola, Mozambique) descendents (11,12). Patients were evaluated using a questionnaire, physical examination and laboratory tests. Diagnosis of DM2 was based on the guidelines from the Expert Committee report (13). Weight and height were measured without shoes and in lightweight clothes; BMI was classified according to the World Health Organization criteria (14). It was not possible to obtain data for these parameters for all patients. The reference group (control) consisted of 190 healthy DM2-free blood donor volunteers at HC-USP (96 EA and 94 AfA, age: 48.28 ± 16.39 years), who were previously described by Errera et al. (11,12). The main clinical and laboratory data are reported in Table 1 and were obtained as recommended previously (11,12). A group of 439 non-diabetic quilombo remnant individuals was also included. Previous to the abolition of slavery in Brazil, many fugitive African slaves founded communities called "Quilombos". Currently, population groups living in Quilombos are referred as quilombo remnants, who are partially genetically isolated (15). Data on systolic blood pressure, diastolic blood pressure, height, weight, BMI, waist circumference, and plasma glucose level were available for the quilombo remnant individuals (15).

The Ethics Committees of the Instituto de Biociências-USP, of HC-USP, and of UFRGS approved the study. All subjects gave written informed consent.

Genomic DNA was extracted from peripheral blood using standard protocols and amplified PCR. Both SNPs were detected using SnuPE method (c.-8503G>A: 5' AAATAGTATTATTTTATTCC 3' and c.10225C>G: 5' GAAA TCTTTGAATGCCAAGT) and a Megabace DNA sequencer

Table 1. Clinical characteristics of type 2 diabetes mellitus patients of European ancestry and African ancestry, and of non-diabetic individuals from quilombo remnant populations.

	European ancestry		African ancestry		Quilombo remnants	
	Mean ± SD	N	Mean ± SD	N	Mean ± SD	N
Age (years)	52.72 ± 15.08	443	50.22 ± 14.74	124	43.42 ± 17.57	439
Systolic blood pressure (mmHg)	142.2 ± 23.4	331	141.28 ± 24.1	51	127.32 ± 24.3	439
Diastolic blood pressure (mmHg)	85.67 ± 12.8	331	86.11 ± 12.3	51	82.92 ± 13.4	439
Body mass index (kg/m^2)	28.5 ± 5.3	387	28.7 ± 5.4	105	24.8 ± 4.4	439
Waist circumference (cm)	98.0 ± 11.9	132	98.4 ± 12.6	26	83.2 ± 10.6	438
Total cholesterol (mg/dL)	210 ± 47.2	437	209 ± 78.2	105	NA	
HDL cholesterol (mg/dL)	44.8 ± 12.9	437	49.6 ± 14.3	105	NA	
LDL cholesterol (mg/dL)	133.8 ± 43.5	437	133.9 ± 47.5	105	NA	
Serum creatinine (mg/dL)	1.31 ± 1.39	380	1.87 ± 2.57	94	NA	
Triglycerides (mg/dL)	186.8 ± 146.7	437	142.2 ± 103.9	101	NA	
HbA1C (%)	8.39 ± 2.6	304	9.22 ± 2.9	86	NA	
Fasting plasma glucose (mg/dL)	176.4 ± 71.3	354	172.0 ± 67.9	94	97.63 ± 31.9	438

N = number of individuals for whom information was available. NA = data not available.

(SNuPe, Amersham, Piscataway, NJ, USA).

Association studies were performed between a set of 315 DM2 patients (246 EA and 69 AfA) and the control group, both within a comparable age range (mean age DM2 patients: 52.39 ± 16.34 years; mean age control group: 48.28 ± 16.39 years). Distribution of genotypes and alleles was compared using the χ^2 or the Fisher exact test. The level of significance adopted was $P < 0.05$. Statistical analyses were carried out with SSPS (Statistical Package for the Social Sciences for Windows, version 10.0). Sample size power was calculated with PS Power and Sample Size Calculations, version 2.1.30 (16).

Genotypic distributions for the SNPs c.-8503G>A and c.10225C>G were in Hardy-Weinberg equilibrium in all groups ($P > 0.05$).

Ethnicity had a significant effect on the distribution of the allelic frequencies of these two SNPs in the DM2 and control groups, in which the G allele of both SNPs was more frequent in AfA than in EA (EA: -8503A = 0.27, AfA: -8503A = 0.16, P = 0.001; EA: 10225G = 0.35; AfA: 10225G = 0.53, $P < 0.001$; Table 2).

