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Influence of *SLCO1B1* polymorphisms on atorvastatin efficacy and safety in Macedonian subjects

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Atorvastatin, as 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor, is a widely prescribed medication for the treatment of dyslipidemia. However, despite its clinical efficacy in reducing major cardiovascular events, a wide inter-individual variability in its response exists. Several studies in this area point to the effect of polymorphisms in the solute carrier organic anion transporter 1B1 (*SLCO1B1*) gene encoding the multiple organic anion-transporting polypeptide 1B1 (OATP1B1) involved in hepatic uptake of atorvastatin. Hence, the aim of this study was to analyze the association between the *SLCO1B1* c.388A>G, c.521T>C, c.571T>C, c.597C>T, c.1086C>T, c.1463G>C and c.*439T>G polymorphisms and lipid-lowering effect and safety of atorvastatin. A hundred and fifty six patients with hyperlipidemia IIa and IIb, all of Macedonian origin, were included in the study receiving atorvastatin 20 – 80 mg/day for 3 months. *SLCO1B1* single nucleotide polymorphisms (SNPs) were genotyped using the TaqMan allelic discrimination assay. As parameters of atorvastatin response, total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), apolipoprotein A (ApoAI), apolipoprotein B (ApoB), lipoprotein(a) (Lp(a)), creatine phosphokinase (CPK), alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were measured, using standard laboratory methods, at baseline and after 3 months of treatment. No statistically significant association between the different *SLCO1B1* SNPs and atorvastatin response was observed. However, the carriers of c.521CC manifested a lower decrease in plasma levels of TG, TC, LDL-C and Lp(a), with percentage difference being 16 %, 7 %, 29 % and 149 %, respectively, compared to the carriers of c.521TT variant. Lower increase in HDL-C (271 %) and ApoAI (293 %) and higher increase in CPK (69 %) in c.521CC carriers were also observed, confirming the lower OATP1B1 activity in carriers of the variant c.521 C allele. Similar results were obtained when a comparison between the percentage of biochemical parameter change was made between **15/*16/*17* heterozygotes and **15/*16/*17* non-carriers. The lack of a statistically significant association between the *SLCO1B1* polymorphism and atorvastatin response can be explained dominantly by the low number of individuals homozygous for the rare c.521C variant allele. Despite this limitation, the study offers valuable information on the influence of the genetic determinant *SLCO1B1* on atorvastatin response in the Macedonian population.

1. Introduction

Organic anion transporter polypeptide 1B1 (OATP1B1), a member of the organic OATPs, transports a number of endogenous and exogenous substances (Pasanen et al. 2007; He et al. 2011; Lopez-Lopez et al. 2011; Niemi et al. 2011; Liu et al. 2013). Being an uptake transporter localized exclusively on the sinusoidal membrane of hepatocytes, its primary role is to remove substrates from the blood into the liver. A number of structurally diverse and clinically important drugs have been identified as substrates of OATP1B1 and its inhibition was considered as an important mechanism in drug interactions (Neuvonen et al. 2006). However, the functional impact of genetic variations on the OATP1B1 encoding gene [solute carrier organic anion transporter 1B1 (*SLCO1B1*) gene] has not been sufficiently studied.

At least 17 single nucleotide polymorphisms (SNPs) have been found within the *SLCO1B1* gene, but three of them only occurred at a frequency of > 0.02 in Caucasian individuals: c.388A>G (rs2306283; Asn130Asp), c.463C>A (rs11045819, Prol155Thr) and c.521T>C (rs4149056; 521T>C, Val174Ala) (Pasanen et al. 2008). Of these, only the non-synonymous c.388A>G and c.521T>C, alone or in combination with each other in three haplotypes, GT (**1b*), AC (**5)* and GC (**15)*, have been associated with an altered transport function and it is these two SNPs/their haplotypes that have been analyzed mostly (Pasanen et al. 2006; 2008; Ho et al. 2007; Rajput et al. 2014; Jiang et al. 2016). The **15* haplotype has been consistently associated with a decreased transport activity, while controversial results have been reported for the **1b* haplotype, suggesting that the *SCLO1B1* genetic risk is drug-specific.

All statins (3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors) are OATP1B1 substrates and their uptake by the transporter is not only the first step of their hepatic elimination, but a delivery system to the liver as a target organ as well. The effects of the most studied *SLCO1B1* SNPs/haplotypes on the transport of certain statins were previously analysed, using both pharmacokinetic (PK) and/or pharmacodynamic (PD) approaches (Pasanen et al. 2006, 2007, 2008; Rodriguez et al. 2011; Superko et al. 2012; Ulvestad et al. 2013; Giannnakopoulou et al. 2014; Prado et al. 2015; Daka et al. 2015). Most of the studies are focused only on the pharmacokinetics of statins, supporting the altered trans-

port function and suggesting that a single copy of either variant haplotype is sufficient to increase the statin plasma concentration. However, the impact of *SLCO1B1* genotypes on lipid-lowering response and induction of adverse reactions remains uncertain.

In a study of Rodrigues et al. (2011), the carriers of c.388GG showed a significantly higher decrease of low-density lipoprotein cholesterol (LDL-C) relative to 388AA+388AG carriers. Japanese hypercholesterolemic patients treated with pravastatin for eight weeks had poor LDL-C reduction as heterozygous carriers of *SLCO1B1*15* allele in comparison with the non-carriers (Takane et al. 2006). In a study of Lee et al. (2010), the bioavailability of atorvastatin was higher in **15/*15* subjects and, consequently, the percentage decrease of total cholesterol (TC) and LDL-C after a four-week treatment was lower in **15/*15* than in **15* heterozygotes and non-carriers of **15* variant. However, the same was not observed by Giannakopoulou et al. (2014), who analyzed the association of *SLCO1B1* 521T>C, 388A>G and 411G>A polymorphisms with response to atorvastatin in 201 adult Greeks with primary hypercholesterolemia.

Based on the assumption that variations in hepatic uptake have greater effect on plasma than on hepatic exposure, a much stronger link between the *SLCO1B1* genotype and development of myopathy, as the most consistent adverse effect of statins, is suggested (Niemi et al. 2011). However, it has been observed mostly among the patients who received simvastatin, in whom the *SLCO1B1* c.521T>C SNP was significantly associated with the gene-dose effect for each adverse effect, including myalgia, with or without elevated creatine phosphokinase (CPK) (SEARCH Collaborative Group 2008). Adverse effects associated with *SLCO1B1* SNPs for other statins have also been reported (Pucceti et al. 2010; Donelly et al. 2011; Marciante et al. 2011), indicating the necessity of further studies in different (ethnic) populations for a wide range of statins in order to firmly conclude the potential association of *SLCO1B1* polymorphisms with a statin response.

Atorvastatin is widely used for the treatment of hypercholesterolemia and hypertriglyceridemia. Prior to its release as a generic in 2011, it was considered the top drug by sales for most of the previous decade and it is still a best-selling prescription drug. This status was obtained due to the well documented benefits to cardiovascular diseases in many groups at a moderate and/or high cardiovascular risk and favorable safety profile. However, as with all drugs, atorvastatin is not completely devoid of potential adverse effects among which the muscle adverse events and hepatic dysfunction are the most reported problems (Golomb and Evans 2008). In addition, a wide inter-individual variability in clinical response exists and among the factors contributing to the variability, genetic variants that affect the hepatic uptake/clearance are depicted. Having this in mind, the aim of the study was to evaluate the effects of variant *SLCO1B1* genotypes and haplotypes on the efficacy, liver function and occurrence of myopathy (and rhabdomyolysis) in patients of Macedonian origin with hyperlipidemia IIa and IIb. Together with the effects of the relevant c.388A>G and c.521T>C SNPs, the effects of 5 other SNPs [c.571T>C (Leu191Leu, rs4149057), c.597C>T (Phe199Phe, rs2291075), c.1086C>T (Tyr362Tyr, rs57040246), c.1463G>C (Gly188Ala, rs59502379) and c.*439T>G (rs4149087)] were evaluated. Although variant alleles of these SNPs in many haplotypes exist, to our knowledge, there are no data for the effects of these SNPs on PK/PD of statins, including atorvastatin.

