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Research Article

Polymorphisms in the Fat Mass and Obesity Associated (FTO) and Transcription factor 7-like 2 (TCF7L2) genes in Euro-Brazilian individuals with type 2 diabetes

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Abstract

Type 2 diabetes (T2D) is a condition that causes a substantial public health burden worldwide. In recent years, several genetic risk factors for diabetes have been identified. To examine the association of polymorphisms rs8050136 and rs9939609 in the FTO (Fat Mass and Obesity Associated) gene, and rs12255372 and rs7903146 in the TCF7L2 (Transcription factor 7-like 2) gene in a sample of Euro-Brazilian individuals with and without T2D. The genotype and allele frequencies for the studied polymorphisms did not demonstrate significant difference (P > 0.05) between the groups. In healthy individuals, the A-allele frequencies (95%CI) for FTO polymorphisms rs8050136 and rs9939609 were 39% (34%–44%) and 38% (34%–43%), respectively, while the T-allele frequencies for TCF7L2 polymorphisms rs12255372 and rs7903146 were 31% (26%– 35%) and 32% (28%–37%), respectively.

Keywords: case-controlled study; FTO; SNP; TCF7L2 polymorphisms; type 2 diabetes mellitus.

Introduction

Type 2 diabetes (T2D) is a common complex disease, and its development is considerably affected by genetic factors [1]. Thus far, a number of studies have identified several validated risk loci for T2D [2], however few genetic variants have been consistently associated with this disease [3-5]. Analysis of the association of T2D with genetic variants in healthy populations has demonstrated that the FTO (Fat Mass and Obesity Associated) gene is linked primarily to body mass index (BMI) and the risk of obesity [6] whereas the TCF7L2 (Transcription factor 7-like 2) gene has a predominant effect on insulin secretion [7].

The FTO gene is expressed in the brain [8], and is associated with cerebrocortical insulin resistance in obese humans [9]. Insulin acts as an adiposity and satiety signal in the brain and is critical for the regulation of normal body weight [10]. Genetic variants in FTO lead to a predisposition to T2D by affecting the BMI of individuals of European [6, 11, 12] and Asian [13] descent. FTO variants rs8050136 and rs9939609 have been associated with obesity in large populations of adults and children [14]. However, FTO polymorphism rs9939609 has been significantly associated with an increased risk of T2D, independent of BMI [15]. Recently, the IRX3 (Iroquois homeobox 3) gene has been shown to be a functional long-range target of obesity-associated FTO variants. Therefore, obesity-associated polymorphisms within FTO are functionally connected with the regulation of IRX3 expression in the brain [16].

The TCF7L2 gene encodes a transcription factor that affects cell proliferation and differentiation via the Wnt signaling pathway [17]. Wnt signaling has been shown to regulate pancreatic β cell proliferation [18], and an impairment in this pathway in the islet is a key process in the development of T2D [19]. Polymorphisms in the TCF7L2 gene, mainly rs12255372 and rs7903146, have been reported to be strongly associated with T2D, as well as with impaired insulin secretion [20].

In this study, we examined the association of FTO polymorphisms rs8050136 and rs9939609, and TCF7L2 polymorphisms rs12255372 and rs7903146 in groups of Euro-Brazilian individuals with or without T2D.

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Materials and methods

Subjects

A total of 402 unrelated Euro-Brazilian subjects, matched by gender, were examined. Subjects were classified as healthy controls (n = 201) and T2D patients (n = 201) according to the criteria of the American Diabetes Association 2014 (ADA) [21] and the Brazilian Diabetes Association 2013 (SBD) [22]. The Control and T2D groups comprised patients from the blood bank at the Clinical Hospital of the Federal University of Paraná (HC-UFPR), Curitiba, Paraná, Brazil. Subjects with overt kidney disease or other severe diabetic complications were excluded from this study.

This research was approved by the Federal University of Parana's Ethics Committee (CAAE 05335612.4.0000.0102).

