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REFERÊNCIA

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Prevalence of the *rs1801282* single nucleotide polymorphism of the *PPARG* gene in patients with metabolic syndrome

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ABSTRACT

Objective: This study aimed to get the genotypic and allelic frequencies of rs1801282 in 179 volunteer donors and 154 patients with Metabolic syndrome (MetS) in Brasilia, Brazil and also examine the association with anthropometric, biochemical and hemodynamic variables in the latter group. MetS comprises a group of diseases resulting from insulin resistance, in-creased risk of type 2 diabetes and atherosclerotic cardiovascular disease. MetS is defined by the presence of increased visceral fat, atherogenic dyslipidemia (elevated triglycerides (TGL)), with decreased high density lipoprotein (HDL) and increased low density lipoprotein (LDL) levels, hypertension (BPH) and disturbances in glucose homeostasis representing a significant burden across the world due to the alarming increase in the incidence over the last decades besides their significant morbidity and mortality. Peroxisome proliferator activated receptor-gamma (PPAR γ) has been mentioned as a candidate gene for determining the risk of MetS. It is a member of the nuclear receptors superfamily and a ligand-activated transcription factor, which regulates the expression of genes involved in the network lipogenesis and adipogenesis, insulin sensitivity, energy balance, inflammation, angiogenesis and atherosclerosis. Among the PPARG genetic variants, single nucleotide polymorphism rs1801282 has been the most extensively studied one since it was first described by Yen and cols. in 1997. This polymorphism is characterized by the replacement of a proline (CCC) to an alanine (GCA) at codon 12 of exon B, due to the exchange of a cytosine with a guanine. The Ala allele frequency varies in different ethnic groups. **Materials and methods:** DNA was extracted using Chelex-100 method and determinations of genotypes were performed by allele-specific chain reaction. **Results:** The distribution of genotype frequency of the MetS group was not statistically different from the frequency in the donor population at large. In the first group, genotype frequency was CC to 0.869 and 0.103 for CG, while allelic frequencies were 0.948 for C and 0.052 for G allele. In the group of donors, the genotype and allele frequencies were 0.882 for CC, 0.117 to CG; and 0.941 to 0.059 for G and C, respectively. GG genotype was not found in any of the two groups. The genotype distribution and allele frequencies were in Hardy-Weinberg equilibrium. No marker could be detected from the analysis of anthropometric, biochemical and hemodynamic variables in the MetS group. **Conclusion:** Our data suggest that this polymorphism is not correlated with predisposition to MetS. The results obtained on a small sample of the population of Brasilia, corroborate the data reported in the literature on the prevalence of this polymorphism in PPAR in populations of different ethnic origins. Arch Endocrinol Metab. 2015;59(4):297-302

Keywords

Single nucleotide polymorphism; metabolic syndrome; rs1801282

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INTRODUCTION

Metabolic syndrome (MetS) comprises a group of related disorders stemming from insulin resistance and leading increased risk of type 2 diabetes and atherosclerotic cardiovascular disease. MetS is defined by the presence of increased visceral adiposity, atherogenic dyslipidemia [increased triglycerides (TGL), with decreased high-density lipoprotein (HDL), and increased low-density lipoprotein (LDL) cholesterol

serum levels], hypertension (HBP), and disturbances in glucose homeostasis (1). It represents a significant worldwide burden due to the alarming increase in its incidence over the last decades, in addition to its significant morbidity and mortality (2).

Environmental and life-style changes are widely accepted as the key factors driving insulin resistance (3) and adiposity (4) and hence the current epidemics of MetS. However, they don't fully explain its occurrence and it is also believed that genetic factors play a major

role in determining the predisposition to the syndrome. *PPARG* is a plausible candidate gene in determining the risk of MetS. It encodes peroxisome proliferator-activated receptor gamma (PPAR γ), a member of the nuclear hormone receptor superfamily of ligand-activated transcription factor, which regulates the expression of network of genes involved in adipogenesis and lipogenesis, insulin sensitivity, energy balance, inflammation, angiogenesis and atherosclerosis (5).

