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ORIGINAL ARTICLE

FREQUENCIES OF SINGLE-NUCLEOTIDE POLYMORPHISMS AND HAPLOTYPES OF THE *SLCO1B1* GENE IN SELECTED POPULATIONS OF THE WESTERN BALKANS

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ABSTRACT

As a membrane influx transporter, organic anion-transporting polypeptide 1B1 (OATP1B1) regulates the cellular uptake of a number of endogenous compounds and drugs. The aim of this study was to characterize the diversity of the solute carrier organic anion transporter family member 1B1 (*SLCO1B1*) gene encoding this transporter in two ethnic groups populating the Western Balkans. The distribution of SCLO1B1 alleles was determined at seven variant sites (c.388A>G, c.521T>C, c.571T>C, c.597C>T, c.1086C>T, c.1463G>C and c.*439T>G) in 266 Macedonians and 94 Albanians using the TaqMan allelic discrimination assay. No significant difference in the frequencies of the single nucleotide polymorphisms (SNPs) was observed between these populations. The frequency of the c.521T>C SNP was the lowest (<13.7 and 12.2%, respectively), while the frequencies of all other SNP alleles were above 40.0%. Variant alleles of c.1463G>C and c.1086 C>T SNPs were not identified in either ethnic group. The haplotype analysis revealed 20 and 21 different haplotypes in the Macedonian and Albanian population, respectively. The most common haplotype in both ethnic groups, **1J/*1K/*1L*, had a frequency of 39.0% and 26.6%, respectively. In both populations, the variant alleles of the functionally significant c.521T>C and c.388A>G SNPs existed in one major haplotype (**15/*16/*17*), with a frequency of 8.6 and 2.4% in the Macedonian and Albanian subjects, respectively. In conclusion, sequence variations of the *SLCO1B1* gene in the studied populations occur at high frequencies, which are similar to that of the Caucasian population. Further studies are needed to evaluate the clinical significance of these SNPs and/or the major SLCO1B1 haplotypes they form for a large number of substrates and for susceptibility to certain diseases.

Keywords: Haplotypes; organic anion-transporting polypeptide 1B1 (OATP1B1); solute carrier organic anion-transporter family member 1B1 (*SLCO1B1*) gene; single nucleotide polymorphisms (SNPs); Western Balkan populations.

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INTRODUCTION

Membrane influx and efflux transporters have a significant role in facilitating or preventing drug movement through biological membranes. Drug responses are largely dependent on their interplay with

phases I and II metabolism and the physicochemical properties of a drug. They function in the selective absorption and elimination of drugs, mediate tissue-specific drug distribution and are also targets of many clinically used drugs. In addition, they play a critical role in the development of resistance to anticancer drugs, anticonvulsants and antiviral agents. When considering drug transport, two major super-families, ABC (ATP *b*inding *c*assette) and SLC (*sol*ute *c*arrier) transporters attract the highest scientific attention.

The SLC super family includes genes that encode facilitating transporters and ion-coupled secondary active transporters that reside in various cell membranes. Genes of the solute carrier organic anion transporter (SLCO) family encode organic anion-transporting polypeptides (OATPs), membrane influx transporters identified mostly in the intestine, liver, kidney, lung, testes, placenta and blood-brain barrier among other organs. The OATP1B1 [previously OATP2, OATP-C and liver specific transporter 1 (LST-1)], expressed in the sinusoidal membrane of the hepatocytes, is known to be involved in the hepatic uptake of a broad array of endogenous compounds (*e.g.,* steroid conjugates, bile acids, eicosanoids and thyroid hormones) and drugs such as methotrexate, fexofenadine, repaglinide and statins [1-6]. Examples of *in vitro* OATP1B1 drug substrates include several HMG-CoA reductase inhibitors, angiotensin-converting enzyme inhibitors and angiotensin II receptor antagonists [6-8]. Many drugs have also been identified *in vitro* as OATP1B1 inhibitors and there are some *in vivo* interactions where OATP1B1 inhibition can be regarded as an important mechanism. Examples include cyclosporine, atorvastatin, gemfibrozil and rifampicin [9,10].