2. Investigations and results

2.1. Genotype and haplotype analyses

The data for genotype distribution, allelic frequency of *SLCO1B1* SNPs and haplotype analysis in patients with hyperlipidemia type IIa and IIb were published previously (Grapci et al. 2015), when the genetic variation of *SLCO1B1* in healthy subjects (n = 110) and patients (n = 156) of Macedonian origin was analyzed. All SNPs in the patients occurred with allele frequency higher than 12 % (except c.1463G>C and c.1086C>T), with the frequency of c.521T>C SNP being the lowest (14 %), c.571T>C variant allele the highest (65 %) and all others above 40 %. No significant difference ($p > 0.05$) in genotype distribution and allelic frequency of all *SLCO1B1* SNPs was observed when comparing the data for female (n = 73) and male (n = 83) subjects. The observed frequency distributions in the patient group did not show significant deviations from the Hardy-Weinberg equilibrium (HWE) ($p > 0.05$; 388A>G, $p = 0.38$; 521C>T, $p = 0.55$; 571T>C, $p = 0.99$ c.597C>T, $p = 0.19$; *439T>G, $p = 0.59$). According to the pair-wise linkage disequilibrium (LD) analysis, the most strongly correlated ($r2 \geq 0.33$) SNP pair was c.597C>T/c.388A>G ($r2 = 0.634$, $D' = 0.802$). The association of the most common SNP pair, c.388A>G/c.521T>C, was relatively weaker ($r2 = 0.135$ and $D' = 0.736$) compared to the other SNP pairs. The c.521T>C SNP showed the strongest correlation with the c.571T>C SNP ($r2 = 0.228$ and $D' = 0.863$), followed by a correlation with the c.597C>T SNP ($r2 = 0.222$, $D' = 0.950$) and c.*439T>G SNP ($r2 = 0.189$, $D' = 1.000$). The haplotype analysis of the patient group revealed 9 different haplotypes, with the most common haplotype (**1JI*1KI*1L*) having a frequency of 40 %. Both variant alleles G and C of the functionally most distinguished SNPs, c.388A>G and c.521T>C, respectively, were present in one haplotype, **15/*16/*17*, with a frequency of approx. 9 % (Grapci et al. 2015).

2.2. Effects of SLCO1B1 SNPs on atorvastatin response

2.2.1. Clinical and laboratory data

The three month-treatment with atorvastatin ($p < 0.05$) decreased significantly the mean plasma levels of triglycerides (TG), TC, LDL-C and apolipoproten B (ApoB) and slightly increased levels of high-density lipoprotein cholesterol (HDL-C) and apolipoprotein AI (ApoAI) within the referent range (Table 1). Specifically, no significant change in the mean level of lipoprotein (a) [Lp(a)] was observed compared to the basal one and this value remained out of the referent range (up to 30 mg/dL) during the whole study. Additionally, no significant increase in the mean values of alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), CPK, myoglobin, sodium and potassium levels as well as in the levels of degradation products was observed, being consistent with the referent values. No reports on intolerance or adverse effects related to atorvastatin therapy were reported by the patients during the study.

Table: Biochemical profile of hypercholesterolemic individuals in response to atorvastatin

Laboratory parameters	Basal values	Change (%)	*p*
TG (mmol/L)	2.32 ± 1.13	-14.41 ± 27.92	< 0.001
TC (mmol/L)	6.81 ± 0.69	-16.37 ± 9.22	< 0.001
LDL-C (mmol/L)	3.76 ± 1.02	-26.39 ± 15.53	< 0.001
HDL-C (mmol/L)	1.51 ± 0.54	2.65 ± 14.60	0.11
ApoAI (g/L)	1.51 ± 0.36	2.91 ± 17.59	0.11
ApoB (g/L)	1.24 ± 0.76	-11.61 ± 16.43	0.04
Lp(a) (g/L)	58.18 ± 49.25	-8.29 ± 19.64	0.23
ALT(U/L)	24.11 ± 10.34	3.82 ± 29.05	0.66
AST(U/L)	21.98 ± 6.77	4.49 ± 37.97	0.55
CPK (U/L)	116.97 ± 64.27	5.24 ± 23.14	0.49
LDH (U/L)	302.74 ± 90.11	3.08 ± 7.42	0.31
Myoglobin (ng/mL)	34.72 ± 13.94	6.22 ± 16.31	0.39
Urea (mmol/L)	5.35 ± 1.92	6.73 ± 2.06	0.32
Serum creatinine (mmol/L)	75.26 ± 23.25	6.88 ± 11.46	0.25
Uric acid (µmol/L)	331.96 ± 80.50	3.20 ± 13.06	0.49
Potassium (mmol/L)	4.81 ± 0.82	10.60 ± 14.79	0.10
Sodium (mmol/L)	138.62 ± 13.01	1.01 ± 1.65	0.45

TG: triglycerides, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, ApoAI: apolipoprotein AI, ApoB: apolipoprotein B, Lp(a): lipoprotein (a), ALT: alanine aminotransferase, AST: aspartate aminotransferase, CPK: creatine phosphokinase, LDH: lactate dehydrogenase.

When the laboratory data were grouped according to the received dose, age, gender, BMI, cigarette smoking, alcohol consumption and physical activity, no significant differences in the mean percentages of all parameter changes were observed. The same was observed when the mean percentages of laboratory parameter changes were compared between the patients with and without diabetes, hypertension, obesity, and menopause (data not shown).

2.2.2. *Effects of SLCO1B1 genotypes and haplotypes*

The association of *SLCO1B1* variants with atorvastatin lipid lowering response is given in Table 2. The impact of *SLCO1B1* polymorphisms on atorvastatin safety is presented by the mean percentage of ALT, AST and CPK changes, having in mind the biochemical profile of the patients, and specificity of CPK for muscle damage and of transaminases for chronic muscle necrosis and liver disorder (Keltz et al. 2014). As the results point out (Table 2), no significant differences between the different genotypes of all *SLCO1B1* SNPs and atorvastatin response were observed.

When the patients were stratified according to the most relevant *SLCO1B1* c.521T>C genotypes, no significant differences in the mean percentage of the biochemical parameter changes were observed after a 3 months treatment with atorvastatin (Table 2). However, the homozygotes for c.521C allele (n = 4) presented a slightly lower mean percentage of TG, TC and LDL-C reduction than carriers of c.521T allele (n = 116), with approx. 16 %, 7 % and 29 % (Fig. a) percentage difference, respectively. The association of c.521T>C SNP with atorvastatin response was more notable in respect to Lp(a). Namely, after 3 months of treatment, a lower decrease in the mean level of this parameter was observed in carriers of c.521CC genotype compared to c.521TT, with the percentage difference being approx. 149 % (Fig. b). The effect of c.521C allele, however, was emphasized mostly for HDL-C and ApoAI. At the end of the study period, HDL-C increase was observed in carriers of the c.521T allele only, while in carriers of the other c.521T>C genotypes, a decrease in this parameter was observed, being higher in carriers of c.521CC allele (approx. 271 % percentage variance) (Fig. c). A similar tendency for ApoAI was observed. While a mean percentage (-2.5 ± 15.3 %) of ApoAI decrease in carriers of c.521CC allele was observed), this parameter was increased in the carriers of the other c.521T>C genotypes, with approx. 293 % and 195 % percentage variance between c.521CC and TT and TC, respectively. All these data point to a lower activity of OATP1B1 in carriers of the variant C allele and this finding is supported by the mean percentage of CPK change. Namely, the mean percentage of CPK increase from basal values was higher in carriers of c.521CC, compared to the other c.521T>C genotypes, with the percentage difference being between approx. 54 % (compared to c.521TC carriers) and 69 % (compared to c.521TT carriers) (Fig. 1d). This tendency was not observed for ALT and AST, where the percentage difference between the different c.521T>C genotypes did not exceed 4 %.

