CYP3A5 genotyping for assessing the efficacy of treatment with simvastatin and atorvastatin

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Abstract

In this work, we examined the impact of polymorphism in the cytochrome P450 (CYP) 3A5 gene, CYP3A5*1 (6986A > G, rs 776746), on the reduction in the lipid levels caused by simvastatin and atorvastatin. We studied 350 hyperlipidemic patients who received 10-40 mg of atorvastatin (n = 175) or simvastatin (n = 175) daily. Genotyping for CYP3A5 was done by PCR-RFLP analysis. Differences in the lipid profile before and after treatment were expressed as the % difference. The frequency of CYP3A5 polymorphism was 13.4% for heterozygotes and 86.6% for homozygotes. Comparison of the responses to same dose of each drug showed that the highest % difference was associated with total cholesterol (TC) in subjects receiving atorvastatin 40 mg compared with simvastatin 40 mg (p = 0.048). However, comparison of the responses to equivalent doses of atorvastatin vs. simvastatin revealed no difference in the % change in any of the lipid parameters examined. In individuals with the same CYP3A5 genotype, a head to head comparison of the efficacy of the same dose of simvastatin vs. atorvastatin revealed an advantage for atorvastatin. For equivalent doses of atorvastatin vs. simvastatin there was no difference in the % change in any of the lipid parameters examined. Within the same genotype there was a significant difference in the % change related to the drug treatment.

Keywords: atorvastatin, cholesterol, CYP3A5 gene polymorphism, simvastatin.

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Introduction

Statins [3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors] are the main drugs for the treatment of high plasma total cholesterol and low-density lipoprotein cholesterol (TC and LDL-C, respectively) concentrations. The recommended plasma concentrations of LDL-C in very high- and high-risk patients are extremely strict (< 70 mg/dL and < 100 mg/dL, respectively), with a trend to < 70 mg/dL also for high-risk patients (Grundy et al., 2004) or, according to new guidelines, a decrease in LDL-C of > 50% (Stone et al., 2014). However, there is considerable variation in the response of TC and LDL-C (25-60% reduction) to statins that remains poorly understood. The most common problems are inappropriate drug category or dose selection, side effects and inadequate dose titration. Genetic factors may contribute to this variability since non-genetic factors (age, sex, smoking, body category weight, race and others) have only a trivial effect on the response to statins.

Three genome-wide association studies (GWAS) of the response to statins and several candidate gene association studies have been reported (Thompson et al., 2005; Donnelly et al., 2008; Thompson et al., 2009; Barber et al., 2010; Deshmukh et al., 2012). From these studies, the consistent finding is that variants in the apolipoprotein E (APOE) gene region are associated with variation in the LDL-C response. In addition, clinical trials of atorvastatin, such as CARDS (Armani and Toth, 2006) and the Anglo-Scandinavian Outcomes Trial (ASCOT), found an association between the LDL-C response to atorvastatin that reached GWAS significance and the Lipoprotein (a) (LPA) and APOE genes (Deshmukh et al., 2012).

Simvastatin and atorvastatin are the most widely used statins, with an enormous amount of research on their lipid-lowering efficacy and other pleotropic actions (Kolovou et al., 2008). These statins are metabolized by two members of the cytochrome P450 superfamily, CYP3A4 and CYP3A5, which are encoded by the CYP3A4 and
CYP3A5 genes, respectively (Prueksaritanont et al., 2003). The CYP3A5 gene is part of a cluster of cytochrome P450 genes on chromosome 7q21.1 and is determined by a single nucleotide polymorphism (SNP; 6986A > G) that results in CYP3A5 (rs 776746) being either present and functional (3A5*1) or entirely absent (3A5*3). This cluster also includes a pseudogene, CYP3A5P1. Polymorphism in CYP3A5, particularly the presence of the CYP3A5*3 allele, is the major factor that modulates gene expression (Kuehl et al., 2001).

Despite the considerable amount of research that has been done on the functions of P450 genes, few studies have assessed the practical relevance of various factors that regulate P450 expression among individuals. As an extension of our previous research (Ragia et al., 2014a,b), in this study we investigated the impact of CYP3A5 gene polymorphism on the effectiveness of simvastatin vs. atorvastatin in patients with hypercholesterolemia.