The OATP1B1 protein is a 691-amino acid glycoprotein with 12 putative membrane-spanning domains and a large fifth extracellular loop. Its encoding gene, solute carrier organic anion transporter family member 1B1 (*SLCO1B1*), is located on chromosome 12 (gene locus 12p12) [11]. A large number of single nucleotide polymorphisms (SNPs), both non synonymous and synonymous, have been discovered in the *SLCO1B1* gene, and several of these have proven to affect a substrate-dependent transport function *in vitro* and *in vivo* [12,13]. While no firm evidence for association between these SNPs and development of certain diseases (*e.g.,* gallstone development, essential hypertension) due to dysregulation of endogenous compounds transport exists, there are numerous research data pointing to their effects on drugs responses.

The SNPs 388 (A>G) (**1b*, rs2306283) and 521 (C>T) (**5*, rs4149056) are considered to be the most prevalent and most relevant variants, encoding a substitution of alanine for valine at amino acid 174 (p.Val174Ala), and amino acid change at position 130 (p.Asn130Asp), respectively. Increased transport activity of pravastatin as well as decreased plasma concentration of ezetimibe in carriers of the *SLCO1B1*1b* allele was observed [14,15], unlike reduced uptake of all statins except fluvastatin in hepatocytes and increased area under curve (AUC) of fexofenadine, repaglinide and irinotecan in carriers of *SLCO1B1*5* [3,4,16,17]. The carriers of the c.521T>C variant were also highlighted by a genome-wide association study as a population with an increased risk for simvastatin-induced myopathy because of the increased plasma and muscle exposure to statins [18]. These findings were further confirmed by Santos *et al.* [19], who suggested that the *SLCO1B1* genetic risk depends on the specific drug that was used. It was also shown that subjects carrying the *SLCO1B1* c.388GG genotype exhibit significantly higher low-density-lipoprotein cholesterol reduction relative to c.388AA+ c.388AG carriers, pointing out that the *SLCO 1B1* c.388A>G polymorphism may be used as an important marker for predicting the efficacy of a lipid-lowering therapy [20].

Recent data point out that these two variants are in linkage disequilibrium (LD) and exist in variable *SLCO 1B1* haplotypes; AT, a haplotype known as **1A* (reference haplotype), GT as **1B*, AC as **5* and GC as **15*, for c.388 A>G and c.521T>C, respectively [13]. The **15* haplotype has been consistently associated with a decreased transport activity, while controversial results have been reported for the**1B* haplotype [21]. It was also demonstrated that the SLCO1B1**17* haplotype (g.-11187G>A, c.388G>A and c.521T>C) was associated with increased plasma concentrations of pravastatin in humans [22], while the **14* haplo type (c.388G-c.463A-c.521T) was characterized with enhanced response to fluvastatin [23].

It is becoming evident that the incidence of sequence variations in the *SLCO1B1* gene is largely dependent on the ethnic background. The c.521T>C variant showed an allele frequency of approximately 10.0-15.0% in Asian populations, 10.0-20.0%

in Caucasians and 1.0-2.0% in African-American populations. The c.388A>G SNP showed an allele frequency of approximately 30.0-45.0% in Caucasians, 70.0-80.0% in African-American/Sub-Saharan African populations and 60.0-90.0% in Asian populations [12,22,24-26]. Therefore, characterization of the genetic variation in this transporting gene is an important step towards understanding the individual variation in drugs-substrates responses and developing a personalized and safer drug therapy.

To the best of our knowledge, there is no evidence about genotyping of OATP1B1 in the populations living in Western Balkans. Also, there is no evidence when considering the populations living in the whole Balkan Peninsula, with exception of one report evaluating association between three *SLCO1B1* SNPs and statin response in the Greek population [27]. In this respect, there has not been any report on the genotype of *SLCO1B1* allelic variants in Macedonian and Albanian populations who are considered Caucasians. The origin of the Macedonians and Albanians is a continuing matter of discussion among historians; they also showed unequivocal signs of a common genetic history. In addition, Western Balkan countries have always been a historical crossroads between Asia, Africa and Europe. Considering all the above, the overall aim was to analyze the diversity of the *SLCO1B1* gene in selected ethnically diverse populations living in the Western Balkans [Republic of Macedonia (RoM) and Republic of Kosovo (RoK)]. In this article, the results from the allele and genotypic frequencies of the several known SNPs in the *SLCO1B1* gene and the haplotypes they form are presented. The results from this study could serve as a baseline clinical data for dosing of all drugs substrates of OATAP1B1 and avoiding the adverse drug reactions.