The effects of *SLCO1B1* haplotypes on the mean percentage of laboratory parameter change are presented in Table 3. Actually, the mean percentage of laboratory data change was compared between two subgroups based on their *SLCO1B1* genotype: **15/*16/*17* non-carriers (n = 92) and **15/*16/*17* heterozygotes (n = 26). **15/*16/*17* Haplotype was chosen as a carrier of the variant c.521C allele. The haplotype analysis confirmed the above-mentioned finding for the effects of individual SNPs on the response

Table 2: Association of *SLCO1B1* SNPs with biochemical parameters in patients treated with atorvastatin

Biochemical parameters	SNP				SNP			
	c.521T>C			*p*	c.388A>G			*p*
Baseline	**TT (n=116)**	**TC (n=36)**	**CC (n=4)**		**AA (n=54)**	**AG (n=80)**	**GG (n=22)**	
TG (mmol/L)	2.30 ± 1.18	2.36 ± 1.02	2.32 ± 0.67	0.77	2.42 ± 1.44	2.25 ± 0.95	2.29 ± 0.87	0.86
TC (mmol/L)	6.83 ± 0.76	6.73 ± 0.36	6.90 ± 0.43	0.68	6.85 ± 0.80	6.75 ± 0.51	6.93 ± 0.99	0.92
LDL-C(mmol/L)	3.72 ± 1.06	3.91 ± 0.88	4.00 ± 0.94	0.56	3.83 ± 0.97	3.70 ± 1.02	3.86 ± 1.13	0.71
HDL-C (mmol/L)	1.52 ± 0.48	1.52 ± 0.57	1.25 ± 0.26	0.49	1.47 ± 0.58	1.54 ± 0.53	1.50 ± 0.50	0.66
ApoAI (g/L)	1.49 ± 0.36	1.57 ± 0.36	1.48 ± 0.25	0.44	1.48 ± 0.34	1.51 ± 0.38	1.54 ± 0.29	0.76
ApoB (g/L)	1.25 ± 0.46	1.23 ± 0.37	1.13 ± 0.32	0.60	1.21 ± 0.41	1.26 ± 0.43	1.25 ± 0.51	0.80
Lp(a) (mg/dL)	64.46 ± 45.08	62.90 ± 53.54	65.31 ± 50.06	0.42	63.80 ± 42.66	57.42 ± 49.90	69.93 ± 64.22	0.49
ALT (U/L)	24.46 ± 11.39	23.84 ± 6.02	24.25 ± 4.24	0.63	23.48 ± 9.30	24.14 ± 10.41	24.70 ± 12.85	0.92
AST (U/L)	22.18 ± 7.07	21.34 ± 6.02	21.81 ± 4.63	0.59	22.34 ± 7.03	21.06 ± 6.16	24.55 ± 7.79	0.16
CPK (U/L)	119.09 ± 69.18	89.00 ± 12.94	89.55 ± 11.94	0.11	125.76 ± 75.14	122.18 ± 62.25	118.68 ± 39.58	0.35
Change (%)								
TG (mmol/L)	-16.48 ± 29.50	-14.16 ± 25.02	-14.08 ± 6.63	0.65	-13.61 ± 31.31	-12.06 ± 27.51	-10.04 ± 21.16	0.11
TC (mmol/L)	-16.55 ± 14.19	-15.82 ± 7.73	-15.39 ± 6.52	0.96	-17.13 ± 10.83	-18.19 ± 15.24	-18.60 ± 9.21	0.52
LDL-C(mmol/L)	-26.59 ± 16.12	-26.47 ± 16.26	-19.96 ± 16.84	0.68	-23.45 ± 14.68	-22.05 ± 16.79	-24.02 ± 13.71	0.38
HDL-C (mmol/L)	1.75 ± 21.75	-1.29 ± 16.67	-3.00 ± 2.89	0.11	-2.45 ± 16.08	-2.24 ± 11.85	-3.02 ± 12.53	0.21
ApoAI (g/L)	4.75 ± 18.26	2.35 ± 18.26	-2.46 ± 15.28	0.12	2.70 ± 11.89	4.30 ± 17.77	3.07 ± 10.34	0.13
ApoB (g/L)	-11.87 ± 16.57	-10.33 ± 17.31	-12.57 ± 4.45	0.39	-7.02 ± 20.46	-8.15 ± 14.35	-7.84 ± 10.60	0.25
Lp(a) (mg/dL)	-8.01 ± 13.83	-5.35 ± 20.74	-1.32 ± 7.47	0.13	-11.98 ± 22.80	-7.45 ± 15.99	-9.65 ± 17.52	0.15
ALT (U/L)	6.82 ± 30.51	5.30 ± 23.02	6.42 ± 15.98	0.41	4.19 ± 32.75	5.72 ± 27.83	4.92 ± 24.54	0.18
AST (U/L)	3.46 ± 40.44	6.14 ± 28.21	5.52 ± 6.35	0.36	2.91 ± 44.61	3.83 ± 36.51	3.53 ± 27.83	0.21
CPK (U/L)	4.21 ± 24.37	4.92 ± 30.96	8.41 ± 18.18	0.15	2.09 ± 19.42	3.25 ± 25.01	6.04 ± 25.02	0.16

All values are presented as a mean ± SD. *p* value of differences in biochemical parameters between different genotype groups of the *SLCO1B1* c.521T>C and c.388A>G SNPs. TG: triglycerides, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, ApoAI: apolipoprotein AI, ApoB: apolipoprotein B, Lp(a): lipoprotein (a), ALT: alanine aminotransferase, AST: aspartate aminotransferase, CPK: creatine phosphokinase.

Table 2 (continue): Association of *SLCO1B1* SNPs with biochemical parameters in patients treated with atorvastatin

Biochemical parameters	SNP c.571T>C				SNP c.597C>T				SNP c.*439T>G			
Baseline	**CC (n=66)**	**CT (n=71)**	**TT (n=19)**	***p***	**CC(n=60)**	**CT (n=67)**	**TT (n=29)**	***p***	**GG (n=32)**	**GT (n=81)**	**TT (n=43)**	***p***
TG (mmol/L)	2.38 ± 1.37	2.17 ± 0.87	2.63 ± 1.05	0.16	2.50 ± 1.38	2.14 ± 0.91	2.33 ± 0.99	0.35	2.15 ± 1.00	2.31 ± 1.13	2.45 ± 1.23	0.50
TC (mmol/L)	6.82 ± 0.84	6.79 ± 0.56	6.84 ± 0.65	0.83	6.94 ± 0.95	6.73 ± 0.48	6.74 ± 0.44	0.78	6.88 ± 0.83	6.75 ± 0.52	6.88 ± 0.87	0.62
LDL-C (mmol/L)	3.92 ± 0.98	3.61 ± 0.97	3.81 ± 1.26	0.21	3.82 ± 1.01	3.73 ± 1.00	3.76 ± 1.08	0.88	3.85 ± 0.78	4.04 ± 0.98	3.92 ± 0.94	0.21
HDL-C (mmol/L)	1.29 ± 0.32	1.42 ± 0.45	1.17 ± 0.25	0.45	1.53 ± 0.62	1.51 ± 0.53	1.46 ± 0.38	0.99	1.49 ± 0.49	1.53 ± 0.55	1.48 ± 0.57	0.86
ApoAI (g/L)	1.50 ± 0.33	1.52 ± 0.39	1.47 ± 0.32	0.84	1.47 ± 0.35	1.50 ± 0.38	1.60 ± 0.31	0.29	1.50 ± 0.30	1.51 ± 0.37	1.50 ± 0.36	0.98
ApoB (g/L)	1.26 ± 0.43	1.22 ± 0.42	1.23 ± 0.50	0.63	1.20 ± 0.41	1.32 ± 0.45	1.13 ± 0.43	0.05	1.24 ± 0.50	1.25 ± 0.40	1.22 ± 0.46	0.73
Lp(a) (mg/dL)	50.84 ± 43.48	66.34 ± 50.35	52.54 ± 62.47	0.36	48.59 ± 38.74	64.51 ± 53.69	65.75 ± 57.99	0.45	64.54 ± 52.27	55.17 ± 49.18	59.03 ± 48.29	0.69
ALT (U/L)	23.24 ± 9.23	24.79 ± 12.04	24.72 ± 6.94	0.54	23.79 ± 10.81	22.94 ± 7.98	27.59 ± 13.41	0.34	25.15 ± 9.59	23.13 ± 9.70	25.19 ± 11.91	0.36
AST (U/L)	22.33 ± 7.78	21.31 ± 5.97	23.27 ± 5.77	0.32	22.08 ± 7.24	20.61 ± 5.82	25.01 ± 7.03	0.11	22.26 ± 5.77	21.53 ± 6.65	22.63 ± 7.69	0.60
CPK (U/L)	119.66 ± 73.59	110.08 ± 44.80	131.05 ± 84.52	0.83	121.96 ± 72.11	113.45 ± 62.67	114.53 ± 51.11	0.79	121.96 ± 72.11	113.45 ± 62.67	114.53 ± 51.11	0.78
Change (%)												
TG (mmol/L)	-19.69 ± 25.13	-11.85 ± 29.29	-18.89 ± 29.92	0.17	-16.14 ± 32.06	-15.74 ± 22.44	-14.96 ± 25.10	0.55	-16.29 ± 25.79	-16.03 ± 28.18	-17.42 ± 29.02	0.32
TC (mmol/L)	-16.20 ± 10.35	-18.22 ± 16.42	-17.35 ± 7.12	0.53	-17.48 ± 10.03	-17.20 ± 17.09	-14.71 ± 8.48	0.50	-16.22 ± 7.82	-15.84 ± 9.43	-20.65 ± 20.70	0.63
LDL-C (mmol/L)	-27.97 ± 15.35	-25.37 ± 16.55	-25.10 ± 10.72	0.43	-26.65 ± 14.10	-29.10 ± 15.11	-24.40 ± 19.86	0.78	-23.18 ± 20.97	-29.61 ± 14.91	-25.12 ± 13.44	0.41
HDL-C (mmol/L)	0.57 ± 14.78	-2.45 ± 12.91	-3.54 ± 14.93	0.15	0.44 ± 17.98	0.70 ± 20.48	-2.83 ± 1.18	0.21	-0.17 ± 16.43	-1.90 ± 14.09	-1.82 ± 13.59	0.19
ApoAI (g/L)	4.03 ± 20.67	4.66 ± 15.06	7.78 ± 9.67	0.38	2.03 ± 20.68	5.90 ± 13.09	1.25 ± 15.86	0.38	4.13 ± 15.73	2.33 ± 21.01	2.71 ± 12.04	0.80
ApoB (g/L)	-12.30 ± 18.84	-11.61 ± 15.12	-8.71 ± 12.02	0.70	-12.66 ± 19.23	-10.61 ± 14.35	-8.81 ± 13.46	0.28	-6.65 ± 11.07	-9.46 ± 19.43	-6.18 ± 13.57	0.20
Lp(a) (mg/dL)	-12.38 ± 24.08	-10.06 ± 15.97	-12.89 ± 9.02	0.43	-10.27 ± 21.16	-12.10 ± 18.24	-9.64 ± 14.89	0.55	-6.36 ± 12.30	-4.50 ± 22.74	-5.20 ± 12.54	0.65
ALT (U/L)	3.98 ± 30.03	0.54 ± 28.75	4.50 ± 23.56	0.10	7.02 ± 33.64	2.60 ± 25.13	2.09 ± 25.93	0.15	2.83 ± 24.88	5.80 ± 29.02	3.82 ± 31.83	0.23
AST (U/L)	2.37 ± 48.56	2.20 ± 23.47	2.70 ± 33.11	0.65	8.11 ± 43.32	4.24 ± 32.00	5.84 ± 39.23	0.30	3.10 ± 31.11	3.34 ± 32.82	4.24 ± 47.63	0.40
CPK (U/L)	9.45 ± 27.04	8.98 ± 12.98	5.46 ± 32.46	0.15	4.19 ± 20.53	3.41 ± 25.84	2.33 ± 23.48	0.24	4.19 ± 20.53	5.41 ± 25.84	2.33 ± 23.48	0.50