Among the genetic variants of *PPARG*, the *rs1801282* single nucleotide polymorphism has been the most extensively studied one since its first description by Yen and cols. in 1997 (6). This variant is characterized by the replacement of a proline (CCA) by an alanine (GCA) at codon 12 of exon B, due to the exchange of a cytosine for a guanine. The Ala allele frequency ranges from 2 to 23% in different ethnic groups (7). Its frequency is low in Asian and African populations (1 to 3%) (6) and higher in Caucasians (20%) (8-10). Deeb and cols. (11) were among the first researches to describe the functional implications of the Ala allele, by demonstrating that this variant displayed decreased binding affinity to the cognate promoter element and reduced ability to transactivate PPAR γ -responsive promoters. These findings suggested that the *rs1801282* variant could contribute to the observed lower body mass index (BMI) and insulin sensitivity among the carriers of this polymorphism (11).

However, subsequent studies in different populations have proposed opposed conclusions. Some describe that the presence of polymorphisms confers a protective effect with respect to type 2 diabetes risk (12), whereas others indicate that it is associated with increased susceptibility to the disease (8,13). There are also studies in which no association was found between this variant and the frequency of type 2 diabetes (14-16). To our knowledge, the association between the *PPARG* variant *rs1801282* and the risk of metabolic diseases has not been addressed in the Brazilian population. The aim of the present study was therefore to describe the genotypic and allelic frequency of the *rs1801282* polymorphism among MetS patients from the University Hospital of Brasilia, in comparison with control subjects from Brasilia, Brazil. We also investigated whether the biochemical and anthropometric variables differed among MetS patients with the *PPARG* Ala variant when compared to those with the wild type Pro/Pro genotype.

MATERIALS AND METHODS

Subjects

All 154 MetS subjects enrolled in this study were from the population treated at the University Hospital of Brasilia. The patients were unrelated, aged over 45 years and had MetS in accordance to the National Cholesterol Education Program/Adult Treatment Panel III (NCEP/ATPIII) criteria (17). The 179 unrelated volunteers, of both sexes, aged over 45, were blood donors from Blood Center Foundation of Brasilia. It was not possible to assess this population in all anthropometric and biochemical variables defined by NCEP ATPIII for MetS. However, the selection criteria helped us to exclude patients with diabetes (glycated hemoglobin < 6.5%) (18), hypertension (BP < 130/85 mmHg) (19) and obesity (BMI < 30) (20). In both groups, the exclusion criteria were: patients who refused to participate in the study, under the age of 45 years and using hyperglycemic medications such as glucocorticoids. Written informed consent was obtained from each participant after a full explanation of the study, which was approved by the Ethics Committee of the Health Sciences School from the University of Brasilia. Written informed consent was obtained from each participant after a full explanation of the study, which was approved by the Ethics Committee of the Health Sciences School from the University of Brasilia.

Anthropometric measurements and blood chemistry

Height, weight, waist circumference (WC), systolic and diastolic blood pressures were measured and the results were averaged. The body mass index (BMI) was calculated by dividing the weight (kg) by the height (m) squared. After an overnight fast, with at least 12 hours, blood samples were taken for determination of laboratory parameters considered for this project: fasting glucose, total cholesterol and fractions, triglycerides. All samples from patients with MetS were analyzed by conventional methods only in the Clinical Laboratory of the University Hospital of Brasilia. The results of biochemical tests were analyzed according to cutoff points proposed by the NCEP/ATPIII criteria used for classification of MetS.

rs1801282 genotyping

A venous blood sample (5 mL) was obtained from peripheral venipuncture using disposable material

and EDTA-containing tubes. Samples from MetS patients were collected at the University Hospital of Brasilia and, from volunteers, at Hemocentro Brasilia Foundation. DNA was extracted by the Chelex-100 method (21), and *rs1801282* genotype assignments were conducted by allele-specific polymerase chain reaction.

Initially, each pair of primers for the *rs1801282* polymorphism was used in PCR reactions with allele specific singleplex, in a final volume of 25 μ l containing 50 ng template DNA; 1 ng of each primer (direct, general and reverse); Syber Green Rox qPCR Master Mix Thermo Scientific and 1x H₂O Milli-Q qs (primers under request).

Amplifications were carried out in Step One Plus (Life Technologies®), which was composed of the following: denaturation at 95°C for 10 minutes; followed by 40 cycles of 15 seconds denaturation at 95°C, 1 minute at 60°C and melting curve 95°C for 15 seconds; 60°C 1 min, 95°C for 15 seconds.

Statistical analysis

The categorical data were described as mean \pm standard deviation. Variables were analyzed using nonparametric analysis (Mann Whitney test and Fisher). Hardy-Weinberg equilibrium, differences in genotype and allele frequencies between MetS and general individuals were analyzed by using Pearson's χ^2 test in GenAlex Program. All statistical tests were carried out using GraphPad Prism version 6.00 for Windows, GraphPad Software, San Diego, California, USA (www.graphpad.com). A p value < 0.05 was considered statistically significant (two-tailed).