MATERIALS AND METHODS

Subjects and Study Protocol. For the aim of this study, a total of 233 Caucasian patients (age 18-72 years, average body mass index (BMI) 26.20 kg/m^2, 109 women and 124 men) with hypercholesterolemia type IIa or IIb, were selected randomly from the outpatients evaluated for coronary heart disease at the University Clinic of Cardiology in Skopje (RoM) and the University Clinical Center in Prishtina, Clinic for Internal Diseases (RoK). Of these, 156 (66.95%) were Macedonians, 64 (27.47 %) Albanians, four (1.72%) Turks and nine (3.86%) Gypsies. Due to the low number of patients, the data for the groups of Turks and Gypsies are not presented in this paper. Therefore, the evaluated group of patients (220 individuals, 105 female and 115 male patients) consisted of 70.91% Macedonians (n = 156, 73 women and 83 men) and 29.09% Albanians (n = 64, 32 women and 32 men).

Initially, the study protocol was approved by the Ethics Committee of the Faculty of Pharmacy and Committee for Clinical Studies of the Faculty of Medicine, University "Ss. Cyril and Methodius" (UKIM), Skopje, RoM, and the Ethics Committee and Committee for Clinical Studies of the Faculty of Medicine, University in Prishtina, RoK. All participants received oral and written information and gave a written informed consent before entering the study. Exclusion criteria (note: not relevant for the results present in this study, but important for the overall aim of the research) included cancer in remission for period shorter than 5 years, Cushing syndrome, hyperthyroidism, positive hepatitis B surface antigen, hepatitis C virus antibody, fibromyalgia, myopathy, rhabdomyolysis, malabsorption syndrome, renal failure, liver disease, McArdle disease, women who are pregnant, nursing or have planned a pregnancy, drugs interacting at the level of SLCO1B1. Data for BMI, cigarette smoking, blood pressure, alcohol consumption, physical activity and pharmacotherapy were also collected and recorded. To evaluate the frequency of genetic variations in genes encoding *SLCO1B1*, one blood sample was obtained from each participant for DNA extraction on the first day of the hospital visit.

In this study, 140 DNA samples obtained from the DNA bank of the Center for Biomolecular Analysis at the UKIM-Faculty of Pharmacy, Skopje, RoM, were also analyzed for the diversity of the *SLCO1B1* gene. These samples were obtained from healthy individuals (of Caucasian ethnicity, 78.57% Macedonians, 21.43% Albanians, 79 males, average age 48.0 ± 12.9, BMI 26.16 kg/m^2) selected by medical history, physical examination and routine laboratory tests before entering the study. Considering that there was no significant difference (p >0.05) in the allelic frequencies of *SLCO1B1* variants and genotype distributions between healthy subjects and patient groups, statistical analysis was also performed on the total population consisted of 360 subjects, of which 73.89% were Macedonians (n = 266, 129

women and 137 men) and 26.11% Albanians ($n = 94$, 42 women and 52 men).

Genomic DNA Extraction and Genotyping Procedures. Three mL venous blood samples drawn with EDTA as anticoagulant were collected and stored at 4 °C prior to DNA isolation. DNA isolation was performed at the Center for Biomolecular Pharmaceutical Analyses, UKIM-Faculty of Pharmacy, Skopje, RoM, using the Qiamp DNA Blood kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer's protocol. The samples were kept at –20 °C until further analysis. The *SLCO1B1* SNPs to be genotyped were selected on the basis of literature data [6,13,20,28,29] and a previous study in which 151 subjects were included [30]. The following variants in the *SLCO1B1* gene were analyzed: c.388A>G (Asn130Asp, rs2306283), c.521T>C (Val174Ala, rs4149056), c.571T>C (Leu191Leu, rs414 9057), c.597C>T (Phe199Phe, rs2291075), c.1086C>T (Tyr362Tyr, rs57040246), c.1463G>C (Gly488Ala, rs5950 2379), c.*439T>G (rs4149087, the position is given with the first nucleotide 3' of the stop codon (TAA) set to *1) using TaqMan allelic discrimination assay (Applied Bio-systems, Foster City, CA, USA).

