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PPARα polymorphisms as risk factors for dyslipidemia in a Brazilian population

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ABSTRACT

Background and aims: Peroxisome proliferator-activated receptor α is a nuclear receptor involved in the regulation of several biochemical pathways. Polymorphisms within its gene have been associated with several metabolic traits. We aimed to investigate the association of L162V and Intron 7G>C polymorphisms with serum level markers and common morbidities affecting an older adult/elderly cohort from Cuiaba City, Mato Grosso State, Brazil, as well as to compare the results with a previously studied population from São Paulo City, Brazil.
Methods and results: The studied population consisted of 570 subjects from Cuiaba City, Brazil, who were subjected to clinical interviews and blood collection for laboratory examinations and DNA extraction. Dyslipidemia was defined when participants were taking oral hypolipemiants or those with total cholesterol above 200 mg/dL, HDL-c below 40 mg/dL, LDL-c above 130 mg/dL and TG above 150 mg/dL. Restriction fragment length polymorphism polymerase chain reaction (RFLP-PCR) was used for polymorphism genotyping. Individual polymorphism and haplotype data were available for analyses. In the studied sample, allele frequencies were 0.052 and 0.292 for 162V and Intron 7C, respectively. In brief, 162V allele was associated with dyslipidemia ($p = 0.025$), and after correction for alcohol consumption and waist-to-rip ratio, a tendency of association could still be observed ($p = 0.050$). In addition, Intron 7C allele was associated with dyslipidemia even after correction for the same variables ($p = 0.029$). When compared to our previous study from São Paulo, we found some divergences regarding these results, which may be explained by differences between the two populations. Haplotype association analyses revealed an association between L/C haplotype and dyslipidemia ($p = 0.021$) and between V/C haplotype and lower LDL-c levels when compared to L/G haplotype ($p = 0.044$).
Conclusion: These results may help to clarify the role of *PPARα* gene in lipid and lipoprotein metabolism and the evaluation of its polymorphisms and haplotypes as being characterized as genetic risk factors for metabolic disturbances.

1. Introduction

Epidemiological data have indicated a continuous increase in the world's life expectancy during the last decades, in parallel to a high prevalence of age-related diseases [1]. Cardiovascular disease represents the leading cause of death in adults worldwide. Moreover, higher prevalence of obesity, type II diabetes, dyslipidemia and other metabolic-related morbidities are considered important healthcare issues [2]. It is well-known that high LDL-cholesterol (LDL-c) and triglycerides (TG) levels and low HDL-cholesterol (HDL-c) levels are strong predictable factors for cardiovascular events [3]. Thus, a dysregulation of metabolic homeostasis, together with an inadequate diet and lifestyle habits leads to alterations in lipid and lipoprotein profiles.

The occurrence of genetic polymorphisms in genes of molecules strictly involved in regulation of fatty acid uptake and β-oxidation can have influence on lipid homeostasis, acting as risk factors for metabolic

Abbreviations: LDL-c, LDL-cholesterol; TG, triglycerides; HDL-c, HDL-cholesterol; PPAR, peroxisome proliferator-activated receptor; RFLP-PCR, restriction fragment length polymorphism polymerase chain reaction; BMI, body mass index; OR, odds-ratio; CI, confidence interval; df, degrees of freedom.

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disturbances [4]. Peroxisome proliferator-activated receptors (PPARs) are nuclear receptors involved in the regulation of several biochemical pathways such as lipid and carbohydrate metabolism, lipoprotein synthesis, adipogenesis and insulin sensitivity [5,6]. Three distinct isoforms of these nuclear receptors (PPARα, PPARβ/δ and PPARγ) are encoded by distinct genes and have specific tissue distribution. Endogenous ligands (fatty acids, eicosanoids or oxidized phospholipids) or synthetic ligands activate PPAR, which heterodimerizes with retinoid X receptor. This complex binds specifically to DNA sequences known as PPAR response elements, thus activating PPAR-regulated gene expression. PPARα is mainly expressed in tissues with extensive fatty acid catabolism and its activation leads to changes in the transcription of multiple genes that regulate lipid and lipoprotein metabolism including *LPL*, *APOC3*, *APOA1* and *APOA5* [2,7].

PPARα is located on 22q13.3 and several single nucleotide polymorphisms described within this gene were associated with metabolic features like insulin resistance, dyslipidemia and cardiovascular risk factors [6]. A leucine to valine transversion in codon 162 (L162V; rs1800206), represented by a C to G substitution in the exon 5, has been associated with changes in TG, total cholesterol, LDL-c, HDL-c and APOA1 plasma concentrations in Caucasian and Indian populations [8,9].

Another polymorphism, a G to C substitution at Intron 7 of the same gene (Intron 7G>C; rs4253778), has been associated with variation in lipid serum levels and cardiovascular risk modulation [6]. Moreover, a previous study from our group has identified an association of this polymorphism with lower TG and VLDL levels and higher HDL-c levels in an elderly cohort from São Paulo City, Brazil [4].

