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PON1 polymorphisms are predictors of ability to attain HDL-C goals in statin-treated patients

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ABSTRACT

Objectives: PON1 plays an important role in inhibiting LDL-C oxidation, which reduces atherosclerosis and cardiovascular disease. Elevated PON1 activity or levels may contribute to increased HDL-C levels, but controversy exists over the hypothesis that genetic variation in the PON1 gene locus modulates HDL-C levels and responses to statin treatment. Therefore, the objective of this study was to investigate the association between two polymorphisms in the *PON1* gene and statin responses in a south Brazilian population.

Design and methods: The study population included 433 dyslipidemic patients who were prescribed statins. Total cholesterol, triglyceride, HDL-C and LDL-C levels were measured in these patients both before and after approximately 6 months of treatment with simvastatin/atorvastatin. Genotypes were assessed by real-time PCR for two *PON1* polymorphisms, Q192R (rs662) and L55M (rs854560).

Results: Baseline lipid levels were not associated with Q192R or L55M polymorphisms. For the Q192R (rs662) polymorphism, we observed that HDL-C goals were attained less often in patients with RR homozygosity than in Q allele carriers (χ^2 P = 0.009, adjusted residual analysis P = 0.003). For the L55M (rs854560) polymorphism, LL homozygotes were underrepresented among subjects that achieved the HDL-C goal (χ^2 P = 0.026, adjusted residual analysis P = 0.008). Analysis by univariate logistic regression confirmed that QQ/QR and MM/ML carriers had an increased chance of attaining HDL-C goals (OR = 2.41, CI95% = 1.32–4.40, P = 0.004 and OR = 1.68, CI95% = 1.15–2.45, P = 0.008). In a multivariate logistic analysis used to assess predictors of attaining an HDL-C goal > 1.55 mmol/L, we observed that gender (OR = 1.71, CI95% = 1.04–2.83, P = 0.036), baseline HDL-C levels (OR = 1.13, CI95% = 1.10–1.16, P < 0.001) and the QQ/QR + MM/ML genotypes increased the chance of achieving HDL-C goals (OR = 2.81, CI95% = 1.35–5.85, P = 0.006).

Conclusions: The results of this study show that the Q192R (rs662) and L55M (rs854560) polymorphisms may play a role in interindividual variation in achievement of HDL-C goals in response to statins.

Introduction

Coronary artery disease (CAD) and other cardiovascular diseases are the leading causes of morbidity and mortality in different regions of the world [1,2]. In Brazil, approximately 32% of all adult deaths are due to cardiovascular disease (CVD) [3]. The onset of these diseases is related to the presence of atherosclerosis, a chronic disease with multifactorial etiology [4,5]. Among the factors contributing to risk for CVD, dyslipidemia has been shown to be prevalent and its association with other factors significantly increases the risk of developing cardiovascular diseases [6].

With regards to genetic influences on coronary heart disease development, there is evidence indicating that changes in paraoxonase family (*PON*) genes contribute to CVD pathogenesis [7–9]. Studies have shown that the paraoxonase1 (PON1) enzyme is associated with the inhibition of lipid peroxidation of high-density lipoprotein cholesterol (HDL-C), reduced oxidative modification of low-density lipoprotein cholesterol (LDL-C) [10,11], and preservation of HDL-C and LDL-C function [12,13]. Serum PON1 activity is also inversely correlated with cardiovascular disease in that individuals with diseases of the carotid artery or coronary and myocardial infarction display lower PON1 activity [14,15]. Thus, paraoxonase 1 has been identified as a candidate gene that may explain individual propensity for cardiovascular disease [16,17].

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Human paraoxonases are a multigene family of polymorphic enzymes that consists of three different genes, *PON1*, *PON2* and *PON3*, which are located adjacent to each other on the long arm of chromosome 7 (between q21.3 and q22.1) in humans [18]. Although many modulators of PON1 have been identified, by far the biggest effect on PON1 activity occurs through *PON1* genetic polymorphisms [19]. The purification and cloning of human *PON1* revealed two common polymorphisms (L55M and Q192R) that have been extensively investigated [20]. The Q192R (rs662) polymorphism is more widely recognized and is a missense substitution that changes a glutamine (Q) to an arginine (R) [21]. It has been shown that the 192R allele is less effective at inhibiting the oxidation of LDL and has a substrate-dependent effect on activity [22]. Furthermore, lower HDL-C and higher LDL-C and triglyceride concentrations were observed in RR homozygous patients [23]. The L55M (rs854560) polymorphism involves the conversion of a leucine (L) to a methionine (M). The 55 M allele results in significantly higher PON1 mRNA and serum protein levels [24] and has been associated with lipid profiles, but these results have been inconsistent [23,25].

