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IMPACT OF CYP3A4*1B ON THE LIPID PROFILE IN INDIVIDUALS WHO USE HMG-COA REDUCTASE INHIBITORS

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ABSTRACT
Statins are part of a broadly effective group of drugs used in the treatment of dyslipidemia. Its mechanism of action is based on inhibition of the 3-hydroxy-3-methyl-glutaryl-CoA reductase enzyme (HMG-CoA reductase), resulting in a decrease in the hepatic synthesis of cholesterol and an increase in LDL receptor expression in hepatocytes. **Objective:** This study aimed to investigate the effect of the polymorphic gene CYP3A4 (−392A>G) on the lipid profile in hypercholesterolemic individuals treated with statins in São Luis (Maranhão State, Brazil). **Methods:** A total of 201 volunteers diagnosed with hypercholesterolemia who were using statin (10 mg/day) were investigated. Total cholesterol, HDL cholesterol, and triglyceride (after 12 hours fasting) concentrations were determined by enzymatic methods. LDL cholesterol was calculated by the Friedewald formula when the triglyceride concentration did not exceed 400 mg/dL. PCR-RFLP (polymerase chain reaction-restriction fragment length polymorphism) was used to detect CYP3A4 –392A>G polymorphism. **Results:** The distribution of genotypes for the variant CYP3A4 (−392A>G) allele was: AA = 75.1%, AG = 21.9%, and GG = 3.0%. Allele and genotype frequencies were in Hardy-Weinberg equilibrium (p = 0.502). The serum levels of total cholesterol (AA = 164.0 ± 37.9 vs. GG = 233.1 ± 66.8, p<0.005) and LDL cholesterol (AA = 92.8 ± 32.4 vs. GG = 150.0 ± 59.4, p<0.05) showed different concentrations among individuals with allele AA vs. GG. **Conclusion:** This study suggests that the total cholesterol and LDL cholesterol levels are influenced by the variant CYP3A4 (−392A>G) allele in individuals who use statins.

KEYWORDS: polymorphism, CYP3A4, statin.

INTRODUCTION
Statins are among the most used drugs worldwide and the first choice in the hypolipemiant treatment. Several studies have reported the effects of statins on primary and secondary prevention of cardiovascular events and mortality, not only due to their hypolipemiant effect, but also because of their pleiotropic characteristics such as anti-inflammatory, antithrombotic, and antioxidant actions [1, 2, 3, 4 5, 6, 7, 8].

The statins' mechanism of action is to inhibit in a competitive and reversible way the endogenous synthesis of cholesterol, by interrupting the conversion of 3-hydroxy-3-methylglutaryl-coenzyme A reductase (HMGCoA) to mevalonate, which is the limiting step of cholesterol biosynthesis resulting in a compensatory increase in the expression of low-density lipoprotein (LDL) receptors in hepatic cells, with a consequent reduction in LDL cholesterol and total cholesterol in the bloodstream (approximately 20% to 50%, respectively) [9, 10]. Also, it gives rise to a modest decrease in triglycerides (TG, 10% to 40%) and a small increase in the levels of high-density lipoprotein (HDL) cholesterol (5% to 15%) [11, 12].

Statins are well tolerated; however, the clinical effectiveness and treatment safety vary considerably from person to person due to a combination of phenotypic characteristics and genotypic factors [13, 14]. To date, pharmacogenetic studies have investigated the relationship between common genetic variants and the lipid responses to statin therapy or adverse events, which could help to optimize statin therapy by identifying individuals with increased risk or benefit.

Statins are metabolized by liver and intestine, through the P450 cytochrome enzyme system, especially by CYP3A family (in which CYP3A4 and CYP3A5

isoforms are included). The CYP3A family plays an important role in human metabolism, and it is involved in over 50% of clinically used drugs. On the other hand, simvastatin, lovastatin, and atorvastatin are metabolized by CYP3A4 [15, 16, 17].

