

Possible gene-gender interaction between the SLC01B1 polymorphism and statin treatment efficacy

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Abstract

OBJECTIVES: A significant inter-individual variability in statin treatment efficacy is likely to have a strong genetic background. A candidate gene with the potential to influence statin treatment efficacy is *SLCO1B1*. This gene codes for the solute carrier organic anion transporter, which has been shown to regulate the hepatic uptake of statins and some other drugs.

MATERIALS AND METHODS: The *SLCO1B1* rs4149056 (T>C) polymorphism was successfully analysed in a group of 253 patients with dyslipidemia (treated with simvastatin or atorvastatin, 10 or 20 mg per day) and 470 healthy normolipidemic controls. The polymorphism was analysed using nested PCR-RFLP. Lipid levels (total, LDL and HDL cholesterol; triglycerides) were analysed before and after 10–13 weeks of treatment.

RESULTS: After treatment, as expected, there was a significant decrease both in the total cholesterol ($7.60 \pm 1.36 \rightarrow 5.37 \pm 1.12$ mmol/L, $p < 0.001$) and LDL cholesterol ($5.04 \pm 1.34 \rightarrow 3.17 \pm 0.99$ mmol/L, $p < 0.001$) levels. The distribution of the individual genotypes in the patients (TT=61.7%, CT=31.6%, CC=6.7%) was similar ($p=0.35$) to that of the normolipidemic controls (TT=64.4%, CT=31.3%, CC=4.3%). Homozygous CC males exhibited the lowest ($\Delta -21.2 \pm 7.2\%$) decrease of total cholesterol in contrast to the females, in whom the same genotype was associated with the highest ($\Delta -33.5 \pm 7.6\%$) decrease ($p=0.04$ for gene-gender interaction).

CONCLUSIONS: The results of our pilot study suggest possible gender-dependent effects of the rs4149056 variant within the *SLCO1B1* gene on statin treatment efficacy.

Abbreviations:

DNA - deoxyribonucleic acid
HDL - high density lipoprotein
LDL - low density lipoprotein
PCR - polymerase chain reaction
SLCO1B1 - solute carrier organic anion transporter

INTRODUCTION

Dyslipidemia (together with arterial hypertension) is the most common risk factor of cardiovascular disease. Treatment of dyslipidemia significantly reduces the risk of vascular events and even mortality.

Statins are the most widely used drugs for treatment of dyslipidemia. By inhibiting the key enzyme of cholesterol synthesis, 3-hydroxy-3-methylglutaryl coenzyme A reductase, these compounds effectively and safely reduce both the levels of atherogenic lipoproteins in the plasma and, most importantly, global cardiovascular risk. However, statin efficacy significantly differs even between patients treated with the same dose of the same statin (Sever *et al.* 2003), and it is likely that these differences are genetically determined.

One possible candidate that contributes to variation in the efficacy of statins is the common variant within the solute carrier organic anion transporter *SLCO1B1* (SEARCH Collaborative Group, 2008).

The *SLCO1B1* (alternative former name *SLC21A6*, OMIM acc No. 604843)-encoding organic anionic transport polypeptide OATP1B1 mediates absorption of different drugs (including statins) by hepatocytes, and its polymorphism (rs4149056) influences the clearance of statins from circulation (Niemi *et al.* 2006, Pasanen *et al.* 2006). OATP1B1 is a 691-amino acid transmembrane receptor, a member of the organic anion transporter family, and is expressed exclusively in the liver. The rs4149056 polymorphism occurs as a four allelic polymorphism with two common alleles (c.521T and c.521C, Val174 and Ala174) and two very rare alleles (c.521A, Glu174 and c.521G, Gly174; http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4149056). Carriers of the C allele have higher plasmatic concentrations of statins in comparison with common TT homozygotes (Niemi *et al.* 2006, Pasanen *et al.* 2006).

We have studied the impact of the *SLCO1B1* gene variant on the magnitude of the decrease in plasma lipids after treatment with commonly used statins.

PATIENTS AND METHODS

Patient selection

Patients with primary dyslipidemia that had been prescribed statin treatment were retrospectively selected from the databases of Lipid Clinics of the 3rd Department of Internal Medicine of the 1st Faculty of Medicine, Charles University and the Institute for Clinical and Experimental Medicine, Prague, Czech Republic. Two hundred fifty-seven patients were included. The average age was 61.4±13.1 years (91 males and 166 females); 21.8% of the patients were diabetics, and 49.8% had hypertension. All patients received standardised lifestyle advice upon their first visit to the clinics and were instructed to maintain a standardised low-cholesterol diet provided by an experienced dietician. Table 1 shows the baseline characteristics of the study group. We com-

pared the pre-treatment lipid levels with the first values obtained after initiation of statin treatment, usually after 12 weeks (range 10 to 13 weeks) of therapy. Patients taking simvastatin (53.7%) or atorvastatin (46.3%) in doses of 10 (~90% of individuals) or 20 mg/day were enrolled in the study. Exclusion criteria comprised i/ weight loss of more than 5% between visits, suggesting a substantial impact of lifestyle changes ii/ diagnosis of familiar hypercholesterolemia based on clinical and laboratory data, and iii/ no change in total and/or LDL-cholesterol, suggesting non-compliance of the patients.