Statins are 3-hydroxymethyl-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors that are widely used to treat dyslipidemic patients, where they have been shown to lower LDL-C and triglyceride levels and slightly increase HDL-C levels [26]. Although statins are usually highly effective and are generally well tolerated, interindividual variation in efficacy has been observed. Considering that 1) lipid and lipoprotein homeostasis gene polymorphisms may be related to drug-response differences, 2) high levels of PON1 activity [27] or levels [28] contribute to increased levels of HDL-C, and 3) Q192R and L55M polymorphisms at *PON1* gene locus are responsible for 48% and 4% of PON1 phenotypic variance, respectively [29], our hypothesis is that these SNPs may modulate lipid levels, and especially HDL-C levels, in patients treated with statins. Thus, the objective of this study was to evaluate the influence of two functional polymorphisms in the *PON1* gene (Q192R and L55M) on the therapeutic efficacy of simvastatin/atorvastatin in a cohort of Southern Brazil.

Methods

Study population

The study population for this analysis consisted of 643 patients with dyslipidemia who received lipid-lowering simvastatin/atorvastatin therapy after medical evaluation. All patients invited to participate in this study were screened with a physical examination, provided a medical history and underwent a clinical laboratory evaluation. The individuals were of European descent and residents in the city of Porto Alegre, Brazil. Exclusion criteria were: age < 20 years, a triglyceride (TG) concentration ≥ 4.52 mmol/L, altered thyroid stimulating hormone levels, impaired hepatic or renal function, the presence of an unstable or uncontrolled disease that influences lipid metabolism, and previous therapy with other lipid-lowering drugs. Physical examinations, clinical data, and clinical laboratory data were obtained by a physician, and the doses administered were determined by a physician according to the clinical characteristics of each patient. Biochemical measurements were analyzed prior to the initiation of treatment with statins (simvastatin/atorvastatin) and approximately six months later to evaluate therapeutic efficacy. Other prescribed medications, such as calcium channel blockers, diuretics, and antithrombotic agents, were continued throughout the study.

After the application of exclusion criteria, four hundred thirty-three patients were analyzed for the lipid-lowering efficacy of the therapy. Therapeutic responses were measured at baseline and after approximately 6 months (6.2 ± 3.2) of treatment. This protocol was approved by the Ethics Committee of Federal University of Health Sciences of Porto Alegre. All participants in the study gave written informed consent.

Biochemical analyses

Peripheral blood samples were collected after 12 h of fasting. Total cholesterol (TC), triglyceride (TG), and glucose concentrations were determined using conventional enzymatic methods on a Mega Merck Analyzer (Merck Darmstadt, Germany), and with specific kits supplied by Labtest (Brazil). HDL-C was determined using a selective immunoseparation-based homogeneous assay, followed by colorimetric quantification. LDL-C was calculated according to Friedewald et al. [30].

Patients with normalized lipid profiles after statin therapy were evaluated according to the following criteria and recommendations of the V Brazilian Guidelines on Dyslipidemia and Prevention of Atherosclerosis [31] for primary prevention of CVD: TC < 5.18 mmol/L, LDL-C < 2.59 mmol/L, HDL-C > 1.55 mmol/L, and TG < 1.70 mmol/L.

DNA extraction and PON1 genotyping

Genomic DNA was isolated from peripheral blood samples using a technique described by Lahiri and Nurnberger [32]. The genotyping of the Q192R (rs662) and L55M (rs854560) polymorphisms in the *PON1* gene was performed by allelic discrimination real-time PCR using TaqMan SNP Genotyping assays from Applied Biosystems Inc., California, USA, according to the manufacturer's recommended protocol.

Statistical analysis

Continuous variables are expressed as the mean ± standard deviation. TG levels were log-transformed before analyses due to their skewed distribution, although non-transformed values are shown in the Results section. Allele frequencies were estimated by gene counting. The agreement of genotype frequencies with Hardy–Weinberg equilibrium expectations was tested using chi-square tests. Haplotype frequencies and linkage disequilibrium were estimated with Multiple Locus Haplotype Analysis version 2.0 and ARLEQUIN software version 3.1. Based on the findings of Kivistö el al. [33], the daily doses of simvastatin were transformed to equivalent doses of atorvastatin at a ratio of 2:1. The mean percentage of change in plasma lipid levels was obtained by taking the difference between pre- and post-treatment lipid levels, multiplying by 100 and then dividing by the pre-treatment level for each parameter. To determine the association between genotypes and baseline lipid levels or mean percentage of change in plasma lipid levels, the means for each variable were compared with a General Linear Model using the type III sums of squares. Age (years), gender, smoking status, standardized statin dosage (mg), treatment period (months), and baseline lipid levels (mmol/L) were included in each model as covariates for follow-up lipid levels and for lipid-lowering response (% of change in plasma lipid levels). For baseline lipid levels, age (years), gender, and smoking status variables were used as covariates. The distribution of genotypes between groups was evaluated by chi-square test, and adjusted residual values [cell-by-cell analyses] were assessed by WINPEPI 2.1, when appropriate. Multiple logistic regression was performed to assess the variables associated with achievement of lipid level goals. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS 19.0 for Windows.

Results

Characteristics of the study population

We evaluated a cohort of 433 individuals of European descent who were residents of southern Brazil and who used simvastatin/atorvastatin lipid-lowering therapy. The main characteristics of the study population are presented in Table 1. The subjects in the study were aged between 25 and 88 years (61.6 ± 10.9 years); 33.3% were males, 83.1% of participants were on simvastatin treatment, and 16.9% participants were atorvastatin users. The average treatment duration was approximately

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