Polymerase chain reaction was performed on the quantitative real-time PCR (q-PCR) system Mx3005P (Strata gene, La Jolla, CA, USA) using TaqMan genotyping protocols (TaqMan®Drug Metabolizing assay; Applied Bio-systems) in total volume of 12.5 μL under following conditions: one cycle of 2 min. at 50 °C, one cycle of 10 min. at 95 °C, and 50 cycles of 15 seconds at 92 °C and 1 min. at 60 °C.

Population Genetics and Statistical Analysis. The study sample alleles and genotype frequencies were estimated with a gene counting method. The agreement with Hardy-Weinberg equilibrium (HWE) of the observed genotypic distribution for the *SLCO1B1* gene was tested with the χ^2 test. The statistical analysis was carried out using the Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software (v. 19.0).

Genetic diversity was quantified between the members of the same ethnic population, between the ethnic populations, and between different ethnic populations and the global population. Population comparisons were also performed with the χ^2 test of population differentiation. Odds ratios (ORs) were calculated with 95% confidence interval (95% CI). For multiple comparisons, Bonferroni's post hoc test was used. Statistically significant differences were those where the p value was less 0.05. Linkage disequilibrium for each pair of SNPs within each population was quantified (correlation r^2 and coefficient of linkage disequilibrium D' values) to find the haplotypes in the study groups. The statistical analyses were carried out using the SHEsis software platform for the analysis of LD, haplotype construction and genetic association at polymorphism loci (http://analysis2.bio-x.cn/myAnalysis.php) [31]. The haplotypes were presented with their previously assigned names, as cited in the study of Pasanen *et al.* [13] in which allelic frequencies at 11 variant sites were determined (g.11187G>A, g.11110T>G, g.10499A>C, c.388A>G, c.411G>A, c.463C>A, c.521T>C, c.571T>C, c.597C>T, c.1929A>C and c.*439T>G). Considering that five of these SNPs and two other SNPs have been analyzed in the present investigation, one haplotype has several names and there are haplotypes that we designated as new.

RESULTS

Genotypes and Allele Frequencies. Genetic variation of *SLCO1B1* was studied in 360 subjects in total, both patients with hyperlipidemia type IIa or IIb and healthy subjects, of which 266 were of Macedonian and 94 of Albanian ethnicity. Observed genotypes and allelic frequencies of *SLCO1B1* gene polymorphisms did not differ significantly ($p > 0.05$) when comparing the data obtained from patients and healthy subjects (Table 1). In addition, the observed frequency distributions did not show significant deviations from HWE ($p > 0.05$) in both populations of the two ethnic groups, the population of both patients and healthy subjects, confirming the random selection of the individuals, *i.e.,* representativeness of the population samples being studied. Taking all this into consideration, genotype and allele frequencies for the total population of Macedonians and Albanians were estimated and the data are presented in Table 2.

Data for distribution of genotypes and allele frequencies of *SLCO1B1* gene polymorphisms between females and males, including both patients and healthy subjects within each ethnic group, are presented in Table 3. No significant differences for all *SLCO1B1* gene polymorphisms were observed between female and male subjects, both within each and between the two ethnic groups.

Table 1. Allelic and genotypic frequencies of *SLCO1B1* in patients with hyperlipidemia type IIa or IIb and healthy subjects.