Materials and Methods

Subjects

We genotyped 350 unrelated Greek subjects (227 men and 123 women) aged 18-75 years old with primary hypercholesterolemia. Additional inclusion criteria were a stable (regular) use of medication, 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. Subjects with a history of renal or thyroid disease and uncontrolled diabetes mellitus were excluded. The subjects were randomly assigned to simvastatin or atorvastatin treatment for at least three months. Statin therapy can have an important effect on the lipid profile within one month of initiating treatment. However, in some patients, the dose of statin needs to be adjusted and an additional measurement of the lipid profile is required in the following months. This additional measurement three months after the first is enough to evaluate the maximum efficacy of statin treatment. The dose of simvastatin (10-40 mg/daily) or atorvastatin (10-40 mg/daily) was adjusted if required, according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) treatment goal for LDL-C based on the risk category (LDL-C < 130 mg/dL, high-risk < 100 mg/dL or very high-risk < 70 mg/dL, which correspond to concentrations of < 3.4, < 2.6 and < 1.8 mmol/L, respectively) (Stone et al., 2005). The % decrease in LDL-C with statin treatment is usually predictable. However, in some cases, higher doses of a particular statin are required to achieve the recommended treatment goal. All subjects were taking simvastatin or atorvastatin as the only lipid-lowering drug. This study was approved by the Institutional Review Board of the Onassis Cardiac Surgery Center.

Determination of blood lipids

Total cholesterol (TC), triglyceride (TG) and high density lipoprotein cholesterol (HDL-C) levels were measured with enzymatic colorimetric methods using a Roche Integra Biochemical analyser and commercially available kits (Roche Diagnostics Gmbh, Hannheim, Germany). The serum LDL-C levels were calculated using the Friedewald formula in subjects with TG levels < 400 mg/dL (4.5 mmol/L).

Genotyping of the CYP3A5*3 polymorphism

Blood samples (5 mL) were collected by direct venipuncture from each patient into a vacutainer tube containing ethylenediaminetetraacetic acid (EDTA). DNA was extracted by using a QIAGEN-FlexiGene DNA kit. The polymerase chain reaction (PCR) was used to amplify the sequence of interest as previously described (Arvanitidis et al., 2007). The reaction mix consisted of 5 µL of 10x buffer, 1.5 µL of 1 mM MgCl2, 0.4 µL of 25 mM dNTPs, 0.5 µL of forward primer (115 pmol/µL), 0.5 µL of reverse primer (134 pmol/µL), 0.5 µL of Taq polymerase (5 U, HyTest) and 3 µL of DNA in a final volume of 50 µL. The reaction involved an initial denaturation at 94 ºC for 5 min followed by 40 cycles of denaturation at 94 ºC for 1 min, annealing at 55 ºC for 1 min and extension at 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. All amplifications were done in a PTC-200 thermocycler (MJ Research, Watertown, MA, USA).

The PCR product (293 bp, 10 µL aliquot) was digested in a 20 µL reaction volume containing 5 U of SspI restriction enzyme (Takara Bio Inc.) at 37 ºC for 2.5 h. The digested products were separated by electrophoresis on 2.5% (w/v) agarose gels and visualized by staining with ethidium bromide in conjunction with a 25 bp molecular weight ladder (Invitrogen). The fragments obtained for the CYP3A5*1 allele were 148 bp, 125 bp and 20 bp in size, while those for the CYP3A5*3 allele were 168 bp and 125 bp. An internal positive control for the CYP3A5*1 allele (rare allele) was included in each PCR-restriction fragment length polymorphism (RFLP) run.

All samples were genotyped in duplicate. In addition, 10% of the samples were randomly selected and genotyped by a different investigator who was unaware of the outcome of previous analysis. The correct genotype was confirmed in all of these blind analyses. We therefore believe that our PCR-RFLP results are valid, with no need for further validation by Sanger sequencing or real-time PCR.

Statistical analysis

All continuous variables were expressed as the median ± interquartile range (25th-75th percentile, IQR) since
they deviated from a normal distribution. All categorical variables were shown as absolute and relative (percentage) frequencies. The differences in TC, TG, HDL-C and LDL-C before and after simvastatin treatment were also expressed as absolute or % differences; % differences were calculated using the relationship = [(variable after – variable before)/variable before] x 100. Mann-Whitney U and Wilcoxon rank-sum statistics were used to assess the differences in continuous variables between different groups and before (baseline) and after treatment, respectively. All tests were two-sided with the level of significance set at p < 0.05. The data were analyzed using the statistical software package SPSS® v.22 (IBM®).

