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Impact of *CYP3A5* Gene Polymorphism on Efficacy of Simvastatin

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Abstract: *Background:* One of the promises of human genetics is individualized therapy. Therefore, we evaluated the impact of *CYP3A5 gene* polymorphism on the effectiveness of simvastatin (a HMG-CoA reductase inhibitor). *Methods:* Patients (n = 191) with hypercholesterolemia were treated with simvastatin for at least 6 months and were genotyped for the *CYP3A5* polymorphism. *Results:* The frequency of *CYP3A5* polymorphism was 0.5% for *WT* (wild-type), 15.6% for *HT* (heterozygous, expressors) and 83.9% for *HM* (homozygous, non-expressors). Differences in lipid profile before and after dose-response of simvastatin treatment were described as % difference {[(variable after-variable before)/variable before]*100}. There was a trend towards the decrease of low density lipoprotein cholesterol (LDL-C) in *HT* individuals who had a -35.2% reduction with a dose of 20 mg simvastatin and *HM* individuals who had a slightly higher decrease (-37.5%) despite the lower dose of simvastatin (10 mg, p = 0.07). Furthermore, *HT* genotype individuals had significantly higher than expected (6-8%) LDL-C % difference between 20 and 40 mg of simvastatin (-35.2 vs -49.2%, p = 0.037). In individuals with *HM* genotype a significant LDL-C % difference was found between 10 and 40 mg of simvastatin (-37.5 vs -48.4%, p = 0.023). *Conclusion:* The individuals with *HM* polymorphism display a trend towards higher LDL-C reductions compared with *HT* polymorphism. Within the same genotype, differences between doses were also observed. These findings need to be confirmed in larger studies.

Keywords: Simvastatin, *CYP3A5* gene polymorphism, low density lipoprotein cholesterol.

INTRODUCTION

HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme A) reductase inhibitors (statins) are the first-line drugs for hypercholesterolemia treatment. However, there is considerable variation in the reduction of plasma low density lipoprotein cholesterol (LDL-C) concentration in response to statin treatment (from - 25 to - 60%) [1]. Even more confusing is the efficacy of statins on triglycerides (TGs) reduction, varying from - 10 to - 50%, and high density lipoprotein cholesterol (HDL-C) varying from - 1 to + 15% [2, 3]. Environmental and genetic factors may contribute to this variability.

Simvastatin is one of the oldest lipophilic statins, which enters and exits hepatocytes through passive diffusion [1, 2]. Simvastatin is extensively metabolized by two enzymes of the cytochrome P450 superfamily that catalyze the oxidation of organic substances, the CYP3A4 and CYP3A5 which are encoded by the *CYP3A4* and *CYP3A5* genes, respectively [4]. The most common reaction catalyzed by cytochromes P450 is a monooxygenase reaction. The P450 proteins are localized in the cell endoplasmic reticulum and their expression is induced by glucocorticoids and various pharmacological agents [5,6]. The *CYP3A5* gene is part of a cluster of cytochrome *P450* genes on chromosome 7q21.1. This cluster includes a pseudogene, *CYP3A5P1* and *CYP3A5*. There are substantial CYP3A expression differences between individuals that contribute to the variation in bioavailability and clearance of CYP3A substrates. A polymorphism in *CYP3A5*, the CYP3A5*3 allele, is the major factor that modulates P450 proteins expression [7]. Also, *CYP3A5* plays an important role in the appearance of adverse effects.

Statins have been reported to increase CYP3A expression *in vitro* as ligands to nuclear receptors (pregnane X receptor and constitutive androsterone receptor) that form heterodimers with retinoid X receptors and bind to responsive elements in the CYP3A4 and CYP3A5 promoter regions [8]. Individuals with at least one *CYP3A5*1* allele express large amounts of CYP3A5. Thus, the *CYP3A5* may be one of the important genetic contributors to inter-individual and interracial differences in CYP3A-dependent drug responses and clearance. Despite the significant research that has been performed to find out the function of P450 genes, few studies have evaluated the practical value of various factors involved in controlling P450 expression and variation among individuals [9].

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The aims of this study were:

1. To investigate the impact of *CYP3A5* gene polymorphism on the effectiveness of simvastatin in patients with primary hypercholesterolemia.
2. To explore the influence of genetic variations of *CYP3A5* gene on the response to simvastatin treatment by examining genotype frequencies in hyper-responders and hypo-responders.