RESULTS

Genotyping was carried out for 333 unrelated subjects (154 MetS patients and 179 individuals from general population) from the University Hospital of Brasilia and from the Hemocentro Foundation of Brasilia, Brazil. In the MetS group, the mean age was 57.9 \pm 6.83 years and 67.53% were female. In the population group, the mean age was 49.33 \pm 4.22 years, however the majority was male (67%). Clinical characteristics of the individuals enrolled in the study are presented in tables 1 and 2.

The genotyping results are summarized in table 3. Deviation from the Hardy-Weinberg equilibrium was not seen in either the MetS ($\chi^2 = 0.462$, df = 1, p =

0.49) or the general group ($\chi^2 = 0.695$, df = 1, p = 0.404). The genotype distribution of the *rs1801282* polymorphism for the MetS was 0.896 for the CC genotype and 0.103 for the CG genotype. For the general group, they were 0.888 and 0.112, respectively. There were no GG genotypes in both groups. The biochemical and anthropometric results for all MetS subjects are shown in table 4. No differences were seen in the mean values of age, height, weight, WC, BMI, plasma triglyceride, plasma total cholesterol, HDL and LDL cholesterol, as well as systolic and diastolic blood pressure among different genotypes, when we analyzed the MetS group individually (Table 5).

Table 1. Baseline characteristics of the metabolic syndrome subjects, University Hospital of Brasilia

n (154)	Mean \pm Standard deviation
Gender (M/F)	50/104
Age (years)	57.9 \pm 6.83
Weight (kg)	79.87 \pm 16.29
Height (cm)	1.62 \pm 0.09
Waist circumference	111.61 \pm 16.24
Systolic BP (mmHg)	131.50 \pm 14.76
Diastolic BP (mmHg)	84.09 \pm 7.97
BMI (kg/m ²)	30.73 \pm 6.57
FBS (mg/dL)	152.81 \pm 73.66
Total-c (mg/dL)	184.89 \pm 39.04
TGL (mg/dL)	180.92 \pm 87.91
HDL-c (mg/dL)	42.25 \pm 22.39
LDL-c (mg/dL)	108.67 \pm 36.20

BP: blood pressure; BMI: body mass index; Total-c: total cholesterol; TGL: triglycerides; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein; FBS: fasting glucose.

Table 2. Baseline characteristics of the volunteers subjects from the Hemocentro Foundation of Brasilia

n (179)	Mean \pm Standard deviation
Gender (M/F)	118/61
Age (years)	49.33 \pm 4.22
Weight (kg)	77 \pm 16.5
Height (cm)	1.72 \pm 0.09
Systolic BP (mmHg)	124 \pm 10.99
Diastolic BP (mmHg)	82 \pm 10.83
BMI (kg/m ²)	26.45 \pm 4.32
Hb A1C (%)	< 6.5%*

BP: blood pressure; BMI: body mass index; Total-c: total cholesterol; TGL: triglycerides; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein; Hb A1C: glycated hemoglobin.

* Criteria defined by the American Association of Diabetes and used by Hemocentro Foundation of Brasilia for screening type 2 diabetes mellitus.

Table 3. Allelic frequency and genotypes distribution of studied population

Groups	Allelic frequencies (%)		Genotypic frequencies n (%)		
	C (Pro)	G (Ala)	CC (Pro/Pro)	CG (Pro/Ala)	GG (Ala/Ala)
MetS	146 (94.8%)	5.2 (8%)	138 (89.61%)	16 (10.38%)	0
General population	168 (94.1%)	5.9 (11%)	159 (88.82%)	20 (11.17%)	0

MetS: metabolic syndrome.