Clinical chemistry data and genotyping of SNPs

Biochemical parameters were determined using routine laboratory methods (Abbott Diagnostics), including immunoturbidimetry for glycated hemoglobin (HbA1c), conducted with an automated system by using reagents, calibrators, and controls provided by the manufacturer (Architect Ci8200, Abbott Diagnostics). The levels of 1,5-anhydroglucitol were measured enzymatically (GlycoMark, Inc).

DNA was extracted from blood samples using the salting out technique [23], and concentrations (NanoDrop, ThermoScientific) were normalized to 20 ng/µl for the assays. Only DNA samples with 280/260 absorbance ratios of 1.8 to 2.0 (NanoDrop, ThermoScientific) were used in this study. Polymorphisms rs9939609, rs8050136, rs7903146, and rs12255372 were genotyped using fluorescent probes (TaqMan®, Life Technologies; codes C_30090620_10, C_20311259_10, C_29347861_10 and C_291484_20 respectively) and the real time PCR StepOnePlus™ System (Life Technologies). All reagents were supplied by Life Technologies. The reaction mixture (6 µl final volume) contained 3.0 µl of Master Mix (DNA polymerase, Mg2+, buffer, additives), 0.3µl of SNP Genotyping Assay (40X), 1.7 µl of ultra-pure water and 1.0 µl of genomic DNA (20 ng/µl). The cycling sequence was: 1 × 30 sec at 60°C (pre-PCR); 1 × 10 min at 95°C, 55 × 15 sec at 95°C followed by 1 min at 60°C; one final cycle of 30 sec at 60°C (final extension). All genotypes were analyzed using the StepOnePlus software (TaqMan® Genotyper software 1.0), and a minimal quality threshold of 95% was maintained for the analysis.

Statistical analysis

Normality was tested using the Kolmogorov-Smirnov test. Parameters with normal distribution were compared using the Student's t-test for independent samples; the Mann-Whitney U test was used for parameters with non-normal distribution. Categorical variables were compared using the chi-square test. Allele frequencies and Hardy-Weinberg (HW) equilibrium were tested with the chi-square test (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl).

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

Results

The clinical characteristics of the study's subjects are detailed in Table 1. T2D patients were significantly older, heavier (higher

Table 1: Anthropometric and experimental characteristics of the study groups.

Characteristics	Controls	T2D patients	P-value
	n = 201	n = 201	
Age, y	53.0 (35.0–62.0)	56.0 (49.0–64.0)	<0.001*
Male/Female,	81/120	73/128	0.412**
Body mass index, kg/m^2	25.7 ± 3.8	31.6 ± 6.3	<0.001
Hypertension, %	16.5	66	<0.001**
Family history of diabetes, %	-	61.7	-
Family history of obesity, %	-	35	-
Fasting glucose, mmol/L	5.1 (4.7–5.4)	7.3 (5.8–10.0)	<0.001*
HbA_{1C}, mmol/mol	35.5 (32.2–37.7)	57.4 (46.4–74.9)	<0.001*
1,5-anhydroglucitol, µmol/L	129.7 (102.3–163.3)	57.2 (25.5–109.0)	<0.001*
Creatinine, µmol/L	70.7 (61.8–79.6)	75.1 (61.8–88.4)	<0.001*
Urea, mmol/L	5.1 (4.3–6.0)	5.5 (4.5–7.1)	0.002*
Total cholesterol, mmol/L	5.1 (4.3–5.7)	4.5 (3.9–5.3)	<0.001*
HDL-cholesterol, mmol/L	1.35 ± 0.37	1.09 ± 0.32	<0.001
LDL-cholesterol, mmol/L	3.0 ± 0.9	2.7± 0.86	<0.001
Triglycerides, mmol/L	1.5 (1.0–2.2)	2.0 (1.7–2.3)	0.066*
Total Protein, g/L	71 (66–74)	74 (70–78)	<0.001*
Albumin, g/L	41 (38–43)	40 (38–42)	0,433*