Ethnic Group	**Macedonian[a]**		**Albanian[b]**	
Number of Subjects	**Patients (*n*=156) (%)**	**Healthy Subjects (*n*=110) (%)**	**Patients (*n*=64) (%)**	**Healthy Subjects (*n*=30) (%)**
Location/Position[c]/dbSNP ID				
Exon 4/c.388A>G/rs2306283				
AA	54 (34.6)	34 (30.9)	20 (31.2)	9 (30.0)
AG	80 (51.3)	58 (52.7)	35 (54.7)	16 (53.3)
GG	22 (14.1)	18 (16.3)	9 (14.1)	5 (16.7)
p Value[d]	0.77369		0.94636	
G allele	124 (40.0)	94 (42.7)	53 (41.4)	26 (43.3)
A allele	188 (60.0)	126 (57.3)	75 (58.6)	34 (56.7)
p Value[e]	0.49074		0.80295	
Exon 5/c.521T>C/rs4149056				
CC	4 (2.6)	5 (4.5)	–	1 (3.0)
CT	36 (23.1)	19 (17.3)	14 (21.9)	7 (23.3)
TT	116 (74.3)	86 (78.2)	50 (78.1)	22 (73.3)
p Value[d]	0.38218		0.33055	
C allele	44 (14.1)	29 (13.2)	14 (11.0)	9 (15.0)
T allele	268 (85.9)	191 (86.8)	114 (89.0)	51 (85.0)
p Value[e]	0.76116		0.42813	
Exon 5/c.571T>C/rs4149057				
CC	66 (42.3)	45 (41.0)	22 (34.4)	12 (40.0)
CT	71 (45.5)	53 (48.2)	22 (53.1)	15 (50.0)
TT	19 (12.2)	12 (11.0)	8 (12.5)	3 (10.0)
p Value[d]	0.89666		0.84958	
C allele	203 (65.0)	143 (65.0)	78 (61.0)	39 (65.0)
T allele	109 (35.0)	77 (35.0)	50 (39.0)	21 (35.0)
p Value[e]	0.98782		0.59224	
Exon 5/c.597C>T/rs229107				
CC	60 (38.5)	40 (36.4)	16 (25.0)	11 (36.7)
CT	67 (42.9)	50 (45.5)	35 (54.7)	14 (46.6)
TT	29 (18.6)	20 (18.2)	13 (20.3)	5 (16.7)
p Value[d]	0.91693		0.50617	
T allele	125 (40.1)	90 (40.9)	61 (47.6)	24 (40.0)
C allele	187 (59.9)	130 (59.1)	67 (52.3)	36 (40.0)
p Value[e]	0.84493		0.32550	
Exon 8/c.1086C>T/rs57040246				
CC	156 (100.0)	110 (100.0)	64 (100.0)	30 (100.0)
CT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
p Value[d]	>0.05		>0.05	
T allele	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
p Value[e]	>0.05		>0.05	

Continue

Table 1. Continued

Exon 10/c.1463G>C/rs59502379				
CC	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
GG	156 (100.0)	58 (100.0)	64 (100.0)	30 (100.0)
p Value[d]	>0.05		>0.05	
C allele	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
p Value[e]	>0.05		>0.05	
3'UTR/c.*439T>G/rs4149087				
GG	32 (20.5)	28 (25.45)	15 (23.4)	9 (30.0)
GT	81 (51.9)	50 (45.45)	31 (48.4)	16 (53.3)
TT	43 (27.6)	32 (29.1)	18 (28.1)	5 (16.7)
p Value[d]	0.52208		0.46337	
G allele	145 (46.5)	106 (48.2)	61 (47.7)	34 (56.7)
T allele	167 (53.5)	114 (51.8)	67 (52.3)	26 (43.3)
p Value[e]	0.69853		0.24882	

dbSNP: database of single nucleotide polymorphism; 3'UTR: 3' untranslated region; NCBI: National Center for Biotechnology Information.
[a] Macedonians populating the RoM.
[b] Albanians populating the RoM and RoK.
[c] The positions of SNPs are given in relation to the NCBI reference sequences NM_006446.2 (cDNA; c.) with the fist nucleotide of the ATG first codon set to 1 and the nucleotide 5' of ATG set to –1. The position of c.*439 is given with the first nucleotide 3' of the stop codon (TAA) set to *1.
[d] The *p* value for the differences of genotype distributions between the patients and healthy subjects within the ethnic group.
[e] The *p* value for the differences of allelic frequencies between the patients and healthy subjects within the ethnic group.