Studies have analyzed polymorphisms of a single nucleotide (SNPs) in candidate genes to understand, at least in part, the variability in the response of these drugs. This fact includes relevant genes such as CYP3A4 and its polymorphism −392A>G (−392A>G rs2740574, being the −392G allele also known as CYP3A4*1B) which have demonstrated to influence the therapeutic response of these statins [18, 16, 15].

Thus, this study aimed to evaluate the effects of the variant CYP3A4*1B on the lipid profile in hypercholesterolemic individuals in treatment with statins.

METHODS

Ethics

This study was approved by the Committee of Ethics in Research of the University Hospital President Dutra of the Federal University of Maranhão, on April 30, 2012 (CEP 007/2012), and informed consent was obtained from all patients enrolled.

Samples

Two hundred one unrelated individuals (92 men and 109 women) from São Luís (Maranhão State, Brazil) with a mean age of 61.8 years (SD ± 15.6 years) with hypercholesterolemia were genotyped. Additional inclusion criteria were age over 18 years old, a regular use of medication (10 mg/day simvastatin or atorvastatin), with drugs that were unlikely to interfere with the lipid profile (patients with chronic heart disease were on cardioselective β-blockers and aspirin, patients with hypertension were on angiotensin-converting enzyme inhibitors), and a routine lifestyle for at least four weeks prior to screening for this study. People with a history of cancer, renal, hepatic or thyroid disease, uncontrolled diabetes mellitus, and pregnant women were excluded.

Determination of blood lipids

Total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) levels were analyzed using commercially available kits (Roche Biochemical 6000 -C501; Roche Diagnostics). Serum LDL-C levels were calculated using the Friedewald formula in individuals with TG levels <400 mg/dL.

DNA sample and extraction

Peripheral blood samples (5 mL) collected by direct venipuncture from each patient were stored at -20 ºC in sterile EDTA vacutainer tubes until DNA extraction. Genomic DNA extraction was performed using the QIA amp DNA Blood Mini kit (Qiagen®) following the manufacturer's instructions.

*Genotyping of the CYP3A4*1B polymorphism*

Polymorphism CYP3A4*1B was genotyped by PCR-RFLP. Selected primers were 5' GGA CAG CCA TAG AGA CAA CTG CA 3' and 5' CTT TCC TGC CCT GCA CAG 3', which produce a 334-base pair (bp) fragment. PCR conditions were: a 25uL reaction mixture containing 50ng of genomic DNA, 1U of HotStarTaq Plus Master Mix (containing HotStarTaq Plus DNA Polymerase, PCR Buffer with 3mM MgCl2, and 400µM each dNTP; Qiagen®) and 10pmol of each primer. After initial denaturation at 94 ºC for 5 minutes, amplification was performed using 40 cycles of 94 ºC for 1 minute, 55 ºC for 1 minute, and 72 ºC for 1 minute, followed by 72 ºC for 10 minutes for final extension. PCR products were digested overnight with restriction endonucleases PstI (Jena Bioscience®), following the manufacturer's recommendations. Restriction fragments were resolved on a 2% agarose gel and visualized by staining with ethidium bromide in conjunction with molecular weight ladder (Invitrogen®). The CYP3A4*1B allele was characterized by four distinctive fragments of 199, 81, 33 and 21-bp, whereas the CYP3A4 wild-type allele was identified by three fragments of 220, 81 and 33-bp.

Statistical Analysis

The chi-square, Fisher exact tests, and ANOVA were used for association analysis, and confirmation was obtained by the Lilliefors test (significance considered when $p<0.05$). Statistical calculations were performed using the Epi Info® v3.4.3, 2007 and Statsoft Statistica® v7.0.61.0 softwares.

RESULTS

Laboratory parameters of lipid profile

Regarding the serum lipid levels of the 201 tested samples, an overall average was found in 170.1 mg/dL (SD ± 45.0) for total cholesterol, 97.1 mg/dL (SD ± 37.3) for LDL cholesterol, and 48.2 mg/dL for HDL cholesterol (SD ± 12.1). When serum lipid levels were evaluated according to gender, women had higher levels of total cholesterol ($p = 0.026$), LDL cholesterol ($p = 0.015$) and HDL cholesterol ($p<0.001$) than men (Table 1).