Results

Patient and treatment characteristics

Table 1 shows the baseline characteristics of the patients. The majority of patients reached the required treatment levels within three months and in only 20% of the patients was the third or fourth measurement done at 4-6 months. The TC, LDL-C and TG concentrations decreased significantly with simvastatin and atorvastatin treatment, whereas HDL-C was unaltered. With regard to the statin dose, 11% of the patients were on 10 mg, 26% were on 20 mg and 13% on 40 mg of simvastatin per day compared to 18%, 21% and 11% for these same doses, respectively, for atorvastatin.

Genotype frequencies

The genotype frequency of the CYP3A5 polymorphism was 86.6% for homozygous (HM, non-expressors) and 13.4% for heterozygous (HT, expressors) individuals. The wild-type (WT) genotype was excluded from the analysis because of the small number of subjects with this genotype (n = 2) and the lack of crucial data [Hardy-Weinberg equilibrium, p = 0.178]. The frequency of CYP3A5 expressors was in accordance with that previously reported for the Greek population (Arvanitidis et al., 2007; Kolovou et al., 2014; Ragia et al., 2014c). Gender was not associated with the CYP3A5 frequency (p = 0.408).
The TC, TG, HDL-C, LDL-C concentrations and % differences before and after treatment based on genotype and regardless of the statin used are shown in Tables 1 and 2 and Figures 1 and 2. The decrease in TC, TG and LDL-C levels was significant for both genotypes, whereas HDL-C levels did not differ significantly. TC and LDL-C decreased significantly with both statins. In subjects with the HM genotype, both statins decreased TG, whereas in subjects with the HT genotype only simvastatin 20 mg and atorvastatin 20 mg decreased TG significantly (p = 0.005 and p = 0.010, respectively). Analysis of the responses to equivalent doses of atorvastatin and simvastatin (20 mg vs. 40 mg and 10 mg vs. 20 mg, respectively) revealed no difference in the % change in any of the lipid parameters examined.

**Dose-response according to CYP3A5 genotype**

**HT genotype**

The highest % differences for TC occurred in subjects receiving atorvastatin 40 mg compared to the other treatment groups (simvastatin 10, 20 and 40 mg: p = 0.011, 0.007 and 0.048, respectively, and atorvastatin 10 and 20 mg: p = 0.006 and 0.028, respectively). Similarly, the highest % differences for LDL occurred in subjects receiving atorvastatin 40 mg compared to the other treatment groups (simvastatin 10 and 20 mg: p = 0.026 and 0.004, respectively, and atorvastatin 10 and 20 mg: p = 0.032 and p = 0.017, respectively), except for simvastatin 40 mg (p = 0.802).

**HM genotype**

The highest % differences for TC occurred in subjects receiving atorvastatin 40 mg compared to simvastatin 10 mg (p = 0.010). The median HDL-C difference was significantly different between subjects treated with 20 mg of simvastatin and those treated with 20 mg of atorvastatin (2 vs. -3, respectively, p = 0.021). The decrease in LDL was significantly higher in subjects receiving simvastatin 40 mg or atorvastatin 40 mg compared to those receiving simvastatin 10 mg (p = 0.046 and 0.002, respectively).

**Discussion**

![Figure 1 - Lipid values according to genotype and before and after treatment with simvastatin. HDL – high density lipoprotein cholesterol, HM – CYP3A5 homozygous, HT – CYP3A5 heterozygous, LDL – low density lipoprotein cholesterol, Simva – simvastatin (10, 20 and 40 mg), TC – total cholesterol and TGL – triglycerides. Y axis describes the values (mg/dl) of TC, TGL, HDL and LDL before and after treatments. The Y axis is labeled from 0 to 1,000.](image-url)
In this study, we compared the efficacy of simvastatin vs. atorvastatin in relation to CYP3A5 polymorphism in Greek patients with hypercholesterolemia. The CYP3A5 gene may be one of the important genetic contributors to inter-individual and inter-racial differences in the response to and clearance of CYP3A-metabolized statins. Willrich et al. (2008) found that the CYP3A5*3A allele was associated with an attenuated cholesterol-lowering response to 10 mg of atorvastatin in 139 non-African individuals. Kim et al. (2007) examined the simvastatin plasma concentration in 22 individuals over 12 h after the ingestion of a single dose of 20 mg and found that the mean area under the plasma concentration-time curve for simvastatin in CYP3A5*1/*1 carriers was significantly lower than in CYP3A5*3/*3 carriers and that the mean oral clearance was also significantly different between CYP3A5*1/*1 and CYP3A5*3/*3 carriers. However, other pharmacokinetic parameters, including the peak plasma concentrations and half-life, did not differ between the genotypes. Kivistö et al. (2004) studied 69 Caucasian patients and found that lovastatin, simvastatin and atorvastatin were significantly less effective in CYP3A5 expressors than in non-expressors [mean % reduction in serum TC was lower in CYP3A5 expressors than in non-expressors, i.e., 17% vs. 31%]. Kajinami et al. (2004) examined whether genetic variation in the CYP system [a promoter (A290G) and two non-synonymous polymorphisms (F189S and M445T) in the CYP3A4 gene locus] affected the statin response in 340 hypercholesterolemic patients who were treated with atorvastatin. The A290G variant allele was significantly associated with higher levels of post-treatment LDL cholesterol, whereas the M445T variant was associated with lower levels of LDL-C before and after treatment. Wang et al. (2011) studied the CYP3A4 polymorphism (rs35599367, C > T) in 235 patients receiving atorvastatin, simvastatin or lovastatin and found that T carriers required significantly lower statin doses than non-T carriers.

