

Interleukin-18 (rs187238) and glucose transporter 4 (rs5435) polymorphisms in Euro-Brazilians with type 1 diabetes

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Genet. Mol. Res. 16 (3): gmr16039755

Received June 20, 2017

Accepted August 11, 2017

Published September 21, 2017

DOI <http://dx.doi.org/10.4238/gmr16039755>

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ABSTRACT. Type 1 diabetes (T1D) is an autoimmune disease with a strong genetic component that has been associated with several genetic loci. Interleukin 18 (IL-18) is a potent proinflammatory cytokine, which is involved in the innate and adaptive immune responses, and in the pathogenesis of various diseases including T1D. Glucose transporter 4 (GLUT4) is known to be an insulin-responsive glucose transporter and has been associated with various diseases, including diabetes mellitus. We investigated the association of the polymorphisms rs187238 (IL-18) and rs5435 (GLUT4) in a case-control study in Euro-Brazilians with T1D (N = 136) and healthy subjects (N = 144). Real-time PCR with TaqMan[®] fluorescent probes were applied for genotyping. All polymorphisms were in Hardy-Weinberg equilibrium. The minor allele frequencies for the G-allele (rs187238; IL-18) in healthy and T1D groups were 28.5% [95%CI = 23-34%] vs 31.6% [95%CI = 26-37%], P = 0.416, and for the T-allele (rs5435, GLUT4) were 33%

[95%CI = 28-39] vs 27% [95%CI = 23-33%], $P = 0.167$, respectively. Genotype comparisons for both polymorphisms showed no significant differences ($P > 0.05$). The polymorphisms rs187238 and rs5435 were not associated with T1D in the studied population. The minor allele frequencies for both polymorphisms were similar to those of other Caucasian populations.

Key words: Type 1 diabetes; Genetic susceptibility; Interleukin-18 gene; Glucose transporter 4 gene; Polymorphisms; SNP

INTRODUCTION

Diabetes mellitus is a heterogeneous group of metabolic diseases characterized by hyperglycemia resulting from insulin secretion, insulin action, or both (Marathe et al., 2017). Type 1 diabetes (T1D) is characterized by T cell-mediated destruction of insulin-producing pancreatic beta cells. T1D is considered one of the most studied genetic disorder (Noble and Valdes, 2011). It accounts for 5-10% of all cases of diabetes, and can occur at any age; however, it is more common in childhood and adolescence (American Diabetes Association, 2017). Autoimmune destruction of beta cells is related to multiple genetic susceptibilities and environmental factors; however, the incidence and prevalence of T1D are increasing worldwide (Atkinson et al., 2014).

The most common human genetic variations in the genome are single nucleotide polymorphisms (SNPs). Over the last few decades, a variety of genetic polymorphisms has been associated with type 1, type 2, and gestational diabetes (Zhan et al., 2013). Since the genotypic and allelic frequencies of these polymorphisms may vary according to the ethnic composition of the population studied, studies are needed to verify these frequencies in distinct populations (Gupta et al., 2012)

Interleukin-18 (IL-18) is a member of the IL-1 family of inflammatory cytokines and is located on chromosome 11 (11q23.1; OMIM 600953). IL-18 was originally known as interferon gamma (INF- γ) inducing factor (Tavares et al., 2013); it is produced by inflammatory cells and can activate the production of other inflammatory cytokines (Elsherbiny and Al-Gayyar, 2016). Higher production of INF- γ has been associated with T1D (Tavares et al., 2013). Several studies have found an association between SNPs in the promoter site of the IL-18 gene and INF- γ production and eventual development of T1D. For example, allelic variations in the promoter regions of cytokine genes can result in changes in cytokine production (Khripko et al., 2008), leading to alteration of the immune response, which is associated with many immunological diseases (Tavares et al., 2013).

IL-18 is a proinflammatory cytokine and plays an important role in the immune response (Tiret et al., 2005). Increased level of IL-18 production has been shown in Crohn's disease and the acute phase of experimental autoimmune encephalomyelitis (Giedraitis et al., 2001).

Increased plasma levels of IL-18 have been shown in T1D patients, which were associated with disease progression. For example, increased expression of IL-18 mRNA by macrophages and the subsequent rise of INF- γ levels was observed in non-obese diabetic mice with induced autoimmune diabetes (Kretowski et al., 2002; Tavares et al., 2013). In the insulinitis stage of T1D, IL-18 production by pancreatic beta cells played a major role in the stimulation of inflammation (Kretowski et al., 2002).