There was also a difference in lipid serum levels when the comparison was made according to age, with a reduction in total cholesterol ($p = 0.002$) and LDL cholesterol ($p = 0.001$) as the age increased; however, this behavior was not observed in HDL cholesterol ($p = 0.226$) (Table 1).

CYP3A4 −392A>G polymorphism

Regarding the CYP3A4 −392A>G polymorphism frequencies, 151 (75.1%) patients were genotyped as wild-type allele (AA), 44 (21.9%) as AG, and 6 (3.0%) as GG. The observed genotype frequencies of CYP3A4 −392A>G polymorphism were in Hardy-Weinberg equilibrium in the population ($p = 0.216$).

The CYP3A4 −392A>G polymorphism showed a significant association with serum levels of total

cholesterol (p = 0.013) and LDL cholesterol (p = 0.025), with a noticeable increase in these values in individuals with the GG genotype. The same association was not significant compared to HDL cholesterol levels (p = 0.692) (Table 1).

Table 1. Relationship between gender, age and CYP3A4*1B variant, and changes in cholesterol levels: LDL-C and HDL-C.

Features	Total (n=201)		COL T, mg/dL		LDL-C, mg/dL		HDL-C, mg/dL	
	Cases	(%)	Mean ± SD	P	Mean ± SD	P	Mean ± SD	P
Gender								
Female	109	(54.2)	175.1 ±41.4	0.026	100.9 ±34.5	0.015	52.1 ±12.3	< 0.001
Male	92	(45.8)	164.2 ±48.4		92.5 ±40.0		43.4 ±9.9	
Age, years								
≤ 40	21	(10.4)	184.9 ±39.9	0.002	110.5 ±31.0	0.001	49.1 ±9.3	0.266
41 - 60	61	(30.3)	184.0 ±49.7		108.9 ±41.7		46.4 ±12.4	
61 - 80	97	(48.3)	160.6 ±38.7		89.5 ±31.6		48.2 ±12.0	
> 80	22	(10.9)	159.2 ±50.5		84.7 ±41.7		51.5 ±13.5	
CYP3A4 −392A>G								
AA	151	(75.1)	164.0 ±37.9	0.013	92.8 ±32.4	0.025	47.9 ±11.7	0.692
AG	44	(21.9)	182.3 ±55.2		104.3 ±43.4		49.5 ±13.8	
GG	6	(3.0)	233.1 ±66.8		150.0 ±59.4		44.8 ±4.2	

DISCUSSION

Cardiovascular diseases (CVDs) are considered one of the major problems of this decade and the main cause of morbimortality among women in many countries such as the USA and Brazil, especially among women over 50 years old. Among them, there are more deaths from CVDs (41.3%) than the next seven causes of death combined, with a six times greater risk of dying from CVDs than from breast cancer. In Brazil, CVDs also rank first among the causes of mortality since the 60s, for both men and women [19].

There is a great interest in understanding the response to hypolipemiant drug and its relationship with genetic variant. Therefore, many studies have been carried out to identify SNPs and recognize the polymorphic frequency associated with the hypolipemiant response.

In the present study, it was sought the association between the presence of mutation in the CYP3A4*1B gene and possible changes in the metabolism of statins, and thereby compromising the effectiveness of treatment in hyperlipidemic people. Both single (AG) and double (GG) mutation showed significant results from the statistical t-test and ANOVA regarding changes in the lipid variables (total cholesterol and LDL cholesterol). This finding demonstrates that hypolipemiant medication is influenced by polymorphism [18].

The influence of genetic variation in CYP3A4 (−392A>G) gene it was studied in 340 people with hypercholesterolemia who were treated with 10 mg/day atorvastatin. The results obtained by these researchers allowed to verify that the −392G variant allele is significantly (p = 0.038) associated with high plasma LDL cholesterol levels after treatment[14]. Another study also showed the effects of the CYP3A4 (−392A>G) gene polymorphism on the effectiveness and safety of simvastatin, but no significant association was found [20].