Table 2. Genetic variation of the *SLCO1B1* gene in Macedonian and Albanian subjects.

Ethnic Group	**Macedonian[a]**			**Albanian[b]**		
Number of Subjects	**Observed Frequency *n*=266 (%)**	**Expected Frequency by HWE (%)**	***p* Value[c]**	**Observed Frequency *n*=94 (%)**	**Expected Frequency by HWE (%)**	***p* Value[c]**
Position[d]/dbSNP ID						
c.388A>G/rs2306283						
AA	88 (33.1)	34.8		29 (30.8)	33.6	
AG	138 (51.9)	48.4	0.99737	51 (54.2)	48.7	0.99358
GG	40 (15.0)	16.8		14 (14.9)	17.7	
p Value[e]			0.91299			
G allele	218 (40.9)			79 (42.0)		
A allele	314 (59.0)			109 (58.0)		
p Value[f]			0.80266			
c.521T>C/rs4149056						
CC	9 (3.4)	1.9		1 (1.1)	1.5	
CT	55 (20.7)	23.7	0.99200	21 (22.3)	21.5	0.99919
TT	202 (75.9)	74.4		72 (76.6)	77.0	
p Value[e]			0.48666			
C allele	73 (13.7)			23 (12.2)		
T allele	459 (86.3)			165 (87.8)		
p Value[f]			0.60597			

Continue

Table 2. Continued

c.571T>C/rs4149057						
CC	111 (41.7)	42.3		34 (36.2)	38.7	
CT	124 (46.6)	45.5	0.99969	49 (52.1)	47.0	0.99408
TT	31 (11.6)	12.2		11 (11.7)	14.3	
p Value[e]			0.61507			
C allele	346 (65.0)			117 (62.2)		
T allele	186 (35.0)			71 (37.8)		
p Value[f]			0.49039			
c.597C>T/rs229107						
CC	100 (37.6)	35.5		27 (28.7)	30.0	
CT	117 (44.0)	48.2	0.99625	49 (52.1)	49.5	0.99864
TT	49 (18.4)	16.3		18 (19.1)	20.4	
p Value[e]			0.27697			
T allele	215 (40.4)			85 (45.2)		
C allele	317 (59.6)			103 (54.8)		
p Value[f]			0.25125			
c.1086C>T/rs57040246						
CC	266 (100.0)	100.0		94 (100.0)	100.0	
CT	0 (0.0)	0.0	>0.05	0 (0.0)	0.0	>0.05
TT	0 (0.0)	0.0		0 (0.0)	0.0	
p Value[e]			>0.05			
T allele	0 (0.0)	0.0		0 (0.0)		
p Value[f]			>0.05			
c.1463G>C/rs59502379						
CC	0 (0.0)	0 (0.0)		0 (0.0)	0 (0.0)	
CG	0 (0.0)	0 (0.0)	>0.05	0 (0.0)	0 (0.0)	>0.05
GG	266 (100.0)	100.0		94 (100.0)	100.0	
p Value[e]			>0.05			
C allele	0 (0.0)			0 (0.0)		
p Value[f]			>0.05			
c.*439T>G/rs4149087						
GG	60 (22.5)	25.5		24 (25.5)	22.2	
GT	131 (49.2)	50.0	0.99993	47 (50.0)	49.8	1.0000
TT	75 (28.2)	24.5		23 (24.5)	27.9	
p Value[e]			0.73126			
G allele	251 (47.2)			95 (50.5)		
T allele	281 (52.8)			93 (49.5)		
p Value[f]			0.42917			

HWE: Hardy-Weinberg equilibrium; dbSNP: database of single nucleotide polymorphism; 3'UTR: 3' untranslated region; NCBI: National Center for Biotechnology Information.

[a] Macedonians populating the RoM.

[b] Albanians populating the RoM and RoK.

[c] The *p* value for the differences between observed and expected frequencies of genotype distributions within the ethnic group.