All values are presented as a mean ± SD. *p* value of differences in biochemical parameters between different genotype groups of the *SLCO1B1* c.571T>C, c.597C>T and c.*439T>G SNPs. TG: triglycerides, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, ApoAI: apolipoprotein AI, ApoB: apolipoprotein B, Lp(a): lipoprotein (a), ALT: alanine aminotransferase, AST: aspartate aminotransferase, CPK: creatine phosphokinase.

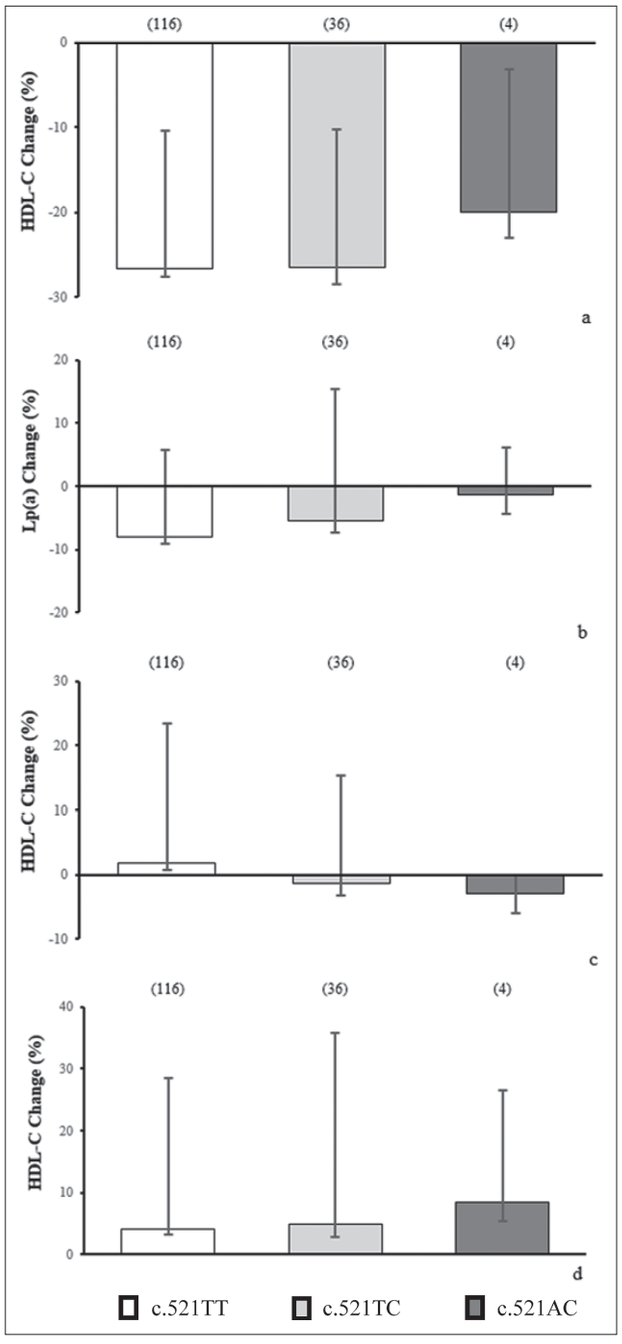

Fig. 1: The effect of the c.521T>C SNP on LDL-C (a), Lp(a) (b), HDL-C (c) and CPK (d) change (%) from baseline in response to atorvastatin. $p > 0.05$, as compared with One-Way ANOVA. Number of individuals in parenthesis.

to atorvastatin treatment. Namely, the mean percentage of TG, TC, LDL-C changes after a 3 month-treatment were slightly lower in **15/*16/*17* heterozygotes (n = 26), with a percentage difference 14 %, 22 % and 2 %, respectively, compared to **15/*16/*17* non-carriers (n = 92). A slight difference in Lp(a) between the haplotypes was also observed, with the change percentage being lower in **15/*16/*17* heterozygotes (percentage difference of approx. 13 %). However, having in mind the mean percentage of HDL-C and ApoAI change, these differences (although statistically non-significant) were more potentiated, being -0.03 ± 22.8 % and 1.9 ± 19.5 % in **15/*16/*17* heterozygotes *vs.* 0.8 ± 21.5 % and 6.6 ± 13.4 %, respectively, in patients non-carriers of **15/*16*/*17.* The same was observed when the mean percentage of CPK change between **15/*16/*17* heterozygotes and **15/*16/*17* non-carriers was compared, with the CPK increase being higher in the first group, with a percentage difference of approx. 63 % (Table 3).

3. Discussion

The limited and inconclusive data for the effect of *SLCO1B1* polymorphism on statin response was a strong motive to investigate the association of variant *SLCO1B1* genotypes and haplotypes present in the Macedonian ethnicity with atorvastatin efficacy and safety. The effects of the relevant c.388A>G and c.521T>C SNPs were evaluated, but also of other SNPs present in the study population at frequencies higher than 40 % (c.571T>C, c.597C>T and c*439T>G). They are considered as "silent" SNPs and their association with the OATP1B1 activity has not been clarified yet. The calculated genotype and allele frequencies of *SLCO1B1* SNPs in the study group were in HWE, confirming the random selection of the patients. For all *SLCO1B1* SNPs, the distribution between the male and female patients did not differ significantly and these data are partially consistent with the results of Hubacek et al. (2012), where no significant difference in the c.521 genotype distribution was observed when comparing the male and female subjects. The association between c.388A>G and c.521T>C was relatively weaker compared to the other SNP-pairs and to the previously reported LD data for this SNP pair (Rodrigues et al. 2011). Variant alleles of c.388A>G and c.521T>C were contained in the haplotype **15/*16/*17*, occurring with a frequency slightly higher but comparable to that of Caucasians as reviewed by Grapci et al. (2015). The most active c.521T>C SNP showed the strongest correlation with c.597C>T and these SNPs, together with c.439T>G SNP, existed in two main haplotypes (*5 and **15/*16/*17*), while the synonymous c.571T>C was found in the most frequent *SLCO1B1* haplotype, **IJ/*IK/*IL*, with frequencies previously reported by Grapci et al. (2015).