METHODS

Subjects

The number of participants needed to achieve 90% statistical power at a significance level of 5% and to evaluate differences higher than 5% in total cholesterol (TC) values after treatment was 190. Therefore, we consecutively selected and genotyped 191 Greek unrelated subjects [125 men and 66 women; median age ± interquartile range (75^{th}-25^{th} percentile, IQR) = 60.0 years ± 17.0)] with primary hypercholesterolemia who attended the Lipid Clinic of the Cardiology Department at Onassis Cardiac Surgery Center. They gave their consent before entering the study. Additional inclusion criteria were a stable medication, which was unlikely to interfere with lipid profile (patients with coronary heart disease were on cardioselective β-blockers and aspirin, whereas patients with hypertension were on angiotensin converting enzyme inhibitors) and routine lifestyle for at least 4 weeks prior to study screening. Subjects with a history of renal or thyroid disease and uncontrolled diabetes mellitus were excluded from the study. Subjects were assigned to simvastatin treatment for at least 6 months [9 ± 2 (SD), Standard Deviation]. The dose of simvastatin (10-40 mg/day) was adjusted according to the National Cholesterol Education Program Adult Treatment Panel III (NCEP ATP III) treatment goal for LDL-C based on risk category (LDL-C <130, < 100 or < 70 mg/dl; < 3.4, < 2.6 or < 1.8 mmol/l) [10]. The lipids were monitored every 3 months in order to titrate simvastatin dose. All subjects were taking simvastatin as the only lipid-lowering drug. Furthermore, according to LDL-C changes, patients were divided to Hyper-responders and Hypo-responders.

Our Institutional Review Board approved the study.

Determination of Blood Lipids and Glucose

Total cholesterol (TC), TGs and HDL-C levels were measured using enzymatic colorimetric methods, on a Roche Integra Biochemical analyzer, with 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 CYP3A5*3 Polymorphism

Five millilitre blood samples were collected by direct venipuncture from each patient and were drawn in a vacutainer tube containing ethylene diaminetetraacetic acid (EDTA). DNA was extracted by using QIAGEN-FlexiGeneDNA kit.

Polymerase chain reaction (PCR) was used to amplify the sequence of interest as described previously [9]. The following pair of primers was used for amplification: 5'-CATCAGTTAGTAGACAGATGA-3' (forward) and 5'-GGTCCAAACAGGGAAGAAATA-3' (reverse). The underlined nucleotide is a mismatch with CYP3A5 sequence, creating an additional restriction site in the PCR product. The reaction mix was composed by: 5 μl 10x buffer, 1.5 μl $MgCl_2$ 1 mM, 0.4 μldNTPs 25 mM, 0.5 μl forward primer 115 pmol/μl, 0.5 μl reverse primer 134 pmol/μl, 0.5 μlTaq polymerase (5U-HyTest), 3 μl DNA (final volume: 50 μl). PCR started with initial denaturation at 94°C/5 min and followed by 40 cycles under the following conditions: denaturation at 94°C/1 min, annealing at 55 °C/1 min and extension at 72 °C/1 min. The final extension was carried out at 72 °C/10 min. All PCR amplifications were carried in the PCR-engine apparatus PTC-200 of MJ Research (Watertown, Mass., USA).

The PCR product (293 bps) of 10 μl was digested in a 20 μl reaction volume with 5 U of SspI restriction enzyme (Takara Bio Inc., Shiga, Japan) at 37 °C for 2.5 h. The digested products were resolved on 2.5% w/v agarose gel electrophoresis, visualized by staining with ethidium bromide and identified with a 25 bps molecular weight ladder (Invitrogen). The fragments obtained for CYP3A5*1 allele were 148, 125 and 20 bps, while for *CYP3A5*3 allele* were 168 and 125bps. An internal positive to *CYP3A5*1* allele (rare allele) control was used in each PCR-RFLP run.

Statistical Analysis

All continuous variables are presented as median ± interquartile range (75^{th}-25^{th} percentile, IQR) since they deviated from normal distribution. All categorical variables are presented as absolute and relative (percentage) frequencies. Mann-Whitney U and Wilcoxon rank-sum statistics were used in order to evaluate the differences in continuous variables between different groups and between baseline and after treatment, respectively. The differences in TC, TGs, HDL-C and LDL-C before and after simvastatin treatment were also described as % difference, based on the following rule: % difference = [(variable after-variable before)/variable before]*100. All tests were two-sided at a significance level of $p < 0.05$. Data were analyzed using STATA™ statistical software (Version 9.0, Stata Corporation, College Station, TX 77845, USA).