Table 4. Comparison of biochemical, anthropometric and hemodynamic variables data of metabolic syndrome subjects between different groups of rs1801282 genotypes

Variables n = 154	Pro12Ala polymorphism		p
	Pro12Pro n (138) CC	Pro12Ala n (16) CG	
Gender (M/F)	46/92	04/12	0.3658
Age (years)	57.9 ± 6.87	58.12 ± 6.69	0.9238
FBG (mg/dL)	155.91 ± 76.59	126.31 ± 30.83	0.1562
Total-c (mg/dL)	183.71 ± 39.26	188.18 ± 47.66	0.4851
TGL (mg/dL)	184.22 ± 90.16	152.87 ± 60.84	0.2626
HDL-c (mg/dL)	42.45 ± 23.44	40.56 ± 9.95	0.4963
LDL-c (mg/dL)	107.49 ± 35.08	118.18 ± 44.40	0.3384
BMI (kg/m ²)	30.94 ± 6.63	28.89 ± 5.28	0.2780
Waist circumference	111.93 ± 16.14	108.81 ± 17.37	0.4368
Systolic BP (mmHg)	131.67 ± 15.11	130 ± 11.54	0.8509
Diastolic BP (mmHg)	84.13 ± 8.17	83.75 ± 6.19	0.7248

BP: blood pressure; BMI: body mass index; Total-c: total cholesterol; TGL: triglycerides; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein; FBG: fasting blood glucose.

Table 5. Association of rs1801282 genotypes and the presence of metabolic syndrome components

MetS components	Pro12Pro n (%) CC	Pro12Ala n (%) CG	p
FBS ≥ 100 (mg/dL)	133 (96.37%)	14 (87.5%)	0.1562
BP ≥ 130/85 mmHg	81 (58.69%)	7 (43.75%)	0.2925
TGL ≥ 150 mg/dL)	77 (55.79%)	8 (50%)	0.7919
HDL-c < 40 mg/dL (men)	24 (17.39%)	3 (18.75%)	0.1147
HDL-c < 50 mg/dL (women)	77 (55.79%)	8 (50%)	0.2251
WC ≥ 88 cm (women)	89 (64.49%)	11 (68.75%)	0.3923
WC ≥ 102 cm (men)	29 (21.01%)	3 (18.75%)	0.6292

MetS: metabolic syndrome; BP: blood pressure; BMI: body mass index; Total-c: total cholesterol; TGL: triglycerides; HDL-c: high density lipoprotein; LDL-c: low density lipoprotein; FBS: fasting glucose; WC: waist circumference.

The analysis of the frequencies of the individual components of MetS also showed no significant difference among the genotypes (Table 6). To see if the effects of the polymorphisms were associated with hypertension (BP > 130/85 mmHg), obese (BMI > 30 kg/m²) or impaired fasting glucose (fasting glucose > 100 mg/dL), we divided the MetS group in three subgroups, but no statistically significant difference was found.

DISCUSSION

The observational of individual differences in the risk of developing MetS has led to an increased interest in genetic factors that might determine the susceptibility to insulin resistance, since it is the core physiopathological component of MetS (22-24). The identification of candidate genes for the syndrome has therefore been a major focus of research over the last decades, and it is expected that these findings will translate into better understanding of the physiopathology of MetS in addition to enabling the development of appropriate therapeutic and preventative strategies.

The initial description of the *PPARG* rs1801282 polymorphism in patients with type 2 diabetes across diverse populations placed the gene as an attractive candidate in studies addressing genetic factors associated with metabolic disturbances. This was reinforced by well established role of PPAR γ in regulating key aspects of energy balance, including adipocyte differentiation, lipogenesis and insulin action, in addition to the inflammatory response, angiogenesis and atherosclerosis (5). Many studies have subsequently investigated the association between rs1801282 and the various individual components of MetS. Nevertheless, few of them have explored this association with the MetS as an entity. Our study is one of the few studies investigating this association, and is the first one addressing this issue in a Brazilian population.

The distribution of rs1801282 polymorphism is quite wide, ranging from 0.630 to 0.956 for the CC genotype, from 0.015 to 0.300 for the CG genotype and from 0 to 0.067 for GG. The frequency of the C allele ranges from 0.780 to 0.977 and that of the G allele varies from 0.023 to 0.220 for the G allele in an Iranian population (25). In the present study, we found that the frequency of the C allele was 0.95 and the frequency of the G allele was 0.05 in both the MetS group and the non-MetS group, and these results are in line with previous results observed in the general population. Indeed, our findings are close to the data from other previously published studies investigating diverse populations, such as the Chinese (26), Italian (27), French (28), African American (29), Korean

Table 6. Comparison of biochemical, anthropometric and hemodynamic variables data of metabolic syndrome subjects between different groups of rs1801282 genotypes in three subdivided groups according to hypertension, diabetes and obesity