The data for TG, TC, LDL-C and ApoB changes from the baselines after three month treatment with atorvastatin are in accordance with the literature data that report a decrease of 12 – 31 %, 17 – 46 %, 25 – 61 % and 17 – 50 %, accordingly, after 6 - 44 week treatment (Gumprecht et al. 2011; Rodrigues et al. 2011; Adams et al. 2015). For HDL-C and ApoAI, controversial data exist, pointing to a decrease (-2.5%) (Rodrigues et al. 2011) or increase (0.5 - 16.5 %) for HDL-C (Adams et al. 2015; Gumprecht et al. 2011; Fu et al. 2013) and a decrease of 4 % (Gumprecht et al. 2011) or increase up to 5 % for ApoAI (Rodrigues et al. 2011). Specifically, no relevant changes in the levels of Lp(a) were observed and the literature data in this regard are inconclusive. While in one study the levels of Lp(a) increased for 36% (Dujovne et al. 2000), in other studies they were not significantly changed even when the basal values had been increased for more than 30 mg/dL (Djordjevic et al. 2011). However, in several other studies, the 3-12-month-treatment resulted in a significant decrease of Lp(a) values (Parsa et al. 2012; Hernandez et al. 2011) and this was also confirmed by a large meta-analysis of 31 prospective studies, including 9870 patients with hyperlipidemia IIa or IIb (Takagi and Umemoto 2011). One of the explanations for the increased Lp(a) was that the early treatment with atorvastatin increases mobilization and clearance of oxidized phospholipids in vessel walls (Tsimikas and Witztum 2008). Considering their vasoactive and pro inflammatory properties, this effect could be considered as added clinical benefit. Against this claim stands the possibility of potentiated pro inflammatory and atherogenic effects, for the same reason (increased content of oxidized phospholipids).Whether these phospholipids can serve as predictors of clinical efficacy of atorvastatin and what would be the effect of *SLCO1B1* polymorphism on this marker remain to be answered in the future.

Considering the actual study, as in most pharmacogenetic studies, no significant association between the studied *SLCO1B1* SNPs and atorvastatin response was observed. However, all data related to the association of c.521T>C SNP and lipid profile point to a lower activity of OATP1B1 in carriers of the variant c.521C allele and this is consistent with most of the research data (Akao et al. 2012; SEARCH Collaborative Group 2008) and those presented in the previous study (Daka et al. 2015), in which the healthy Macedo-

Table 3: Influence of the *SLCO1B1*15* variant on the change of laboratory data in response to atorvastatin

Biochemical parameters	*SLCO1B1*15* variant			*SLCO1B1*15* variant		
	**15/*16/*17 heterozygotes (n=26)*	**15/*16/*17 non carriers (n=92)*		**15/*16/*17 heterozygotes (n=26)*	**15/*16/*17 non carriers (n=92)*	
	Baseline		*p*	% Change		*p*
TG (mmol/L)	2.47 ± 1.36	2.33 ± 1.27	0.70	-13.63 ± 26.34	-15.59 ± 31.29	0.21
TC (mmol/L)	6.70 ± 0.33	6.80 ± 0.72	0.56	-13.05 ± 5.53	-16.18 ± 10.07	0.16
LDL-C (mmol/L)	3.95 ± 0.83	3.84 ± 1.03	0.46	-27.31 ± 17.59	-28.00 ± 15.32	0.18
HDL-C (mmol/L)	1.51 ± 0.44	1.50 ± 0.57	0.65	-0.03 ± 22.84	0.82 ± 21.50	0.14
ApoAI (g/L)	1.47 ± 0.35	1.52 ± 0.35	0.11	1.88 ± 19.53	6.59 ± 13.39	0.10
ApoB (g/L)	1.27 ± 0.41	1.25 ± 0.43	0.68	-11.85 ± 18.61	-12.89 ± 18.43	0.47
Lp(a) (mg/dL)	67.05 ± 51.12	57.40 ± 42.41	0.16	-11.46 ± 15.21	-13.01 ± 22.29	0.24
ALT (U/L)	22.62 ± 6.80	23.76 ± 10.52	0.55	6.59 ± 19.67	3.32 ± 31.36	0.23
AST (U/L)	21.81 ± 6.53	22.34 ± 7.43	0.39	5.25 ± 18.11	8.70 ± 43.28	0.19
CPK (U/L)	102.12 ± 45.45	115.37 ± 68.36	0.12	10.93 ± 19.06	5.93 ± 23.10	0.10

All values are presented as a mean ± SD. *p* value of differences in biochemical parameters between **15/*16/*17* heterozygotes and **15/*16/*17* non-carriers. TG: triglycerides, TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, ApoAI: apolipoprotein AI, ApoB: apolipoprotein B, Lp(a): lipoprotein (a), ALT: alanine aminotransferase, AST: aspartate aminotransferase, CPK: creatine phosphokinase.

nian carriers of the variant C allele had markedly higher values for maximal plasma concentration (C_{max}) and observed plasma area under curve (AUC_{0-48}) of atorvastatin (140 % and 67 %, respectively) compared to the carriers of the c.521TT genotype (Daka et al. 2015). Absence of statistical significance can be attributed to the significantly lower number of c.521CC carriers (n = 4) in comparison with the other c.521 genotypes (n = 36 for c.521CT; n = 117 for c.521TT). The same effect of c.521T>C SNP on the pharmacokinetics of atorvastatin has been confirmed in other studies as well, with an increase of AUC by 144 % (Wilke et al. 2012) and decrease of oral clearance by 45 % (Ulvestad et al. 2013) in homozygotes for the C allele, relative to the wild-type TT genotype.

The effect of variant c.521CC allele on the mean lipid parameter percentage change was more emphasized for HDL-C and ApoAI than for LDL-C or TG. These differences can be explained with the differences in the mechanisms with which atorvastatin affects the lipid parameters, their basal values and in dose-response ratios. However, not all mechanisms with which atorvastatin affects the lipid parameters are known (e.g. TG) and there is inconsistent evidence considering the basal values-atorvastatin response ratio (DALI Study Group 2001). A review of 254 studies (n = 33505 subjects), aimed to evaluate a dose-dependent atorvastatin response after a 3-12-week treatment, pointed to a linear response in respect to TG, TC and LDL-C (Adams et al. 2015). Each dose doubling resulted in TG, TC and LDL-C decrease by 4.1 % (95 %CI 2.5 - 5.0), 3.6 % (95 %CI 3.2 - 4.0) and 4.9 % (95 %CI 4.5 - 5.5), respectively. However, when all available data from a dose range of 2.5 - 80 mg/day were considered, it was concluded that lower doses increase HDL-C, unlike higher doses that diminish the atorvastatin effect (Adams et al. 2015). In this case, each dose doubling decreases the HDL-C increase by 0.9 % (95 % CI, 0.3 - 1.5). Hence, decreased OTAP1B1 activity in c.521CC and c.521CT genotypes cannot be compensated by higher doses, which can explain the profile of HDL-C (and probably ApoAI) in carriers of variant C allele. In addition, non-significant differences in the mean percentage of all parameter changes were observed when the patients were grouped according to the received dose.

The c.388A>G that encodes amino acid change at position 130 (p.Asn130Asp) is consistently related to an increased OATP1B1 activity (He et al. 2011; Prado et al. 2015). In the actual study, this effect was observed only in respect to TC and HDL-C, with a percentage decrease of these parameters being higher (without statistical significance) in c.388GG carriers (n = 22) compared to AG (n = 80) and AA carriers (n = 54). Similar to our finding are the results from several studies conducted in the European population, which report a small or lack of association between c.388A>G SNP and a lipid-lowering response to statins (Hopewell et al. 2013; Bailey et al. 2010; Giannakopoulou et al. 2014). The same was observed in a study of Fu et al. (2012) and Yang et al. (2010) in which Chinese population was involved. Opposite to these findings are the results coming from the studies conducted in South American populations. In a study of Rodrigues et al. (2011), *SLCO1B1* c.521T>C SNP had no influence on atorvastatin response in Brazilians, while in carriers of *SLCO1B1* c.388A>G variant allele significantly increased response to atorvastatin was observed, suggesting that it could be an important marker for predicting the efficacy of lipid lowering therapy. In addition, Prado et al. (2015) reported the association between *SLCO1B1* c.388A>G and HDL-C levels in response to atorvastatin in Chilean individuals.