The IL-18 gene has six exons, and the polymorphism rs187238, also known as -137C>G, is located in the promoter region and has been associated with decreased IL-18 expression (Szeszko et al., 2006). Polymorphism rs187238 has been indicated to be a susceptibility loci for various diseases, including coronary artery disease in Asian and Indian populations (Bao et al., 2016), atopic eczema, and seasonal allergic rhinitis (Szeszko et al., 2006). Furthermore, rs187238 has been associated with T1D in other populations, including Brazilian, Polish, Chinese, and Japanese populations (Tavares et al., 2013).

The glucose transporter 4 (GLUT4) gene (17p13.2; OMIM 138190) encodes the GLUT4 solute carrier (Corrêa-Giannella and Machado, 2013). The gene product is a glucose transporter, which can be found in adipose, skeletal, and cardiac tissues, and is known to be an insulin-responsive glucose transporter (Mueckler and Thorens, 2013). GLUT4 is considered responsible for approximately 75% of all human body glucose uptake mediated by glucose transporters (Malodobra-Mazur et al., 2016). A deficiency of the insulin-mediated translocation of GLUT4 to the plasma membrane, causing insulin resistance, along with a defect in insulin secretion from pancreatic beta cells can lead to the development of diabetes mellitus (Mueckler and Thorens, 2013).

The polymorphism rs5435 (C>T) in the GLUT4 gene is located in exon 4 and is an equivalent variation (Asn130Asn). rs5435 has been associated with type 2 diabetes in a South Indian population (Bodhini et al., 2011b). GLUT4 has been associated with obstructive sleep apnea syndrome in a Chinese population (Yin et al., 2014). To our knowledge, no analysis of this polymorphism has been performed in a Brazilian population.

We studied the association of rs187238 (IL-18) and rs5435 (GLUT4) with T1D in a Brazilian population. The gene map and polymorphism locations are shown in Figure 1.

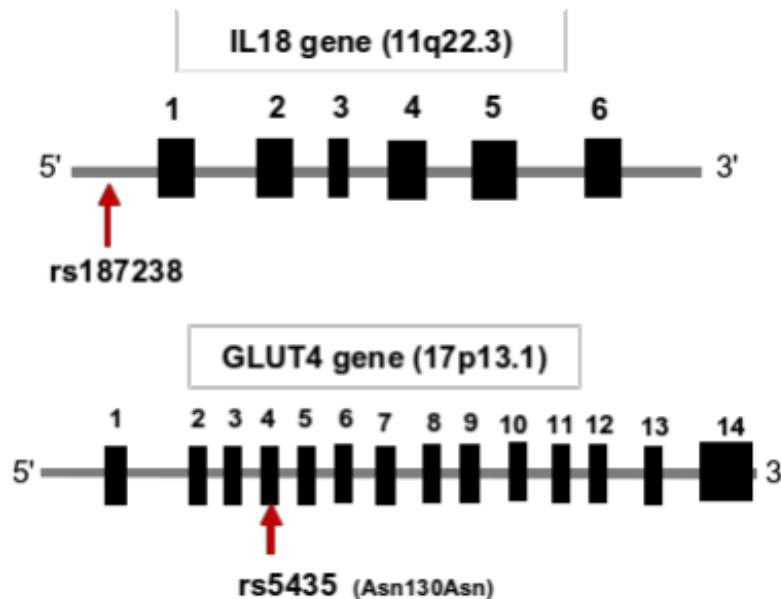


Figure 1. Genomic structure of the studied genes and the location of the selected polymorphisms. The structures of the IL-18 gene with the position of polymorphism rs187238 (-137C>G) in the promoter region and GLUT4 with the position of polymorphism rs5435 (Asn130Asn) in exon 4 are shown.

MATERIAL AND METHODS

Subjects

A total of 280 adults of Euro-Brazilian descent were examined. The sample was classified as healthy individuals (control group, N = 144), and individuals diagnosed with T1D (N = 136) according to the criteria of the American Diabetes Association (2017) and Brazilian Diabetes Association (SBD - Diretrizes da Sociedade Brasileira de Diabetes, 2015-2016). The control group was obtained from blood bank donors. The groups were matched by gender and age. The project was approved by the Ethics Committee of the Federal University of Paraná Health Science Sector (CAAE 01038112.0.0000.0102).