There were no significant differences in genotypic and allelic frequencies of SNPs -8503 and c.10225 between DM2 patients and the control population: [EA (SNP c.-8503G>A: DM2 group -8503A = 0.26; control group -8503A = 0.30; P = 0.14; SNP 10225C>G: DM2 group 10225G = 0.37; control group 10225G = 0.32; P = 0.40)] or [AfA (SNP c.-8503G>A: DM2 group -8503A = 0.16; control group -8503A = 0.15; P = 0.34; SNP 10225C>G: DM2 group 10225G = 0.51; control group 10225G = 0.52; P = 0.50)], suggesting that these SNPs are not associated with DM2 in these groups.

The effect of the genotypes on quantitative laboratory data and anthropometric features of DM2 patients was compared by the unpaired Student *t*-test and ANOVA. These parameters were not associated with any genotype of the SNP c.-8503G>A/*ADIPOR1* [total cholesterol (EA: P = 0.69; AfA: P = 0.50), HDL cholesterol (EA: P = 0.23; AfA: P = 0.26), LDL cholesterol (EA: P = 0.85; AfA: P = 0.43), serum creatinine (EA: P = 0.13; AfA: P = 0.89), triglycerides (EA: P = 0.31; AfA: P = 0.31), systolic blood pressure (EA: P = 0.83; AfA: P = 0.29), diastolic blood pressure (EA: P = 0.55; AfA: P = 0.95), HbA1c (EA: P = 0.16; AfA: P = 0.97), plasma glucose (EA: P = 0.10; AfA: P = 0.28), height (EA: P = 0.36; AfA: P = 0.90), weight (EA: P = 0.79; AfA: P = 0.15), BMI (EA: P = 0.92; AfA: P = 0.18), and waist circumference (EA: P = 0.68; AfA: P = 0.91)].

In contrast, analysis of SNP 10225C>G in 156 DM2 patients with BMI information showed an association between the 10225G allele in EA and low BMI with borderline level of significance (P = 0.037), but not with any other parameter (data not shown). We genotyped an additional

Table 2. Genotypic frequencies of two *ADIPOR1* gene single nucleotide polymorphisms (SNP) in the studied populations and P value for different pairs of population comparisons.

SNP	EA			AfA			Quilombo remnants	P value			
	DM2	Control	Total	DM2	Control	Total		EA *vs* AfA	Control *vs* DM2		AfA *vs* Quilombo remmants
									In EA	In AfA	
c.-8503G>A								0.001	0.14	0.34	–
GG	135 (54.9%)	49 (51%)	184 (53.8%)	47 (68.1%)	56 (71.8%)	103 (70.1%)	–				
AG	95 (38.6%)	36 (37.5%)	131 (38.3%)	22 (31.9%)	20 (25.6%)	42 (28.6%)	–				
AA	16 (6.5%)	11 (11.5%)	27 (7.9%)	0 (0%)	2 (2.6%)	2 (1.4%)	–				
c.10225C>G								<0.001	0.78	0.65	0.27
GG	51 (11.5%)	9 (9.8%)	60 (11.2%)	29 (23.4%)	20 (21.3%)	49 (22.5%)	126 (28.7%)				
GC	196 (44.2%)	40 (43.5%)	236 (44.1%)	69 (55.6%)	59 (62.8%)	128 (58.7%)	207 (47.2%)				
CC	196 (44.2%)	43 (46.7%)	239 (44.7%)	26 (21%)	15 (15.9%)	41 (18.8%)	106 (24.1%)				

EA = European ancestry; AfA = African ancestry; DM2 = type 2 diabetes mellitus; Control = DM2-free blood donor volunteers.

set of 207 DM2 subjects and the group of 439 non-diabetic individuals from quilombo remnants (age*:* 43.42 ± 17.57 years) in order to increase the power of the study.