Values are presented as mean ± SD, median (interquartile range) or %, Controls and T2D patients
P-values, t-test (independent variables), *Mann-Whitney U test or **Chi-square test

BMI), and more hypertensive compared with healthy individuals (controls). The median value for HbA1C (57.4 mmol/mol) and median value for 1,5-anhydroglucitol (57,2 μmol/L) suggested that the T2D group had poor glycemic control. The mean total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) levels were lower in the T2D group than the control group. Triglyceride, total protein and albumin levels did not significantly differ between the groups. Levels of the renal function markers (creatinine and urea) were higher in T2D subjects than in controls; however, none of the patients exhibited overt symptoms of kidney disease.

The allele and genotype frequencies of the FTO and TCF7L2 polymorphisms were in accordance with HW equilibrium expectations in both groups (P > 0.05), The genotype and allele frequencies for the studied polymorphisms were not significantly differ (P > 0.05) between the groups (Table 2).

Table 2: Genotype and allele frequencies of the FTO and TCF7L2 polymorphisms in the absence (Control) or presence of type 2 diabetes (T2D).

Polymorphism	Control (n = 201)	T2D (n = 201)	P
FTO rs8050136			0.879
C/C	78 (38.8)	82 (40.8)	
C/A	89 (44.3)	84 (41.8)	
A/A	34 (16.9)	35 (17.4)	
A allele frequency, %	39	38	(χ^2) 0.828
[95%CI]	[34–44]	[34–43]	
FTO rs9939609			0.943
T/T	79 (39.3)	80 (39.8)	
T/A	89 (44.3)	86 (42.8)	
A/A	33 (16.4)	35 (17.4)	
A allele frequency, %	38	39	(χ^2) 0.942
[95%CI]	[34–43]	[34–44]	
TCF7L2 rs12255372			0.523
G/G	97 (48.3)	105 (52.3)	
G/T	84 (41.8)	73 (36.3)	
T/T	20 (9.9)	23 (11.4)	
T allele frequency, %	31	30	(χ^2) 0.701
[95%CI]	[26–35]	[25–34]	
TCF7L2 rs7903146			1
C/C	95 (47.3)	95 (47.3)	
C/T	82 (40.8)	82 (40.8)	
T/T	24 (11.9)	24 (11.9)	
T allele frequency, %	32	32	(χ^2) 1
[95%CI]	[28–37]	[28–37]	

Ctrl, healthy subjects; T2DM, type 2 diabetes patients, Values of genotypes are presented as n (%), P-value, Fisher exact test (two-tailed) or Chi-square test (χ2) 95%CI, 95% confidence interval

Discussion

The overall predisposition to T2D is affected by the individual contributions of several genes [24]. Thus, the identification and characterization of gene variants present in particular ethnic groups that play a significant role in T2D are crucial. Studying populations of different ancestry would assist in the global identification and understanding of the genetic and environmental factors associated with T2D [25].

The T2D group represented well-established risk factors for this pathology, such as obesity, age, and a family history of diabetes (Table 1). The obtained results were similar to previous studies [26]; consistent with other published reports, the frequency of hypertension was high (66%) in the T2D group [27]. T2D patients exhibited poor glycemic control, which was assessed by HbA1C (>53 mmol/mol) and 1,5-anhydroglucitol (<60.9 μmol/L) levels; this was in agreement with the glycemic control exhibited by Brazilian patients with T2D, who were treated by the public healthcare system [28].

The association between T2D and several polymorphisms (single-nucleotide polymorphisms –

SNPs) in the TCF7L2 gene has been confirmed by multiple genome-wide association studies conducted In different ethnic groups [3]. These findings were also demonstrated in several human population studies [29, 30].