Genotyping

DNA was extracted from leukocytes (buffy coat) by using the “salting-out” method of Lahiri and Nurnberger (1991). DNA samples with an A_{260}/A_{280} absorbance ratio between 1.6 and 1.9 were used. The DNA concentrations of all samples were normalized to 20 ng/ μ L as determined by NanoDrop (Thermo Fisher Scientific, Waltham, MA, USA). Polymorphisms rs187238 (C_1223317_10) and rs5435 (C_2552981_10) were genotyped using TaqMan[®] fluorescent probes using the Applied Biosystems[®] 7500 Fast Real-Time PCR System (Thermo Fisher Scientific). Briefly, the reaction mixture (final volume 8 μ L) contained 3.0 μ L Master Mix (Taq polymerase, 0.1 μ L SNP Genotyping Assay Mix (40X), 1.9 μ L ultrapure dH₂O, and 3.0 μ L DNA (20 ng/ μ L). The reaction conditions used for all polymorphisms were: 10 min at 95°C (1 cycle), followed by 50 cycles of 15 s at 95°C and 90 s at 60°C. The genotyping quality was determined to be >98% for all samples by the 7500 Fast System software.

Statistical analysis

We used the Kolmogorov-Smirnov test for normality to verify the continuous variables. The Student *t*-test (two-sided) was used to compare the continuous variables with normal distribution. Mann-Whitney U-test was used to compare the continuous variables without normal distribution. One-way analysis of variance (ANOVA) was applied to verify the association of genotypes with laboratory biomarkers. Categorical variables were compared with the chi-square test. Allele frequencies and Hardy-Weinberg equilibrium (HWE) were determined with the DeFinetti program (<http://ihg.gsf.de/cgi-bin/hw/hwa1.pl>).

Statistical analyses were performed with the Statistic for Windows version 8.0 software (StatSoft Inc., Tulsa, OK, USA). A P value less than 5% ($P < 0.05$) was considered significant.

RESULTS

Characteristics of the samples of this study are shown in Table 1. The groups were matched by age and gender. T1D subjects were determined to be leaner than control subjects as assessed by body mass index. All diabetic subjects had the disease for more than a decade (mean = 16.2 years), and 68% had a family history of diabetes.

Table 1. Anthropometric and laboratory marker characteristics of the study groups.

Parameters	Control (N = 144)	T1D (N = 136)	P
Age (years)	44 ± 5	43 ± 12	0.324
Gender (M/F)	50 / 95	45 / 91	0.529**
Weight (kg)	74.5 ± 13.7	72.3 ± 17.1	0.124
Height (m)	1.7 ± 0.1	1.6 ± 0.1	0.024
BMI (kg/m ²)	26.6 (23.5-99.8)	26.0 (22.8-30.1)	<0.001*
Time of diabetes (years)	-	16.2 ± 10.7	-
Family history of diabetes (%)	-	68.6	-
Non-fasting glucose (mM)	5.3 (4.7-5.8)	10.0 (5.9-13.4)	<0.001*
HbA1c (%)	5.4 ± 0.3	8.7 ± 1.9	<0.001
1,5-anhydroglucitol (µM)	149.8 ± 39.6	40.8 ± 52.4	<0.001
Albumin (g/L)	39 ± 1.5	40 ± 3.9	<0.001
Creatinine (µM)	49.5 (42.4-57.4)	76.9 (70.7-88.4)	<0.001*

Control, healthy subjects; T1D, type 1 diabetic subjects; M, male; F, female; BMI, body mass index. Values are reported as means ± SD or median (interquartile range) unless otherwise indicated. -, no information available. Independent Student *t*-test (two-sided), *Mann-Whitney U-test, or **chi-square test. P values in bold are significant.

As expected, all glyceamic marker levels were higher in the T1D group than in healthy subjects. Poor glyceamic control is characterized by concentrations of the biomarkers hemoglobin A1c (HbA1c) and 1,5-anhydroglucitol at >7% and <61 µM, respectively. In T1D subjects, the mean concentration was 8.7% and 40.8 µM for HbA1c and 1,5-anhydroglucitol, respectively, indicating that these subjects had poor glyceamic control.