[d] The positions of SNPs are given in relation to the NCBI reference sequences NM_006446.2 (cDNA; c.) with the fist nucleotide of the ATG first codon set to 1 and the nucleotide 5' of ATG set to –1. The position of c.*439 is given with the first nucleotide 3' of the stop codon (TAA) set to *1.

[e] The *p* value of differences in genotype distributions between the ethnic groups.

[f] The *p* value of allele frequencies between the ethnic groups.

Table 3. Distribution of genotype and allele frequencies of the *SLCO1B1* gene polymorphisms in female and male groups separately, within each ethnic group.

Ethnic Group	**Macedonian**		**Albanian**	
Number of Subjects	**Females (*n*=129) (%)**	**Males (*n*=137) (%)**	**Females (*n*=42) (%)**	**Males (*n*=52) (%)**
Position/dbSNP ID				
c.388A>G/rs2306283				
AA	42 (32.6)	46 (33.6)	14 (33.3)	15 (28.8)
AG	65 (50.4)	73 (53.3)	24 (57.1)	27 (51.9)
GG	22 (17.0)	18 (13.1)	4 (9.5)	10 (19.2)
p Value[a]	0.66841		0.41932	
G allele	109 (42.2)	109 (39.8)	32 (38.1)	47 (45.2)
A allele	149 (57.8)	165 (60.2)	92 (61.9)	57 (54.8)
p Value[b]	0.56309		0.32702	
c.521T>C/rs4149056				
CC	6 (4.6)	3 (2.2)	–	1 (2.0)
CT	25 (19.4)	30 (21.9)	9 (21.4)	12 (23.1)
TT	98 (76.0)	104 (75.9)	33 (78.6)	39 (75.0)
p Value[a]	0.49822		0.64576	
C allele	37 (14.3)	36 (13.1)	9 (10.7)	14 (13.5)
T allele	221 (85.7)	238 (86.9)	75 (89.3)	90 (86.5)
p Value[b]	0.68707		0.56765	
c.571T>C/rs4149057				
CC	53 (41.2)	58 (42.3)	14 (33.3)	20 (38.5)
CT	61 (47.3)	63 (46.0)	23 (54.8)	26 (50.0)
TT	15 (11.6)	16 (11.7)	5 (11.9)	6 (11.5)
p Value[a]	0.97571		0.82901	
C allele	167 (64.7)	179 (65.3)	51 (60.7)	66 (63.5)
T allele	91 (35.3)	95 (34.7)	33 (39.3)	38 (36.5)
p Value[b]	0.88472		0.69928	
c.597C>T/rs229107				
CC	49 (38.0)	51 (37.2)	12 (28.6)	15 (28.8)
CT	57 (44.2)	60 (43.8)	22 (52.4)	27 (51.9)
TT	23 (17.8)	26 (19.0)	8 (19.0)	10 (19.2)
p Value[a]	0.97042		0.99902	
C allele	103 (39.9)	112 (40.9)	38 (45.2)	47 (45.2)
T allele	155 (60.1)	162 (59.1)	46 (54.8)	57 (54.8)
p Value[b]	0.82278		0.99500	
c.1086C>T/rs57040246				
CC	129 (100.0)	137 (100.0)	42 (100.0)	52 (100.0)
CT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
TT	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
T allele	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
c.1463G>C/rs59502379				
CC	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
CG	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
GG	129 (100.0)	137 (100.0)	42 (100.0)	52 (100.0)
C allele	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Continue

Table 3. Continued

c.*439T>G/rs4149087				
GG	30(23.3)	30 (21.9)	11 (26.2)	13 (25.0)
GT	64 (49.6)	67 (48.9)	19 (45.2)	28 (53.8)
TT	35 (27.1)	40 (29.2)	12 (28.6)	11 (21.2)
p Value[a]	0.92239		0.64414	
G allele	124 (48.1)	127 (46.4)	41 (48.8)	54 (51.9)
T allele	134 (51.9)	147 (53.6)	43 (51.2)	50 (48.1)
p Value[b]	0.69266		0.67119	

[a] The *p* value of differences in genotype distributions between females and males within the ethnic group.
[b] The *p* value of differences of allele frequencies between females and males within the ethnic group.