RESULTS

The TC, LDL-C and TG levels were significantly decreased with simvastatin treatment (-33.1, - 42.9 and - 20.2%, respectively, $p<0.001$), while HDL-C concentration increased (+ 1.6%, $p = 0.01$), (Table **1**).

Simvastatin Dose

The majority of the individuals (51.4%) were on 20 mg daily, whereas 22.9% were on 10 mg and 25.7% on 40 mg daily.

Genotype Frequencies

The genotype frequency of the *CYP3A5* polymorphism was for homozygous (*HM*, non-expressors) 83.9%, for heterozygous (*HT*, expressors) 15.6% and for wild-type (*WT*) 0.5%. The *WT* genotype was excluded from analysis due to small number (n = 1).

Table 1. Patients characteristics and lipid values before and after simvastatin treatment.

		N	%
Gender	Men	125	65
	Women	66	35
CYP3A5	*HT* (Expressors)	30	15.6
	HM (Non-expressors)	161	83.9
Simvastatin dose	10 mg	41	23
	20 mg	92	51
	40 mg	46	26
		Median	IQR
Age (years)		60.0	17.0
BMI (kg/m^2)		27.4	4.1
TC (mg/dl) before		275.0	57.0
TC (mg/dl) after		186.0	39.0
TC difference (mg/dl)		-91.0	56.0
TC difference (%)		-33.1	14.8
TG (mg/dl) before		150.0	95.0
TG (mg/dl) after		116.0	62.0
TG difference (mg/dl)		-29.0	65.5
TG difference (%)		-20.2	32.1
HDL-C (mg/dl) before		47.0	17.0
HDL-C (mg/dl) after		47.0	17.0
HDL-C difference (mg/dl)		+0.9	10.9
HDL-C difference (%)		+1.6	23.0
LDL-C (mg/dl) before		189.0	51.0
LDL-C (mg/dl) after		110.0	36.0
LDL-C difference (mg/dl)		-82.0	53.0
LDL-C difference (%)		-42.9	19.4

BMI = Body Mass Index; TC = Total Cholesterol; TG = Triglycerides; HDL-C = High Density Lipoprotein Cholesterol; LDL-C = Low Density Lipoprotein Cholesterol.
WT genotype was excluded from the analysis due to the small number (n = 1).

Patient Characteristics

Baseline characteristics of the total study patients are shown in (Table **1**).

TC, TGs, HDL-C and LDL-C concentrations as well as % differences before and after simvastatin treatment based on genotype are shown in (Table **2A** and Table **2B**).

The % differences within *HM* and *HT* genotypes are shown in (Table **3**).

Dose-response According to CYP3A5 Genotypes

The individuals with *HM* polymorphism displayed a trend towards higher LDL-C reductions compared with *HT* polymorphism (p=0.07). The *HT* genotype individuals (expressors) had a - 35.2% LDL-C decrease with 20 mg simvastatin, whereas *HM* individuals (non-expressors) displayed a relatively higher decrease (- 37.5%) with the half dose of simvastatin (i.e. 10 mg).

The *HT* genotype individuals had significantly higher than expected (as doubling the statin dose is adding further lowering of the LDL-C only by 6-8%, additionally to the first dose [11]) LDL-C % difference between 20 and 40 mg of simvastatin (- 35.2 vs - 49.2%; p = 0.037). In individuals with *HM* genotype the significant LDL-C % difference was between 10 and 40 mg (- 37.5 vs - 48.4%; p = 0.023).

Hyper-responders and Hypo-responders

Hyper-responders were considered those in the upper percentile (Min-25th percentile; from - 80.3 to - 51.5%) and as Hypo-responders were considered those in the lower percentile (75th percentile-maximum; from - 32.1 to 93.5%). No % differences were found according to dose of

Table 2A. Descriptive statistics by genotype.