MetS components	All subjects with MetS		Subjects with MetS and hypertension (BP ≥ 130/85 mm hg)		Subjects with MetS and diabetes (FBS ≥ 100 mg/dL)		Subjects with MetS and obesity (BMI ≥ 30 kg/m ²)	
	PRO12PRO CC (N = 138)	PRO12ALA CG (N = 16)	PRO12PRO CC (N = 81)	PRO12ALA CG (N = 7)	PRO12PRO CC (N = 133)	PRO12ALA CG (N = 14)	PRO12PRO CC (N = 69)	PRO12ALA CG (N = 7)
Systolic BP (mmHg)	131.67 ± 15.11	130 ± 11.54	140.72 ± 12.68	140 ± 8.16	131.66 ± 15.28	130.71 ± 12.06	132.59 ± 14.36	125.71 ± 9.75
Diastolic BP (mmHg)	84.13 ± 8.17	83.75 ± 6.19	87.29 ± 6.90	90 ± 0	83.99 ± 8.25	84.28 ± 6.46	84.21 ± 7.16	82.85 ± 4.87
FBS (mg/dL)	155.91 ± 76.59	126.31 ± 30.83	158.02 ± 87.87	127.85 ± 29.57	158.35 ± 76.97	131.64 ± 29.15	143.17 ± 49.01	131.71 ± 40.43
TGL (mg/dL)	184.22 ± 90.16	152.87 ± 60.84	191.27 ± 88.11	174.42 ± 63.80	185.54 ± 90.72	163.44 ± 57.46	177.44 ± 87.63	139.14 ± 47.92
HDL-c (mg/dL)	42.45 ± 23.44	40.56 ± 9.95	43.20 ± 28.66	38.71 ± 8.55	42.66 ± 23.82	39.28 ± 10.01	39.73 ± 11.14	42.14 ± 7.71
Waist circumference	111.93 ± 16.14	108.81 ± 17.37	112.50 ± 15.53	105.71 ± 17.19	111.56 ± 15.61	107.28 ± 15.10	119.72 ± 14.56	123.71 ± 14.04

MetS: metabolic syndrome; HDL-c: high density lipoprotein; BP: blood pressure; BMI: body mass index; TGL: triglycerides. P-value and the values given refer to the crude analysis (i.e., not adjusted for age and sex).

(15), Scottish (30), German (31), Finn (28) and Hispanic (32) populations. Additionally, in the first five populations listed above, the Ala12Ala genotype was not found, reinforcing the rarity of this variant worldwide.

Similar to other studies, the association between the *rs1801282* polymorphism and MetS risk was not found in this small sample from Brasilia population. Moreover, this variant was not associated with any of the investigated biochemical, anthropometric or hemodynamic variables among the MetS group. A French population-based study involving 1,155 individuals, including 279 men and women with MetS defined according to NCEP/ATPIII, concluded that none of the polymorphisms studied, including *rs1801282*, was associated with MetS risk (8). A small study involving 253 Korean women diagnosed with MetS (also defined by NCEP/ATPIII recommendations) did not find any association of this syndrome with the genotypes analyzed. There were also no differences between BMI, blood pressure, fasting glucose levels, fasting insulin levels and insulin resistance defined by the homeostatic model assessment in Ala carriers when compared with dominant Pro allele (15).

Rooki and cols. investigated the association between two *PPARG* genotypes polymorphisms (*rs3856806* and *rs1801282*) and their haplotypes in patients with MetS in 340 individuals from Iran (175 patients with MetS and 165 healthy controls), and found a significant difference between the frequency of *rs3856806* frequencies among individuals with MetS and healthy controls, although no association was found between the frequency of *rs1801282* and the occurrence of MetS (33). Yang

and cols. also investigated the *rs3856806* and *rs1801282* polymorphisms in 423 Chinese individuals with MetS and found that only *rs3856806* concluded that only the first one was associated with MetS (34). Another Chinese study with 792 subject from Beijing analyzed the correlations between the occurrence of insulin resistance and MetS and the *PPARG* variants *rs1801282* and *rs3856806*, and found both polymorphisms were associated with insulin results, but not with the components of MetS (35). Finally, results from a recent meta-analysis indicated that the *rs1801282* polymorphism might not be related to MetS as an entity, but could affect the risk of the individual components of this syndrome (14). In our study, there was no association between the frequency of the *rs1801282* polymorphism and either MetS as an entity or its individual components.

In conclusion, the *rs1801282* polymorphism in *PPARG* does not seem to be involved with MetS risk in a Brazilian population. We emphasize that a limitation of our study is the relatively small sample size. Thus, further studies with larger population bases are needed to clarify this issue.

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