We were unable to reproduce the association between the two intronic TCF7L2 polymorphisms (rs7903146 and rs12255372) and T2D in this study (P > 0.05; Table 2.). Since Brazilians form an admixed population, differences in their genetic background could explain the conflicting results between our study and previous studies, especially with regard to SNP rs7903146. Therefore, further studies utilizing lar ger sample sizes are required to clarify the association between this polymorphism and T2D in the Brazilian population.

Additional studies conducted in Brazilian cohorts have also demonstrated conflicting results for SNP rs7903146 and T2D. Barra et al. [31] showed an association between this polymorphism and T2D in a Euro-Brazilian population; the T-allele frequency of controls (27.0%) and T2D (35.8%) subjects were similar to those observed in our study. However, a study conducted by Marquezine et al. [32] evaluating groups of patients with known coronary disease enrolled in the MASS II Trial, and residents of Vitoria City, with a T2D prevalence of 31.0% and 7.9%, respectively, identified an association only with the MASS II group (1.126 OR); the T-MAF (minor allele frequency) for control individuals (47.4%) and T2D (39.1%) carriers were not in agreement with our findings. These inconsistencies could be attributed to the subtle differences within our population, such as ethnic variety and genetic heterogeneity.

The T-allele rs7903146 frequencies for healthy subjects (32%, 95%CI 28%– 37%) in our study were similar to those reported for African (27%) and non-Hispanic white American (29%) individuals [33], British (30.7%) cohorts [34], and Tunisian Arab individuals (39%) [29]. On the other hand, studies in Japanese (3.3%), Chinese (2.9%) (35), Indian (21%) [36], and Mexican (12%) [37] populations exhibited T-allele frequencies substantially lower than that observed in our study.

The frequencies for TCF7L2 T-allele rs12255372 observed in the healthy group in our study (31.0%, 95%CI 26%–35%) were similar to those of African (28%) and non-Hispanic white American (27.5%) individuals [33], British cohorts (29.8%) [34], and Tunisian Arab (34%) subjects [29]; however, the frequencies were considerably higher than those of Japanese (2.2%), Chinese (4.0%) [35], Indian (18%) [36], and Mexican (11.4%) [37] populations.

FTO gene polymorphisms have been associated with T2D (not adjusted for BMI) [38] and obesity [6, 11]. However, results have been variable in other ethnic populations such as Hispanic [11], Asian [13, 39], Oceanic [40], and African-American groups [12]. Therefore, the effect of FTO gene polymorphisms in other ethnic populations should be further examined. Our results indicate that the frequencies of both FTO polymorphisms (rs9939609 and rs8050136) were not associated with T2D or metabolic traits such as obesity ($P > 0.05$), although Silva et al. [44] were able to reproduce the previously determined association between FTO gene variant rs9939609 and increased BMI in a small cohort of Brazilian children and adolescents. The sample sizes in our study were smaller than those used in previous studies conducted in European populations [6, 8], which may explain the lack of significant association in the present study or in the study by Ramos et al. [41]. A robust study performed by Li et al. [39] could not demonstrate any association between rs8050136 and rs9939609 and obesity in a Chinese population. In addition, when only rs9939609 was analyzed in cohorts of African ancestry, their association with obesity was not detected [11, 42]. Grant et al. [42] suggested that both rs3751812 and rs9939609 should be tested in cohorts around the world in order to thoroughly assess the global effect of the FTO locus. The frequency of the rs9939609A-allele (38%) was similar to the frequencies identified in other studies in individuals of European descent (40%) [6, 8, 43], Brazilian children (40%) [44], and adults (35%) [41]; the frequency was higher than that exhibited by Chinese (12%) [13, 39] and Japanese (19%) cohorts [45]. The frequencies of the

FTO polymorphism rs8050136 observed in the healthy group (39%, 95%CI 34%–44%) were similar to those reported in European individuals (39%) [43] and higher than those observed in Asian populations (12%) [39, 46].

In conclusion, TCF7L2 polymorphisms rs7903146 and rs12255372, and FTO polymorphisms rs9939609 and rs8050136 were not found to be associated with T2D in the Euro-Brazilian population studied.

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