Normolipidemic controls

As a control group, a subset of 470 individuals (188 males and 282 females) selected from the Czech post-MONICA study (2,559 individuals, 1,191 males, average age 49 years) was used (Thunsdall-Pedoe *et al.* 2003). The selection criteria were i/ no history of cardiovascular disease, ii/ no lipid-lowering treatment and iii/ the plasma lipid values were below 5.0 mmol/L for total cholesterol, below 2.0 mmol/L for plasma TG and over 0.75 mmol/L (for males) or 0.8 mmol/L (for females) for HDL cholesterol (Table 1).

All participants of the study were of Caucasian ethnicity. Written informed consent was obtained from all study participants, and the local ethics committee approved the design of the study according to the Declaration of Helsinki of 1975.

Genotype analysis

DNA was isolated using the standard salting out method (Miller *et al.* 1988) from 3 ml of whole EDTA blood. The rs4149056 variant was genotyped using nested polymerase chain reaction (PCR) and restriction analysis. A DYAD (MJ Research, Waltham, MA) thermal

Tab. 1. Basic characteristics of the analyzed groups. *p*-value is given for the differences before and after treatment in the patients group.

Character	Patients		Controls	<i>p</i> -value
Number	257		470	
Age	61.4±13.1		42.5±10.2	
% of males	35.4		40.0	
	Before	After		
Total cholesterol	7.60±1.36	5.37±1.12	4.35±0.42	0.001
LDL cholesterol	5.04±1.34	3.14±0.99	n.a.	0.005
HDL cholesterol	1.56±0.46	1.50±0.39	1.38±0.35	n.s.
Triglycerides	2.07±1.06	1.61±0.87	1.03±0.37	0.05
Smoking prevalence	28.1%		24.9%	
Diabetes prevalence	21.8%		1.5%	
Hypertension	49.8%		16.8%	

cycler was used to perform the PCR reaction at a total volume of 25 μ l. In the first step, the DNA was amplified using the primers 5' TTG TTG AGG AAT TTT GCA CCT A and 5' TTC GCT AGT GTG CAA AGA GGG under the following conditions: initial denaturation of 96 °C for 3 min, followed by 35 cycles of 95 °C for 15 sec, 55 °C for 30 sec and 72 °C for 30 sec. The last amplification step was extended for 3 min at 72 °C. For the second PCR, products from the first reaction were diluted with sterile water in a ratio of 1:19 and 1 μ l was amplified using the primers 5' ATA TTC ACC AGG GAT ATT GGC CTG TTG G and 5' AAA CCC TGA ACA CTA ATA TAG AGT TCC AA under the following conditions: initial denaturation of 96 °C for 3 min, followed by 35 cycles of 95 °C for 15 sec, 56.5 °C for 30 sec and 72 °C for 30 sec. The last amplification step was extended for 3 min at 72 °C. A PCR product of 10 μ l was digested in a total volume of 20 μ l with 5 U of the restriction enzyme KpnI at 37 °C overnight in KpnI buffer. The restriction fragments were separated on a 10% polyacrylamide gel.

Analysis of plasma lipids

The lipoprotein parameters in fasting plasma samples were assessed using autoanalysers and conventional enzymatic methods with reagents from Boehringer Mannheim Diagnostics and Hoffmann-La Roche in CDC Atlanta accredited local laboratories. The LDL

cholesterol levels were calculated using the Friedewald formula ($LDLC = TC - HDLC - (TG/2.2)$).

Statistical analysis

The Hardy-Weinberg test (<http://www.tufts.edu/~mcourt01/Documents/Court%20lab%20-%20HW%20calculator.xls>) was applied to confirm the independent segregation of alleles. A Chi-square test (http://www.physics.csbsju.edu/cgi-bin/stats/contingency_form.sh?nrow=2&ncolumn=3), an ANOVA and an ANCOVA for adjustments were used for statistical analysis. Adjustment for age, type and dose of statin was performed. All tests were two tailed, and a significance level of 0.05 was used. Differences in lipid decreases between subjects with different genotypes were expressed and analysed as a percent of the decrease.

RESULTS

As expected, we have observed significant changes in the lipid spectrum in patients treated with statins. Namely, the total cholesterol levels decreased by 29.5% ($7.60 \pm 1.36 \rightarrow 5.37 \pm 1.12$ mmol/L, $p < 0.001$), the LDL cholesterol levels decreased by 37.2% ($5.04 \pm 1.34 \rightarrow 3.17 \pm 0.99$ mmol/L, $p < 0.001$), and the TG levels decreased by 23.5% ($2.07 \pm 1.06 \rightarrow 1.61 \pm 0.87$ mmol/L, $p < 0.05$). The plasma levels of HDL cholesterol remained unchanged (decreased by 4%) (Table 1).