The purpose of this study was to investigate the association of L162V and Intron 7G>C polymorphisms with protein and lipid serum levels, fasting glucose, anthropometric measures and common morbidities affecting an older adult/elderly cohort from Cuiaba City, Mato Grosso state, Brazil. In addition, we aimed to compare the genotype and allele frequencies as well as the association results of these polymorphisms verified in the population from São Paulo City previously studied by our group. This is the first study that associated haplotypes of these two polymorphisms with lipid metabolism alterations.

2. Materials and methods

2.1. Studied population

The studied population consisted of 570 subjects (147 males and 423 females) from Cuiaba City, Brazil. Recruitment took place after advertising on newspapers and television, as well as through third-age community events such as meetings and leisure activities. The mean age of this population was 66.27 ± 8.04 years (ranging from 47 to 90 years) and it was composed of 42.4% of whites, 38.1% of mulattos, 17.2% of blacks, 1.8% of Asiatic origin and 0.5% of other origins (self-reported assessment). The volunteers were subjected to standardized interviews, life habits questionnaires including the CAGE Assessment for alcohol abuse (the individuals were classified as non-drinkers, non-alcoholics and alcoholics) [10] and International Physical Activity Questionnaire [11] as well as clinical evaluation. Information about previous chronic diseases and current medication use was assessed using clinical inquiries or self-informed past history, confirmed with physical examinations or physician reports. Hypertension was considered positive for subjects with systolic blood pressure above 140 mm Hg or diastolic above 90 mm Hg or currently taking anti-hypertensive medication [12]. Positive cases for type II diabetes were considered when currently taking insulin or oral hypoglycemics or those with fasting glucose above 126 mg/dL [13]. Individuals were considered positive for dyslipidemia when they were taking oral hypolipemiants or those with total cholesterol above 200 mg/dL, HDL-c below 40 mg/dL, LDL-c above 130 mg/dL and TG above 150 mg/dL [14]. Depression was assessed using the Geriatric Depression Scale and a score higher or equal to 5 was considered positive [15]. All participants were communicated about the study protocol and gave us informed consent according to the Helsinki Declaration. The Research Ethics Committee from Universidade Federal de São Paulo approved this study.

2.2. Laboratory tests

Lipids and lipid fractions were measured by routine enzymatic tests. Urea, creatinine and albumin serum levels and fasting glucose were measured by colorimetric, kinetic and ultraviolet tests.

2.3. Polymorphism detection

Whole blood was collected in tubes containing 0.1% EDTA and genomic DNA was obtained from peripheral lymphocytes. DNA extraction was performed according to Lahiri and Numberger [16] or using GFX™ Genomic Blood DNA Purification Kit (GE Healthcare Life Sciences, United Kingdom), following manufacturers protocol. L162V and Intron 7G>C polymorphisms were detected by polymerase chain reaction followed by restriction fragment length polymorphism (RFLP-PCR) using primers [9,17] and conditions [4] previously described.

2.4. Statistical analyses

Allele and genotype frequencies were calculated for each polymorphism and the χ^2 test was used to investigate deviation from Hardy–Weinberg equilibrium. Mann–Whitney or Student *t* test was performed to verify polymorphism association with serum levels concerning lipids, lipid fractions, creatinine, albumin and urea as well as fasting glucose, body mass index (BMI), abdominal circumference and waist-to-hip ratio. Logistic regression analysis was performed to verify polymorphism association with disease using sex, age, alcohol consumption status (non-drinkers, non-alcoholics and alcoholics), physical activity status (yes or no), BMI, abdominal circumference and waist-to-hip ratio as covariates in the model. Odds-ratio (OR) and 95% confidence interval (95% CI) were also calculated. LV and VV genotype carriers for L162V polymorphism were grouped together due to a low number of VV homozygous in this sample. Individual polymorphism data analyses were performed using SPSS 15.0. Linkage disequilibrium between L162V and Intron 7G>C polymorphisms was measured by D′ using Linkage Disequilibrium Analyzer [18] and haplotype association analysis was performed using SNPStats web tool [19]. The comparison of the genotype frequencies between populations from Cuiaba City and São Paulo City was performed by a χ^2 test. The *p*-value lower than 0.050 was considered statistically significant. Genetic Power Calculator [20] was used to estimate the statistical power for the statistically significant results concerning individual polymorphism data and showed power >80% to detect the genetic effects regarding the association with dyslipidemia found for the allele frequencies and sample size in the present study.

3. Results

Table 1 shows the characteristics of the studied population, including disease prevalence, mean serum and plasma levels as well as anthropometric measures. Five-hundred and fifty seven individuals were genotyped for L162V and five-hundred and fifty nine for Intron 7G>C polymorphism. 162V allele frequency was 0.052 and Intron 7C allele was 0.292. Both polymorphisms were within Hardy–Weinberg equilibrium ($p = 0.201$ and $p = 0.763$, respectively for L162V and Intron 7G>C polymorphisms). Linkage analysis showed modest but significant linkage disequilibrium between these polymorphisms ($D' = 0.374$; $r^2 = 0.019$; $p < 0.001$) and haplotype frequencies were 0.684 for L/G, 0.263 for L/C, 0.030 for V/C and 0.023 for V/G haplotypes.

Logistic regression analysis showed a significant association between 162V allele and dyslipidemia ($p = 0.025$; Table 2). After an