Considering the atorvastatin safety, a recent report submitted to FDA (Hoffman et al. 2012), classified atorvastatin as a drug with intermediary risk for occurrence of myopathy (CPK ≥ 10 ULN). However, the study of Chang et al. (2013) points to the highest incidence of muscle toxicity with atorvastatin among all statins (1.08; 0.67 - 1.73), except rosuvastatin. Similar were the findings obtained from other randomized control studies and meta-analyses where atorvastatin in a dose range of 10 - 80 mg/day had been administered (Athyros et al. 2010; Newman et al. 2006). It was estimated that a dose increase from 40 to 80 mg/day results in a 4-5-fold increase in the incidence of muscle adverse effects. However, when in the actual study the patients were stratified according to the received dose, no significant difference in the mean percentage of CPK change was observed.

To our knowledge, a limited number of studies have evaluated the effects of different c.521 and c.388 genotypes on CPK levels (Brunham et al. 2012; Rodrigues et al. 2011; Santos et al. 2012). In these studies, the c.521TC+CC and c.388AG+GG genotypes were not associated with increased levels of CPK, even after a dose adjustment as co-variant. However, the differences in CPK between c.521CC and c.521TT carriers observed in the actual study, although statistically non-significant, confirm a lower OATP1B1 activity in the c.521CC genotype. This finding is supported by the previous pharmacogenetic/kinetic study (Daka et al. 2015) and by the study of Niemi (2010) in which a higher CPK increase in c.521CC carriers and lower effect of atorvastatin on the incidence of myopathy in c.521TC and c.521TT carriers were observed.

Other *SLCO1B1* SNPs investigated in the study are synonymous and their relation with the OTP1B1 function is still unknown.

However, the c.571T>C and c.597C>T SNPs have shown to be highly polymorphic and in relatively strong correlation (LD) with c.521C>T in Macedonian subjects. This finding together with the one for weak correlation between c.521T>C and c.388A>G suggest that other *SLCO1B1* haplotypes exist that could modify the lipid lowering effect. This was confirmed by the association between the *SLCO1B1*15* variants and atorvastatin response observed in the study. These data are similar to those observed by several research groups (Rodrigues et al. 2011; Lee et al. 2010) and correlate well with the previous finding for larger AUC_{0-48} (by 37 %) of atorvastatin in the group of **15/*16/*17* heterozygotes (incl. subjects with c.521CT genotype) compared to the AUC_{0-48} in the group of **15/*16/*17* non-carriers (incl. subjects with c.521TT) (Daka et al. 2015). Similarly, in the study of Pasanen et al. (2007), the AUC of atorvastatin was higher in c.521TC heterozygotes with **16* and **17* haplotypes than in the carriers of **15* haplotype, while in a study of Lee et al. (2010), a significantly higher AUC for atorvastatin in **15/*15* carriers than in carriers with **1a/*15*, **1b/*15*, **1a/*1a*, **1a/*1b* and **1b/*1b* haplotypes was detected.

In summary, this study demonstrates the effect of different *SLCO1B1* genotypes and haplotypes on the atorvastatin efficacy and safety in the population of Macedonian origin for first time. No significant association between the different genotypes of *SLCO1B1* c.388A>G, c.571T>C, c.597C>T and *439T>G SNPs and atorvastatin response was observed. The carriers of c.521CC genotype manifested a lower decrease in plasma levels of TC, LDL-C, TG and Lp(a), lower increase in HDL-C and ApoAI and higher increase in CPK compared to carriers of c.521TT variant, suggesting lower hepatic uptake and higher systemic exposure due to the decreased activity of AOTP1B1 in carriers of C-allele. The same tendency was observed when the mean percentage of lipid parameters and CPK changes were compared between **15/*16/*17*-heterozygotes and non-carriers. The actual study has certain limitations that should be consider when interpreting the results. A major limitation is not the total sample size but rather the restricted number of individuals homozygous for the rare c.521C variant allele. In addition, the subjects were recruited without previous information on their genotypes. Also, the interactions of other atorvastatin-lipid-related gene polymorphisms (e.g. CYP3A polymorphisms) were not taken into consideration. There are literature data pointing to the possibility of other transporters compensating a transporter failure and this could be relevant for atorvastatin, having in mind its substrate behavior in respect to OATP1A4 and OATP1B2 (Braeuning et al. 2014).

4. Experimental

4.1. Subjects and study design

A total of 174 patients with hyperlipidemia IIa and IIb (males 52 %, females 48 %, average age 59.9±10.8 yrs, average body mass index (BMI) 28.7±4.4), all of Macedonian ethnicity, were initially selected randomly among the outpatients from the Clinic of Cardiology at the Faculty of Medicine, University "Ss Cyril and Methodius" (UKIM) in Skopje, Republic of Macedonia (RoM). The study was conducted according to the international and local requirements for GCP (Note for Guidance on Good Clinical Practice CPMP/ICH/135/95, Rulebook for pharmacological, toxicological and clinical studies of drugs, Official Gazette of RoM, No. 73/09) and ethical principles regulated by the Declaration of Helsinki (Seoul 2008). All participants received oral and written information and gave a written informed consent before entering the study. The final clinical study protocol as well as the informed consent and other information that required pre-approval were reviewed and approved by the Independent Ethics and Review Committee according to the specifications outlined in the applicable regulations.

Individuals with known hypersensitivity to atorvastatin or some of the drug's excipients, diagnosed with thyroid, liver, kidney and heart diseases, systemic inflammatory diseases, McArdle's disease, cancer history (remission shorter than 5 years), myopathy, rhabdomyolysis or muscle pain of unknown origin, HIV patients, pregnant and nursing women or have planned pregnancy, blood-donors (4 wks. before initiation of therapy) and patients with ALT and AST levels 1.5-fold above the upper limit, increased CPK, serum creatinine above 130 mmol/L and TG higher than 4.5 mmol/L were not included in the study. Other exclusion criteria included patients with alcohol and drug abuse as well as patients under previous treatment with lipid-lowering drugs, drugs known to affect CYP3A4 and OATP1B1 activity and increase LDL-C. Information on age, BMI, gender, hypertension, obesity, menopause status, cigarette smoking, physical activity, alcohol consumption and family history of coronary artery disease were recorded. During the study, 18 patients were excluded due to the treatment with CYP3A4 inhibitors (10), atorvastatin-non-CYP3A4 interacting drugs (6) and hyperthyroidism (2). Therefore, 156 hypercholesterolemic individuals completed the study [83 (53 %) males and 73 (47 %) females, average age 59.7±10.1, average BMI 28.5±4.1]. Hypertension was identified in 63 % of the patients, diabetes type 2 in 19 %, coronary artery disease in 42 %, cardiomyopathy in 13 % and atrial fibrillation in 5 % of the patients.

Atorvastatin was administered in daily doses of 20 mg to 43 % of the patients, 49 % of them received a dose of 40 mg, while the rest of the patients (8 %), a dose of 80 mg. It was considered that the patients adhered to the drug treatment when 80 % of dosage units were used. In addition to atorvastatin, antihypertensive drugs (e.g. ACE inhibitors, selective beta blockers) and oral antidiabetics (e.g. biguanides, sulfonylurea derivatives) were prescribed to 75 % and 15 % of the patients, respectively, while 80 % of the patients were treated with aspirin or clopidogrel to prevent blood clots. Concomitant ingestion of these drugs did not affect the atorvastatin response ($p > 0.05$) as evaluated by the Chi-square test (data not shown). In accordance with the physical examination and laboratory data, at the beginning and during the study the liver and kidney function was preserved in all patients. At the end of the protocol, the patients had appointments with the cardiologist and the response to atorvastatin and adverse reactions were evaluated.

4.2. Genotyping and haplotype analysis

Genomic DNA was extracted from EDTA-anticoagulant blood by a procedure reported previously (Daka et al. 2015; Grapci et al. 2015). Briefly, polymorphisms of the studied *SLCO1B1* SNPs were detected using the TaqMan allelic discrimination assay (Applied Biosystems, Foster City, CA, USA). A polymerase chain reaction was performed on the Real-Time PCR system Mx3005P (Stratagene, La Jolla, CA, USA) using TaqMan genotyping protocols (TaqMan®Drug Metabolizing assay; Applied Biosystems Foster City, Ca, USA) in total volume of 12.5 µL under the following conditions: one 2-minute-cycle at 50 ºC, one 10-min-cycle at 95 ºC, and 50 cycles of 15 s at 92 ºC and 1 min at 60 ºC. DNA isolation was performed at the Center for Biomolecular Pharmaceutical Analyses, UKIM-Faculty of Pharmacy, RoM), using the QiagenQiamp DNA Blood kit (QiagenGmbh, Hilden, Germany) according to the manufacturer's protocol.