Genotypes were successfully analysed in 98.5% of the patients and in 99.3% of the controls. Genotype distributions were in HW equilibrium ($p = 0.13$ for patients and $p = 0.67$ for controls), and the minor allele frequencies, which were close to the distributions found in another Caucasian population (22.5% in patients and 19.9% in normolipidemic controls), are among the highest in the world.

The distribution of the genotypes did not differ significantly between controls and patients (see Table 2 for more details).

Male CC homozygotes exhibited the lowest ($\Delta -21.2 \pm 7.2\%$) decrease in total cholesterol levels in contrast to females, in whom the same genotype was associated with the highest ($\Delta -33.5 \pm 7.6\%$) decrease ($p = 0.04$ for gene-gender interaction, Table 3). Changes in other plasma lipids were not associated with the *SLCO1B1* rs4149056 variant.

DISCUSSION

Statins are the most commonly prescribed lipid-lowering drugs today, and their prescription rates continue to grow. They are generally very effective (decreases of plasma LDL-cholesterol up to 60%), but the efficiency differs significantly between individuals.

Treatment response to statins has a significant genetic component. Surprisingly, despite dozens of publications describing candidate genes for statin treatment efficacy, only the three allelic apolipoprotein E

Tab. 2. Genotype distributions within the analyzed groups.

rs4149056	N	TT		TC		CC		p-value
		N	%	N	%	N	%	
Patients (total)	253	156	61.7	80	31.6	17	6.7	0.35
Controls	467	301	64.4	146	31.3	20	4.3	

Tab. 3. Changes of the lipid parameters according the individual genotypes in percentages of the decrease.

Males			
rs4149056	TT	TC	CC
T-C	30.3 \pm 10.8	28.7 \pm 10.5	21.2 \pm 7.3*
LDL-C	33.9 \pm 16.4	29.8 \pm 25.6	32.0 \pm 6.0
HDL-C	-3.2 \pm 25.5	-5.0 \pm 23.2	-13.1 \pm 19.0
TG	20.5 \pm 31.7	7.6 \pm 48.6	0.9 \pm 37.4
Females			
rs4149056	TT	TC	CC
T-C	28.4 \pm 9.3	29.5 \pm 9.8	33.5 \pm 7.6*
LDL-C	37.6 \pm 12.0	37.4 \pm 15.9	39.8 \pm 7.9
HDL-C	3.4 \pm 23.1	1.3 \pm 18.3	2.6 \pm 23.3
TG	16.7 \pm 28.5	24.1 \pm 27.7	18.5 \pm 27.3

* $p < 0.05$ for differences in decrease between males and females

polymorphisms (E2, E3, E4) seem to have a consistent effect (Hubacek and Vrablik, 2011).

Additionally, genome-wide association studies that have focused on the detection of variants associated with the treatment efficacy of statins have not been very successful (Thompson *et al.* 2009, Barber *et al.* 2010, Daly 2010). Thus, positive associations were mostly detected within the genes selected on the basis of the candidate gene approach (Hubacek *et al.* 2009, Mangravite *et al.* 2010, Hubacek *et al.* 2012, Akao *et al.* 2012).

A promising candidate for the prediction of statin treatment efficacy seems to be the *SLCO1B1* gene and its rs4149056 (T>C) variant, which was originally detected as a genetic determinant of statin induced myopathy. The frequency of the low-activity 521C allele varied markedly between different ethnicities (Passanen *et al.* 2008), with the highest frequency in Europeans (~17%) and the lowest (~3% only) among Africans. Interestingly, all four nucleotides can occur at the same position, and each different codon is translated into a different amino acid. Such a situation is not extremely rare and, intriguingly occurs in another gene playing a role in statin treatment efficacy (apolipoprotein E gene) (Minnich *et al.* 1995, Hubacek *et al.* 2000, Hubacek *et al.* 2005).

Patients treated with fluvastatin and rosuvastatin were not included in the study, as the pharmacokinetics of these statins seem not to be markedly influenced by OATP1B1, which primarily transports atorvastatin and simvastatin (Niemi *et al.* 2006, Passanen *et al.* 2007).

Our results, together with some other published studies (Zhang *et al.* 2007, Generaux *et al.* 2011, Sortica *et al.* 2012), support the notion that future examinations of polymorphism(s) of the *SLCO1B1* gene may be a useful clinical aid in decision making regarding the statin dose. Our pilot study further suggests that there may be a gender-specific effect of the rs4149056 *SLCO1B1* variant in plasma lipid changes. The results need to be verified in a larger group of patients, especially because of the low frequency of the potentially unfavourable genotype.

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