In the Genetic Effects On STATins (GEOSTAT-1) Study, which was a genetic substudy of Secondary Prevention of Acute Coronary Events-Reduction of Cholesterol to Key European Targets (SPACE ROCKET), a randomized, controlled trial comparing simvastatin to rosuvastatin that
### Table 2 - Descriptive statistics according to the heterozygous (HT) and homozygous (HM) CYP3A5 genotypes and statin dose.

| Genotype  | Statin dose (mg) |        |        |        |        |        |        |
|-----------|------------------|--------|--------|--------|--------|--------|--------|
| HT CYP3A5 |                  |        |        |        |        |        |        |
| Age (y)   | 64 (16)          | 62 (9) | 57 (25) | 60 (30) | 56 (5)  | 69 (14) |
| BMI (kg/m²) | 27 (1)          | 26 (1) | 27 (2)  | 26 (2)  | 27 (3)  | 26 (5)  |
| TC (mg/dL) before | 243 (46) | 254 (60) | 242 (45) | 270 (98) | 276 (60) | 295 (116) |
| TGL (mg/dL) before | 123 (34) | 111 (12) | 233 (211) | 165 (118) | 119 (43) | 98 (91)  |
| HDL (mg/dL) before | 55 (10) | 50 (15) | 40 (6)  | 38 (13) | 52 (20)  | 64 (45)  |
| LDL (mg/dL) before | 170 (43) | 185 (45) | 161 (26) | 183 (60) | 203 (46) | 204 (96) |
| TC (mg/dL) after | 181 (25) | 190 (16) | 178 (61) | 164 (86) | 184 (33) | 144 (34) |
| TGL (mg/dL) after | 103 (45) | 101 (17) | 100 (95) | 102 (84) | 91 (99)  | 98 (97)  |
| HDL (mg/dL) after | 54 (12) | 51 (9)  | 41 (14) | 36 (20) | 52 (18)  | 59 (43)  |
| LDL (mg/dL) after | 109 (17) | 111 (40) | 109 (59) | 98 (74)  | 105 (35) | 72 (17)  |
| TC (difference in mg/dL) | -78 (49) | -73 (16) | -78 (43) | -87 (53) | -91 (76) | -151 (82) |
| TGL (difference in mg/dL) | -10 (47) | -16 (35) | -52 (184) | -59 (107) | -7 (46)  | -39 (45)  |
| HDL (difference in mg/dL) | -1 (16) | 1 (4)   | 0 (5)   | -3 (12) | 0.476   | 1 (18)   |
| LDL (difference in mg/dL) | -78 (44) | -68 (27) | -52 (49) | -75 (56) | -96 (49) | -139 (86) |
| TC (difference in %) | -29 (19) | -27 (8)  | -33 (11) | -30 (17) | -33 (19) | -51 (8)   |
| TGL (difference in %) | -9 (32) | -13 (29) | -26 (46) | -44 (33) | -8 (33)  | -21 (49)  |
| HDL (difference in %) | -1 (28) | 1 (10)   | 1 (13)   | -6 (31) | -3 (16)  | 4 (26)    |
| LDL (difference in %) | -43 (19) | -37 (7)  | -32 (25) | -34 (24) | -49 (17) | -68 (11)  |
| HM CYP3A5 |                  |        |        |        |        |        |        |
| Age (y)   | 59 (15)          | 54 (19) | 63 (17) | 58 (20) | 62 (18) | 55 (20) |
| BMI (kg/m²) | 28 (4)          | 26 (3)  | 28 (5)  | 27 (4)  | 27 (4)  | 25 (4)  |
| TC (mg/dL) before | 259 (62) | 276 (53) | 275 (59) | 295 (62) | 292 (59) | 307 (65) |
| TGL (mg/dL) before | 131 (78) | 141 (80) | 157 (89) | 186 (146) | 159 (131) | 152 (139) |
| HDL (mg/dL) before | 49 (15) | 44 (23) | 45 (17) | 47 (17) | 47 (17) | 45 (13) |
| LDL (mg/dL) before | 176 (61) | 193 (49) | 190 (56) | 196 (68) | 204 (53) | 217 (76) |
| TC (mg/dL) after | 184 (56) | 189 (46) | 185 (36) | 180 (41) | 187 (42) | 192 (48) |
| TGL (mg/dL) after | 114 (57) | 108 (68) | 116 (60) | 115 (65) | 127 (74) | 96 (68) |
| HDL (mg/dL) after | 46 (13) | 48 (15) | 47 (17) | 43 (21) | 47 (14) | 44 (19) |
| LDL (mg/dL) after | 110 (46) | 112 (33) | 110 (34) | 110 (39) | 107 (43) | 121 (41) |
recruited 601 patients after myocardial infarction, the patients were genotyped for genes encoding the CYP450 [CYP2C9*2 (430C > T), CYP2C9*3 (1075A > C), CYP2C19*2 (681G > A), CYP3A5*1 (6986A > G)] and other genes. The LDL-C levels and the proportion of patients reaching the LDL-C target of < 70 mg/dL (< 1.81 mmol/L) were assessed after three months. An enhanced response to rosuvastatin was seen in patients with CYP3A5 genotypes (Bailey et al., 2010). In contrast, Rosales et al. (2012) found no differences in the lipid profiles in relation to CYP3A5 polymorphism in Chilean subjects treated with atorvastatin. Gao et al. (2008) studied 217 patients who prospectively received atorvastatin and 199 patients who received simvastatin for four weeks and found that carrying CYP3A4*1G increased the lipid-lowering efficacy of atorvastatin and probably had no significant effect on the response to simvastatin treatment.