4.3. Biochemical profile

Blood samples for a biochemical profile were collected in the morning after an overnight fast, ca. 12 hours after the last dose, one day before and 3 months after the atorvastatin treatment. The response to atorvastatin was evaluated according to the percentage change in TC, LDL-C, HDL-C, TG, ApoAI, ApoB, Lp(a) and the adverse effects were evaluated by measuring the activity of CPK, ALT and AST using standard laboratory procedures and methods (COBAS INTEGRA tests) established by the International Federation of Clinical Chemistry (IFCC), Societe Francaise de Biologie Clinique (SFBC), Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (SCE) and Deutsche Gesellschaft fur Klinische Chemie (DGKC). Other laboratory parameters were determined as well (Table 1) to evaluate the status of eliminatory organs, at the beginning and end of the study period.

4.4. Statistical analysis

Allele and genotype frequencies were estimated with a gene counting method, as described previously by Daka et al. (2015) and Grapci et al (2015). The agreement with HWE of the observed genotypic distribution for the *SLCO1B1* gene was tested with the Chi-square test, while for the analysis of LD for each pair of SNPs, haplotype construction and genetic association at polymorphism loci, the SHEsis software platform was used (http://analysis.bio-x.cn/myAnalysis.php) (Shi and He 2005). The laboratory data (continuous variables) were expressed as a mean±SD, while the category variables as absolute numbers and percentages.

All data were analyzed with the statistical program IBM SPSS Statistics for Windows, Version 19.0 (Armonk, NY: IBM Corp., USA). The data showing the normal distribution and homogeneity of variance were compared using one-way ANOVA (three or more variables) and paired t-test (two variables). Continuous variables deviating from the normal distribution were compared with the Mann-Whitney test or Kruskal-Wallis test as appropriate. Differences were considered statistically significant when p was below 0.05.

Conflicts of interest: None declared.

References

Adams SP, Tsang M, Wright JM (2015) Lipid lowering efficacy of atorvastatin (Review). Cochrane Database Syst Rev 12: CD008226.

Akao H, Polisecki E, Kajinami K, Trompet S, Robertson M, Ford I, Jukema JW, de Craen AJ, Westendorp RG, Shepherd J, Packard C, Buckley BM, Schaefer EJ (2012) Genetic variation at the SLCO1B1 gene locus and low density lipoprotein cholesterol lowering response to pravastatin in the elderly. Atherosclerosis 220: 413–417.

Athyros VG, Tziomalos K, Gossios TD, Griva T, Anagnostis P, Kargiotis K, Pagourelias ED, Theocharidou E, Karagiannis A, Mikhailidis DP; GREACE Study Collaborative Group (2010) Safety and efficacy of long-term statin treatment for cardiovascular events in patients with coronary heart disease and abnormal liver tests in the Greek Atorvastatin and Coronary Heart Disease Evaluation (GREACE) Study: a post-hoc analysis. Lancet 376: 1916-1922.

Bailey KM, Romaine SP, Jackson BM, Farrin AJ, Efthymiou M, Barth JH, Copeland J, McCormack T, Whitehead A, Flather MD, Samani NJ, Nixon J, Hall AS, Balmforth AJ; SPACE ROCKET Trial Group (2010) Hepatic metabolism and transporter gene variants enhance response to rosuvastatin in patients with acute myocardial infarction: the GEOSTAT-1 study. Circ Cardiovasc Genet 3: 276–285.

Braeuning A, Bucher P, Hofmann U, Buchmann A, Schwarz M (2014) Chemically induced mouse liver tumors are resistant to treatment with atorvastatin. BMC Cancer 14: 766.

Brunham LR, Lansberg PJ, Zhang L, Miao F, Carter C, Hovingh GK, Visscher H, Jukema JW, Stalenhoef AF, Ross CJ, Carleton BC, Kastelein JJ, Hayden MR (2012) Differential effect of the rs4149056 variant in SLCO1B1 on myopathy associated with simvastatin and atorvastatin. Pharmacogenomics J 12: 233-237.

Chang CH, Kusama M, Ono S, Sugiyama Y, Orii T, Akazawa M (2013) Assessment of statin-associated muscle toxicity in Japan: a cohort study conducted using claims database and laboratory information. BMJ Open 3:e002040.

Daka A, Dimovski A, Kapedanovska A, Vavlukis M, Eftimov A, Labachevski N, Jakjovski K, Geshkovska MN, Nebija D, Mladenovska K (2015) Effects of single nucleotide polymorphisms and haplotypes of the SLCO1B1 gene on the pharmacokinetic profile of atorvastatin in healthy Macedonian volunteers. Pharmazie 70: 480-488.

Diabetes Atorvastin Lipid Intervention (DALI) Study Group (2001) The effect of aggressive versus standard lipid lowering by atorvastatin on diabetic dyslipidemia: the DALI study: a double-blind, randomized, placebo-controlled trial in patients with type 2 diabetes and diabetic dyslipidemia. Diabetes Care 24: 1335-1341.

Djordjević VB, Cosić V, Stojanović I, Kundalić S, Zvezdanović L, Deljanin-Ilić M, Vlahović P, Popović L (2011) Lipoprotein(a) is the best single marker in assessing unstable angina pectoris. Cardiol Res Pract 2011: 175363.

Donnelly LA, Doney AS, Tavendale R, Lang CC, Pearson ER, Colhoun HM, McCarthy MI, Hattersley AT, Morris AD, Palmer CN (2011) Common nonsynonymous substitutions in SLCO1B1 predispose to statin intolerance in routinely treated individuals with type 2 diabetes: a go-DARTS study. Clin Pharmacol Ther 89: 210-216.

Dujovne CA, Harris WS, Altman R, Overhiser RW, Black DM (2000) Effect of atorvastatin on hemorheologic-hemostatic parameters and serum fibrinogen levels in hyperlipidemic patients. Am J Cardiol 85: 350-353.

Fu Q, Li YP, Gao Y, Yang SH, Lu PQ, Jia M, Zhang LR (2013) Lack of association between SLCO1B1 polymorphism and the lipid-lowering effects of atorvastatin and simvastatin in Chinese individuals. Eur J Clin Pharmacol 69: 1269–1274.

Giannakopoulou E, Ragia G, Kolovou V, Tavridou A, Tselepis AD, Elisaf M, Kolovou G, Manolopoulos VG (2014) No impact of SLCO1B1 521T>C, 388A>G and 411G>A polymorphisms on response to statin therapy in the Greek population. Mol Biol Rep 41: 4631-4638.

Golomb BA, Evans MA (2008) Statin adverse effects: a review of the literature and evidence for a mitochondrial mechanism. Am J Cardiovasc Drugs 8: 373–418.

Grapci AD, Dimovski AJ, Kapedanovska A, Vavlukis M, Eftimov, Geshkovska NM, Labachevski N, Jakjovski K, Gorani D, Kedev S, Mladenovska K (2015) Frequencies of single-nucleotide polymorphisms and haplotypes of the SLCO1B1 gene in selected populations of the western balkans. Balkan J Med Genet 18: 5-21.

Gumprecht J, Gosho M, Budinski D, Hounslow N (2011) Comparative long-term efficacy and tolerability of pitavastatin 4 mg and atorvastatin 20-40 mg in patients with type 2 diabetes and combined (mixed) dyslipidemia. Diabetes Obes Metab 13: 1047-1055.

He J, Qiu Z, Li N, Yu Y, Lu Y, Han D, Li T, Zhao D, Sun W, Fang F, Zheng J, Fan H, Chen X (2011) Effects of SLCO1B1 polymorphisms on the pharmacokinetics and pharmacodynamics of repaglinide in healthy Chinese volunteers. Eur J Clin Pharmacol 67: 701-707.

Hernández C, Francisco G, Ciudin A, Chacón P, Montoro B, Llaverias G, Blanco-Vaca F, Simó R (2011) Effect of atorvastatin on lipoprotein (a) and interleukin-10: a randomized placebo-controlled trial. Diabetes Metab 37: 124-130.