	CYP3A5				
	***HT* (Expressors)**		***HM* (Non-expressors)**		
	Median	**IQR**	**Median**	**IQR**	**p (HT-HM)**
Age (years)	57.0	16.0	61.0	17.5	0.907
BMI (kg/m^2)	27.0	2.2	27.6	4.2	0.458
TC (mg/dl) before	262.0	48.0	279.0	51.0	0.099
TC (mg/dl) after	181.0	33.5	187.0	41.0	0.392
TC difference (mg/dl)	-81.0	56.0	-91.0	53.5	0.180
TC difference (%)	-32.0	18.3	-33.2	14.8	0.408
TG (mg/dl) before	127.0	82.0	154.0	95.0	0.317
TG (mg/dl) after	95.0	60.0	116.0	62.5	0.174
TG difference (mg/dl)	-13.5	48.0	-30.0	67.0	0.400
TG difference (%)	-10.2	31.0	-21.1	33.0	0.551
HDL-C (mg/dl) before	48.8	18.0	46.5	17.0	0.537
HDL-C (mg/dl) after	48.5	19.5	46.5	16.0	0.931
HDL-C difference (mg/dl)	-1.0	9.0	1.0	10.8	0.386
HDL-C difference (%)	-1.7	16.5	2.2	24.8	0.346
LDL-C (mg/dl) before	181.0	60.0	190.5	49.5	0.342
LDL-C (mg/dl) after	108.0	38.0	110.0	36.0	0.256
LDL-C difference (mg/dl)	-79.0	45.0	-82.0	50.6	0.533
LDL-C difference (%)	-42.5	17.3	-42.9	19.0	0.717

BMI = Body Mass Index; TC = Total Cholesterol; TG = Triglycerides; HDL-C = High Density Lipoprotein Cholesterol; LDL-C = Low Density Lipoprotein Cholesterol.
WT genotype was excluded from the analysis due to the small number (n = 1).

Table 2B. Significance of differences [baseline (b), afterwards (a)] for each genotype.

	CYP3A5	
	HT* (E*xpressors)	***HM* (*Non-expressors)***
	p	**p**
TC b-a	< 0.001	< 0.001
TG b-a	0.003	< 0.001
HDL-C b-a	0.629	0.202
LDL-C b-a	< 0.001	< 0.001

TC = Total Cholesterol; TG = Triglycerides; HDL-C = High Density Lipoprotein Cholesterol; LDL-C = Low Density Lipoprotein Cholesterol.
WT genotype was excluded from the analysis due to the small number (n = 1).

simvastatin and genotypes in Hyper-responders and Hypo-responders (Table **4**).

All patients were on hypolipidemic diet before entering the study; therefore the lipid profile changes after simvastatin treatment were not influenced by diet. Furthermore, the BMI was constant during the whole study.

DISCUSSION

We evaluated the impact of *CYP3A5 gene* polymorphism on the effectiveness of simvastatin treatment in subjects with primary hypercholesterolemia. The genotype frequencies for the *CYP3A5 gene* polymorphism in our study cohort were similar to those reported in other Caucasian [8,12,13] and dyslipidemic populations [14]. Concerning the impact of *CYP gene* polymorphism on effectiveness of simvastatin treatment, the results are still not clear [15, 16]. Li *et al.* [15] compared the lipid lowering efficacy of simvastatin (202 patients) and atorvastatin (177 patients) according to *CYP3AP1*3* (non-expressors) variant allele in Chinese hyperlipidemic patients. They reported that in women treated

Table 3. ***CYP3A5 genotype*** **and simvastatin dose.**

			Simvastatin Dose		
			10 mg	**20 mg**	**40 mg**
CYP3A5	*HT* (Expressors)	N	9	10	9
		%	22%	11%	20%
CYP3A5	*HM* (Non-expressors)	N	32	82	37
		%	78%	89%	80%

p = 0.186 between *HT* and *HM* genotypes according to simvastatin dose. % = frequency of CYP3A5 genotypes within simvastatin dose. WT genotype was excluded from the analysis due to the small number (n = 1).

Table 4. Low density lipoprotein cholesterol response by ***CYP3A5*** **genotypes.**

	CYP3A5		***CYP3A5***	
	HM **(Non-expressors)**		***HT*** **(Expressors)**	
	N	%	N	%
Hyper-responders	31	51%	6	43%
Hypo-responders	30	49%	8	57%

p = 0.591, between frequency of *CYP3A5* genotypes in Hyper-responders and Hypo-responders. Hyper-responders: Difference in LDL-C (Low Density Lipoprotein Cholesterol) values (%) after treatment: from - 80.3 to - 51.5% (minimum value and 25th percentile, respectively).
Hypo-responders: Difference in LDL-C values (%) after treatment: from - 32.1 to 93.5% (75th percentile and maximum value, respectively).

with simvastatin, the % reduction of LDL-C level was greater in the *CYP3AP1*3/*3* compared with *CYP3AP1*1* genotypes. Kivisto *et al.* [16] also evaluated whether the expression of *CYP3A5* is associated with an impaired lipid-lowering response to statins in 69 Caucasian patients. They found that lovastatin, simvastatin and atorvastatin were significantly less effective in *CYP3A5* expressors than in non-expressors. The mean serum TC concentration at 1 year was 23% higher and the mean serum LDL-C concentration was 24% higher in subjects possessing the *CYP3A5* expressors than in non-expressors. The mean % reduction in serum TC from baseline was significantly smaller in *CYP3A5* expressors than in non-expressors.