In the total sample of DM2 patients (N = 567), we confirmed the lack of association between this SNP and DM2 both in EA (DM2 group: 10225G = 0.34; control group: 10225G = 0.31; P = 0.78) and AfA (DM2 group: 10225G = 0.51; control group: 10225G = 0.53; P = 0.65) individuals. Except for HDL cholesterol, clinical and anthropometric features in DM2 [total cholesterol (EA: P = 0.31; AfA: P = 0.33), LDL cholesterol (EA: P = 0.17; AfA: P = 0.11), serum creatinine (EA: P = 0.49; AfA: P = 0.89), triglycerides (EA: P = 0.53; AfA: P = 0.49), systolic blood pressure (EA: P = 0.43; AfA: P = 0.48), diastolic blood pressure (EA: P = 0.25; AfA: P = 0.79), height (EA: P = 0.73; AfA: P = 0.90), weight (EA: P = 0.83; AfA: P = 0.58), BMI (EA: P = 0.72; AfA: P = 0.86), waist circumference (EA: P = 0.22; AfA: P = 0.74), HbA1c (EA: P = 0.42; AfA: P = 0.60), and plasma glucose (EA: P = 0.09; AfA: P = 0.74)] were not associated with different genotypes of the SNP c.10225C>G/*ADIPOR1*. Thus, the previous correlation we found between allele 10225G and lower BMI was not confirmed after increasing sample size. We observed that HDL cholesterol was significantly higher in AfA heterozygotes (GC = 53.75 ± 17.26 mg/dL) than in homozygotes (CC = 44.75 ± 10.93 mg/dL; GG = 46.00 ± 12.52 mg/dL; P = 0.02; of which, P = 0.06 between GC and CC genotypes, P = 0.11 between GC and GG genotypes and P = 1.0 between CC and GG genotypes, after performing the *post hoc* Bonferroni test). This association was not found with EA (P = 0.49).

In quilombo remnant individuals, the allele frequency of 10225G was 0.52. Clinical and anthropometric features in these individuals were not associated with any genotype of the SNP c.10225C>G/*ADIPOR1* [systolic blood pressure (P = 0.23), diastolic blood pressure (P = 0.42), height (P = 0.66), weight (P = 0.27), BMI (P = 0.52), waist circumference (P = 0.18), and plasma glucose level (P = 0.17)].

Thus, the present study characterizes for the first time *ADIPOR1* SNPs c.-8503G>A and c.10225C>G in groups of the Brazilian population, including quilombo remnants.

The low allele frequency of both SNPs in our EA sample (-8503A = 0.27 and 10225G = 0.32) was very similar to the frequency found in American Caucasoid, Finnish, German, French, UK, and HapMap Caucasian populations (-8503A = 0.25-0.3 and 10225G = 0.31-0.33) (4,6-9,17); but our SNP 10225G frequency was different from Old Order Amish (-8503A = 0.27 and 10225G = 0.132) (3), probably because they represent an isolated population. This result thus suggests that the Caucasian population is relatively homogeneous worldwide for these SNPs.

Although quilombo remnants form a semi-isolated population (15), no significant difference in SNP c.10225C>G minor allele frequency was found compared to the Brazilian AfA groups. Minor allele frequency in our AfA sample (-8503A = 0.16 and 10225G = 0.52) was different from that in African-Americans (-8503A = 0.36 and 10225G = 0.42). Different origins of African-derived populations in the US and in Brazil or different proportions of admixture of the AfA population in Brazil could explain this. These data indicate the importance of analyzing EA and AfA separately in case-control studies, as we have already suggested (12). Moreover, the data suggest that these SNPs could represent valuable ancestry markers, which can be used to distinguish between chromosomal segments of subjects of AfA and EA.

We did not observe any association of *ADIPOR1* SNPs with DM2 in any of the groups studied here and with HbA1c or BMI, which is consistent with several previous reports (3-9). Siitonen et al. (9) reported an association of *ADIPOR1* SNPs and some metabolic traits according to gender. Our negative results cannot be explained by gender because the genotype distribution did not differ between male and female (data not shown).

The only positive significant association detected was in the DM2 AfA group, in which SNP c.10225C>G heterozygotes showed significantly higher HDL cholesterol levels than homozygotes. This cannot yet be explained at the functional level, but it is known that adiponectin levels correlate positively to HDL cholesterol levels and insulin-stimulated glucose disposal (18,19). However, the difference found between heterozygotes and homozygotes (8.33 mg/dL) was lower than the minimum detectable difference (9.67 mg/dL) with an acceptable power level (P > 0.8) in this sample size. Hence, validation of these data in other samples of African ancestry is necessary to exclude the possibility of false positive.

Our data show that *ADIPOR1* gene variants do not represent major risk factors for DM2, increased BMI or any of the traits analyzed in European and African descendants, even though it should be noted that our sample size had statistical power to detect 1.5 times or greater difference between groups for both populations. On the other hand, these markers may be considered to be informative markers for admixture mapping.

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