In a previous study, we examined the relationship between POR*28 (polymorphic enzyme P450 oxidoreductase, POR) polymorphism, which is associated with increased activity of CYP3A enzymes, and the response to atorvastatin and simvastatin (Ragia et al., 2014b). The POR*28 allele was not associated with the lipid-lowering effect of atorvastatin and the results were replicated in an independent simvastatin-treated population. We also analyzed inter-individual variability in relation to CYP3A4 intron 6 C > T polymorphism (CYP3A4*22 allele, rs35599367) and the response to atorvastatin and simvastatin (Ragia et al., 2014a); there was no association with the lipid-lowering response to both drugs.

In the present study, comparison of equivalent doses of atorvastatin vs. simvastatin revealed no significant difference in the % change in any of the lipid parameters examined. However, comparison of the same drug dose showed higher % differences for TC in individuals receiving atorvastatin compared with simvastatin. A limitation of this study is the rather small number of subjects used. However, few studies have examined the relationship between the statin (simvastatin or atorvastatin)-mediated decrease in LDL-C and the subjects genotype, or between the CYP3A5 polymorphism and the efficacy of these two statins.

The gap between pharmacogenomics and personalized therapy in clinical practice is still very wide, with no guarantee that it will ever be bridged. For this reason, studies of the type described here are very important. Individualized therapy could be practical when the efficacy and side effects of a drug are simple and well-defined and a major goal could be reached when a phenotype-genotype association with a drug response is evaluated. In practical terms, personalized therapy is designed to identify patients who are hyper- or hypo-responders to drugs, as well as individuals who are prone to drug toxicity. However, genomic treatment has not moved as fast as expected, primarily because of scientific and economic difficulties. For treatment with statins, it is much cheaper to change the type of statin, to in-

![Table 2. (cont.)](image-url)
crease the dose or to use combination therapy together with statin treatment rather than perform expensive genotyping, although the cost of DNA sequencing is decreasing radically. Even for well-defined genetic variants such as APOE or cholesteryl ester transport protein (CETP) gene polymorphisms that have reproducible and significant consequences for drug therapy, general recognition of this relationship by the medical community can be slow.

In conclusion, in a head to head comparison of the efficacy of the same dose of simvastatin vs. atorvastatin in individuals with the same CYP3A5 genotype, we found an advantage for atorvastatin, although equivalent doses of atorvastatin vs. simvastatin showed no difference in the % change in any of the lipid parameters examined. Of particular interest was the finding that within the same genotype there was an important difference in the % change according to drug treatment.

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