Similarly to Kivisto *et al.* [16], we also found a trend towards lower % reduction in LDL-C of the *HT* (expressors) compared with *HM* (non-expressors). The *HT* individuals had a -35.2% decrease with a dose of 20 mg simvastatin while the *HM* individuals had slightly higher decrease (-37.5%) in spite the lower dose of simvastatin (10 mg).

In contrast, Shin *et al.* [17] evaluated the efficacy of atorvastatin and found that the *CYP3A5* genotype has minimal effects on the pharmacokinetic parameters of atorvastatin and its interaction with clarithromycin. However, Willrich *et al.* [13] reported that *CYP3A5*3A* allele was associated with reduced cholesterol-lowering response to atorvastatin in non-African individuals. Moreover, Kim *et al.* [18] investigated the effect of *CYP3A5*3* genotype on the pharmacokinetics of simvastatin in 22 men; they found that the area under the plasma concentration-time curve for simvastatin in the *CYP3A5*1/*1* carriers was significantly lower than in *CYP3A5*3/*3*. Wang *et al.* [19] studied the *CYP3A4* polymorphism (*rs35599367, C>T*) in 235 patients taking atorvastatin, simvastatin or lovastatin and found that *T* carriers required significantly lower statin doses than *non-T* carriers.

Noteworthy to mention that, in our study, we found that the % difference in LDL-C within the same genotype according to dose of simvastatin was higher than expected. Many studies [11] have documented that doubling the dose of statin further decreases LDL-C levels by 6-8%. In our study we found a further 14% difference in LDL-C after doubling simvastatin dose from 20 to 40 mg.

A limitation of this study is the relatively small number of subjects. On the other hand, our study has some strengths. We performed a second PCR to avoid a misclassification of heterozygotes as homozygotes. In addition, we studied a reasonably homogenous group of individuals according to geographical and ethnic origin (the majority of patients were from Athens). Ethnic differences in gene distribution have been reported [20,21].

Generally, this type of studies has numerous limitations. Among them are gender differences (in our study the influence of gender on simvastatin treatment was not evaluated due to the relatively small study cohort), the role of absorption of cholesterol, diet, BMI, adherence to treatment, subgrouping of a small population to different doses of simvastatin, interference with other drugs or food supplements and different pathogenesis of dyslipidaemia (including combinations of primary and secondary causes). However, many of these factors should be balanced out within the groups we studied. Assessing some of these factors would also require costly and complex techniques. It follows that some of these measurements are likely to be missing from several studies.

However, polymorphisms may affect the pharmacokinetic and pharmacodynamic profiles of statins, thus creating

a potential application in the personalized prescribing of statin therapy. Genotype evaluation may help to identify individuals with better response to treatment. It follows that the prospective identification of subjects who have a weak or enhanced response to statins may improve the risk-to-benefit ratio of statin therapy. It is still cheaper to try different statins and to increase the dose of a statin or proceed to combination therapy than to perform expensive genotyping. However, we need to understand these interrelationships because they may help to design more effective drugs. Furthermore, if we detect substantial ethnic variations then we could have different statins as first choice. Noteworthy to mention is that there is a lot more to statin action than just *CYP3A5*. For example, upregulation of cholesterol absorption and cellular transport after giving a statin involved genes such as ABCA1, ABCB1, ABCG2, SLCO1B1 [22], new players such as PCSK-9 response to a statin [23] and other factors.

CONCLUSIONS

In the present study, simvastatin was less effective in *CYP3A5* expressors compared with non-expressors. The individuals with *HM* displayed a trend towards higher LDL-C decrease with the half dose of simvastatin compared with *HT* individuals. Within the same genotype, differences between doses were also found. Further large studies are required to clarify the contribution genes to statin-induced lipid lowering effects.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

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Declared none.

LIST OF ABBREVIATIONS

LDL-C = Low density lipoprotein cholesterol

HDL-C = High density lipoprotein cholesterol

TG = Triglyceride

TC = Total cholesterol

PCR = Polymerase chain reaction

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