The interindividual differences in the metabolic profile of many drugs are mainly due to the variant sequence of genes that encode drug-metabolizing enzymes. Therefore, understanding the prevalence of SNPs in a given population is essential for estimating the possibility of interindividual differences in drug response. Thus, the influence of an ethnic group on pharmacogenetic response should be considered. Moreover, differences in the response to these drugs must be considered, due to the possibility of causing hepatotoxicity (dose-dependent) and myopathies (dose-dependent, with the possibility of rhabdomyolysis) [21].

From genotyping, it was found that the polymorphisms in CYP3A4*1B presented frequencies of 75.1% for AA, 21.9% for AG, and 3% for GG alleles. These results corroborate those found by Cavalli and cols [22], who verified CYP3A4*1B variant of 86% for AA, 12% for AG, and 2% for GG alleles. In a study carried out with 94 hypercholesterolemic Chilean people, who were using atorvastatin (10mg/1day/1month), genotypic frequencies were found, for −392A>G variants of the CYP3A4 gene, in 73 (77.7%) individuals non-carrier or carrier of the wild allele, and 21 (22.3%) heterozygous carriers, corroborating the results found in this study (AA = 75.1% and AG = 21.9%) [18]. However, the polymorphism in homozygous was not found in Chilean people, whereas in the present study it was found in 6 (3%) individuals.

A population study of Caucasians (1,198 individuals), aged over 55 years, in hypolipemiant treatment with simvastatin (925 patients) and atorvastatin (273 patients) showed a genotypic frequency of 1,102 (91.9%) non-

carrier of the mutation, 95 (7.92%) heterozygous carriers, and only 1 (0.08%) homozygous [23]. The CYP3A4 −392A>G polymorphism was also studied in Rio de Janeiro (Brazil), where it was found 59.5% (106 participants) of homozygous genotype (45.3% and −392AA and 14.2% −392GG) and 40.5% of heterozygous genotype [24]. Another study conducted in Rio Grande do Sul (Brazil) evidenced that 94.8% of the participants were carrier of −392AA genotype and 5.2% had −392AG genotype. However, the −392GG genotype was not observed in these individuals [25].

Thus, our research is in accordance with other studies conducted in Brazil and elsewhere concerning the variation and frequency of CYP3A4*1B polymorphic genotypes, being the heterozygous the most frequent variants in the population, whereas the homozygous variants are finding in a small number or are not noticeable in some studies of population analysis. Given that genetic polymorphisms of CYP3A4 were unknown until 1996, and since then, more than 20 SNPs have already been identified in its nucleotide sequence, comprising from the CYP3A4*1 to CYP3A4*21 polymorphism, it is verified that there was significant progress in the study of these mutations. Therefore, most of these variations are rare; and therefore unknown, requiring further studies so that we can reach more accurate conclusions about the influence of polymorphisms on the metabolic activity of this enzyme.

In this study, the results demonstrated that for CYP3A4*1B polymorphism, the alleles (A) and (G) had frequencies of 0.86 and 0.14, respectively. By comparing the frequency of allele G (0.14) to those reported in other studies, there is a similarity with the Brazilian population (0.13) [22, 26]. It was also found by the Hardy-Weinberg principle that the allele frequencies in this population are in equilibrium; this fact was evidenced by the chi-square adhesion test results (X^2 = 1.38 and p = 0.5026). Given this result, previous studies show a similarity (X^2 = 1.48 and p = 0.22) indicating the polymorphism in the studied population is in equilibrium [26].

CONCLUSION

From the results of this study, it can be concluded that the studied population showed a higher frequency of individuals with the wild-type allele, and the medication used by the participants showed significantly different results in total cholesterol and LDL cholesterol for homozygous (GG) and heterozygous (AG) genotypes.

This study contributes to the documentation of relevant genetic variations for the metabolization of several compounds, besides allowing the analysis of the evolution of different populations. Pharmacogenetic studies increase the impact on the individualization of treatment and could therefore significantly contribute to optimizing the safety and effectiveness of the medication regimen.

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