Ho RH, Choi L, Lee W, Mayo G, Schwarz UI, Tirona RG, Bailey DG, Stein CM, Kim RB (2007) Effect of drug transporter genotypes on pravastatin disposition in European- and African-American participants. Pharmacogenet Genomics 17: 647–656.

Hoffman KB, Kraus C, Dimbil M, Golomb BA (2012) A survey of the FDA's AERS database regarding muscle and tendon adverse events linked to the statin drug class. PloS One 7: e42866.

Hopewell JC, Parish S, Offer A, Link E, Clarke R, Lathrop M, Armitage J, Collins R; MRC/BHF Heart Protection Study Collaborative Group (2013) Impact of common genetic variation on response to simvastatin therapy among 18 705 participants in the Heart Protection Study. Eur Heart J 34: 982–992.

Hubacek JA, Dlouha D, Adámkova V, Lanska V, Ceska R, Vrablik M (2012) Possible gene-gender inte200raction between the SLCO1B1 polymorphism and statin treatment efficacy. Neuro Endocrinol Lett 33: 22-25.

Keltz E, Khan FY, Mann G (2014) Rhabdomyolysis. The role of diagnostic and prognostic factors.Muscles Ligaments Tendons J 3: 303-312.

Lee YJ, Lee MG, Lim LA, Jang SB, Chung JY (2010) Effects of SLCO1B1 and ABCB1 genotypes on the pharmacokinetics of atorvastatin and 2-hydroxyatorvastatin in healthy Korean subjects. Int J Clin Pharmacol Ther 48: 36-45.

Jiang J, Tang Q, Feng J, Dai R, Wang Y, Yang Y, Tang X, Deng C, Zeng H, Zhao Y, Zhang F (2016) Association between SLCO1B1 -521T>C and -388A>G polymorphisms and risk of statin-induced adverse drug reactions: A meta-analysis. Springerplus 5:1368.

Liu J, Long J, Zhang S, Fang X, Luo Y (2013) Polymorphic variants of SLCO1B1 in neonatal hyperbilirubinemia in China. Ital J Pediatr 10: 39-49.

Lopez-Lopez E, Martin-Guerrero I, Ballesteros J, Piñan MA, Garcia-Miguel P, Navajas A, Garcia-Orad A (2011) Polymorphisms of the SLCO1B1 gene predict methotrexate-related toxicity in childhood acute lymphoblastic leukemia. Pediatr Blood Cancer 57: 612-619.

Marciante KD, Durda JP, Heckbert SR, Lumley T, Rice K, McKnight B, Totah RA, Tamraz B, Kroetz DL, Fukushima H, Kaspera R, Bis JC, Glazer NL, Li G, Austin TR, Taylor KD, Rotter JI, Jaquish CE, Kwok PY, Tracy RP, Psaty BM (2011) Cerivastatin, genetic variants, and the risk of rhabdomyolysis. Pharmacogenet Genomics 21: 280-8.

Neuvonen PJ, Niemi M, Backman JT (2006) Drug interactions with lipid-lowering drugs: mechanisms and clinical relevance. Clin Pharmacol Ther 80: 565-581.

Newman C, Tsai J, Szarek M, Luo D, Gibson E (2006) Comparative safety of atorvastatin 80 mg versus 10 mg derived from analysis of 49 completed trials in 14 236 patients. Am J Cardiol 97: 61–67.

Niemi M (2010) Transporter pharmacogenetics and statin toxicity. Clin Pharmacol Ther 87: 130-133.

Niemi M, Pasanen MK, Neuvonen PJ (2011) Organic anion transporting polypeptide 1B1: a genetically polymorphic transporter of major importance for hepatic drug uptake. Pharmacol Rev 63: 157-181.

Parsa ZA, Ashoori S, Abdollahi A (2012) The effect of two different doses of atorvastatin on lipoprotein-a on patients with acute coronary syndrome. Iranian J Pathol 7: 101 – 106.

Pasanen MK, Fredrikson H, Neuvonen PJ, Niemi M (2007) Different effects of SLCO1B1 polymorphism on the pharmacokinetics of atorvastatin and rosuvastatin.Clin Pharmacol Ther 82: 726-733.

Pasanen MK, Neuvonen M, Neuvonen PJ, Niemi M (2006) SLCO1B1 polymorphism markedly affects the pharmacokinetics of simvastatin acid. Pharmacogenet Genomics 16: 873-879.

Pasanen MK, Neuvonen PJ, Niemi M (2008) Global analysis of genetic variation in SLCO1B1. Pharmacogenomics 9: 19-33.

Prado Y, Saavedra N, Zambrano T, Lagos J, Rosales A, Salazar LA (2015) SLCO1B1 c.388A>G Polymorphism is associated with HDL-C levels in response to atorvastatin in Chilean individuals. Int J Mol Sci 16: 20609-20619.

Puccetti L, Ciani F, Auteri A (2010) Genetic involvement in statins induced myopathy. Preliminary data from an observational case-control study. Atherosclerosis 211: 28-9.

Rajput TA, Naveed AK, Khan S, Farooqi ZU (2014) Frequencies of two functionally significant SNPs and their haplotypes of organic anion transporting polypeptide 1B1 SLCO1B1 gene in six ethnic groups of Pakistani population. Iran J Basic Med Sci 17: 441-447.

Rodrigues AC, Perin PM, Purim SG, Silbiger VN, Genvigir FD, Willrich MA, Arazi SS, Luchessi AD, Hirata MH, Bernik MM, Dorea EL, Santos C, Faludi AA, Bertolami MC, Salas A, Freire A, Lareu MV, Phillips C, Porras-Hurtado L, Fondevila M, Carracedo A, Hirata RD (2011) Pharmacogenetics of OATP transporters reveals that SLCO1B1 c.388A>G variant is determinant of increased atorvastatin response. Int J Mol Sci 12: 5815-5827.

Santos PC, Gagliardi AC, Miname MH, Chacra AP, Santos RD, Krieger JE, Pereira AC (2012) SLCO1B1 haplotypes are not associated with atorvastatin-induced myalgia in Brazilian patients with familial hypercholesterolemia. Eur J Clin Pharmacol. 68: 273–279.

SEARCH Collaborative Group, Link E, Parish S, Armitage J, Bowman L, Heath S, Matsuda F, Gut I, Lathrop M, Collins R (2008) SLCO1B1 variants and statin-induced myopathy-a genomwide study. N Engl J Med 359: 789-799.

Shi YY, He L (2005) a powerful software platform for analyses of linkage disequilibrium, haplotype construction, and genetic association at polymorphism loci. Cell Res 15: 97-98.

Superko HR, Momary KM, Li Y (2012) Statins personalized. Med Clin North Am 96: 123-139.

Takagi H, Umemoto T (2012) Atorvastatin decreases lipoprotein (a): a meta-analysis of randomized trials Int J Cardiol 154: 183-186.

Takane H, Miyata M, Burioka N, Shigemasa C, Shimizu E, Otsubo K, Ieiri I (2006) Pharmacogenetic determinants of variability in lipid-lowering response to pravastatin therapy. J Hum Genet 51: 822–826.

Tsimikas S, Witztum JL (2008) The role of oxidized phospholipids in mediating lipoprotein (a) atherogenticity. Curr Opin Lipidol 19: 369-377.

Ulvestad M, Skottheim IB, Jakobsen GS, Bremer S, Molden E, Asberg A, Hjelmesæth J, Andersson TB, Sandbu R, Christensen H (2013) Impact of OATP1B1, MDR1, and CYP3A4 expression in liver and intestine on interpatient pharmacokinetic variability of atorvastatin in obese subjects. Clin Pharmacol Ther 93: 275-282.

Wilke RA, Ramsey LB, Johnson SG, Maxwell WD, McLeod HL, Voora D, Krauss RM, Roden DM, Feng Q, Cooper-Dehoff RM, Gong L, Klein TE, Wadelius M, Niemi M; Clinical Pharmacogenomics Implementation Consortium (CPIC) (2012) The clinical pharmacogenomics implementation consortium: CPIC guideline for SLCO1B1 and simvastatin-induced myopathy. Clin Pharmacol Ther 92: 112-117.

Yang GP, Yuan H, Tang B, Zhang W, Wang LS, Huang ZJ, Ou-Yang DS, Zhang GX, Zhou HH (2010) Lack of effect of genetic polymorphisms of SLCO1B1 on the lipid-lowering response to pitavastatin in Chinese patients. Acta Pharmacol Sin 31: 382-386.