Serum albumin and creatinine levels were significantly higher in the T1D group compared with the control group, but the concentrations of both markers were within the reference intervals, suggesting that no instances of kidney failure or malnutrition were present in the study groups.

The genotyping report is shown in Table 2. All polymorphisms were in HWE. Genotypes and allele frequencies did not differ between the groups ($P < 0.05$). Analysis with dominant (frequent genotype in homozygous versus others) and recessive (minor genotypes in homozygous versus others) models showed no significant differences.

Table 2. Genotype and allele frequencies for rs187238 and rs5435 polymorphisms in controls and type 1 diabetic (T1D) subjects.

Genes/Polymorphisms		Control (N = 144)	T1D (N = 136)	P
IL-18, rs187238				0.624
	C/C	76 (52.8%)	64 (47.1%)	
	C/G	54 (37.5%)	58 (42.6%)	
	G/G	14 (9.7%)	14 (10.3%)	
MAF	G-allele (95%CI)	28.5% (23-34%)	31.6% (26-37%)	0.416
Dominant model	CC vs CG+GG	76/68	64/72	0.339
Recessive model	GG vs CC+CG	14/130	14/122	0.873
GLUT4, rs5435				0.208
	C/C	67 (46.5%)	77 (56.6%)	
	C/T	58 (40.2%)	42 (30.8%)	
	T/T	19 (13.2%)	17 (12.5%)	
MAF	T-allele (95%CI)	33% (28-39%)	27% (23-33%)	0.167
Dominant model	CC vs CT+TT	67/77	77/59	0.091
Recessive model	TT vs CC+CT	19/125	17/119	0.862

Values are N (%); MAF, minor allele frequency. All polymorphisms were in Hardy-Weinberg equilibrium. Chi-squared test for genotype and allele frequencies, 95%CI: 95% confidence interval.

The associations of all genotypes, in both groups, with the laboratory markers for glyceamic control (glycemia, HbA1c, and 1,5-anhydroglucitol), creatinine, and albumin were not significant ($P > 0.05$), as verified by analysis of variance (data not shown).

DISCUSSION

Diabetes is considered one of the most dominant health problems worldwide, causing various complications and significantly raising mortality (Jensen et al., 2014).

The polymorphisms rs187238 (IL-18 gene) has been associated with autoimmune disease and rs5435 (GLUT4 gene) with glucose regulation, and both are relevant elements in T1D. Few studies have analyzed these polymorphisms in the Brazilian population.

The IL-18 polymorphism rs187238 was associated with susceptibility to T1D in a Northeast Brazilian population (Tavares, Santos et al., 2013). This study reported an association of rs187238 genotypes/allele ($P < 0.001$) with T1D in children ($N = 181$) compared with healthy individuals ($N = 122$). The minor allele frequencies (C-allele) (43.9%; 95%CI: 38-50%) for the control group were higher than we found in our study (G-allele, 28.5%; 95%CI: 23-34%). Northeast Brazilian populations are composed predominantly of African descent, while our study used a Euro-Brazilian population; therefore, differences in population backgrounds could explain this finding.

The SNP analyzed in our study was not associated with T1D (Table 2). The frequency of the minor G-allele observed for the control group was 28.5% (95%CI: 23-34%), which is similar to that reported for a European (Caucasian) population (26%) according to data from the International HapMap Project (<https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/>), and is significantly higher than that reported for a Japanese population (5.3%). It is also similar to Chinese and Indian populations (27.7 and 29.8%, respectively) (Szeszko et al., 2006).

The GLUT4 polymorphism rs5435 C>T was not associated with T1D (Table 2). This polymorphism has been studied in an Indian population and did not show association with T2D (Bodhini et al., 2011a). The frequency of the minor T-allele for the control group was 33% (95%CI: 28-39%), which is similar to those reported for European (30%), Caucasian (37%), Japanese (37%), and Chinese (34%) populations. African (12.5%) and Yoruba/Nigeria (6.9%) populations showed T-allele frequencies lower than in those in the Brazilian population, according to data from the International HapMap Project (<https://www.ncbi.nlm.nih.gov/variation/tools/1000genomes/>).

In conclusion, the polymorphisms rs187238 (IL-18) and rs5435 (GLUT4) were not associated with T1D in adults of Euro-Brazilian descent. The minor allele frequencies for both polymorphisms were similar to those of other European Caucasian populations.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the Araucaria Foundation and CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico).

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