All SNPs, except c.1463G>C and c.1086C>T, occurred at an allele frequency higher than 12.0%. Variant alleles of SLCOB1 c.1463G>C and c.1086C>T polymor- phisms were not identified in either ethnic group in this study. The frequency of the c.521T>C SNP was the lowest, 13.7 and 12.2% for Macedonians and Albanians, respectively, while the frequencies of all other SNPs alleles were above 40.0%, with frequency of the c.571T>C variant allele being highest in both populations (65.0 and 62.2% for Macedonians and Albanians, respectively). No significant differences ($p > 0.05$) in allelic frequencies and genotype distributions of the analyzed SNPs were observed between the two ethnic groups. The SNP variant allele frequencies in the ethnic groups separately compared to data reported from various ethnic groups are presented in Table 4.

Pairwise Linkage Disequilibrium. Pairwise LD profiles for single SNPs using r^2 and D' values for Macedonians and Albanians separately, are shown in Figures 1 and 2, respectively. Generally, the correlations of SNP pairs in the Albanian population were weaker than those of the Macedonian population. The most strongly correlated ($r^2 \geq 0.33$) SNP pair in the Macedonian population was c.597 C>T/c.388A>G ($r^2 = 0.531$, $D' = 0.740$), followed by c.597C>T/c.*439T>G ($r^2 = 0.373$, $D' = 0.699$). Other pairs showing a significant association were c.388A>G/c.*439 T>G ($r^2 = 0.289$, $D' = 0.613$) and c.521T>C/c.571T>C ($r^2 = 0.233$, $D' = 0.919$). The correlation of the most common SNP pair, c.388A>G/c.521T>C, in the Macedonians was relatively weaker compared to other SNP pairs, with $r^2 = 0.113$ and $D' = 0.698$. The c.521T>C showed the strongest correlation with c.571T>C, followed by c.597 C>T ($r^2 = 0.178$, $D' = 0.872$), c.388A>G and c.*439 T>G ($r^2 = 0.072$, $D' = 0.645$).

In the Albanian population, the same SNP pairs, c.597C>T/c.388A>G and c.597C>T/c.*439T>G, showed the strongest correlation with $r^2 = 0.221$, $D' = 0.498$ and $r^2 = 0.214$, $D' = 0.505$, respectively. The correlation between c.388 A>G and c.521T>C in the Albanian population was weaker ($r^2 = 0.009$, $D' = 0.219$) compared to the same SNP pairs in the Macedonian population. Similar data for the LD of c.521T>C with other SNPs were obtained, with the strongest correlation of this SNP with c.571T>C ($r^2 = 0.091$, $D' = 0.635$), followed by c.597C>T ($r^2 = 0.097$, $D' = 0.746$), c.388A>G and c.*439T>G ($r^2 = 0.008$, $D' = 0.238$).

Haplotypes. The haplotype analysis revealed 20 different haplotypes in the Macedonian population and 21 in the Albanian population (Tables 5 and 6). Nine haplotypes in each of the two populations were designated as new. Nine other haplotypes that occurred in the Macedonian and Albanian populations had the same sequence of the actually investigated SNPs as in the newly identified haplotypes presented in the study of Pasanen *et al.* [13].

In the Macedonian population, eight haplotypes occurred at a frequency equal to or greater than 3.0% (Table 5). The most common haplotype in this ethnic group, **1J/ *1K/*1L*, had a frequency of 39.0%, containing variant allele C at position c.571 and having referent nucleotides at all other investigated positions. The variant allele C at position c.571 existed in eight haplotypes, with a frequency between 0.3 and 39.0%. The variant allele G at position c.388 and T at c.597C>T SNP existed in 11, while the variant allele G at c.*439T>G in 12 haplotypes, all occurring with frequencies between 0.3 and 11.6%. The c.521T>C SNP existed in six haplotypes, with a frequency between 0.3 and 8.6%. The variant alleles of the functionally most distinguished SNPs, c.388A>G

Table 4. Allelic frequencies of *SLCO1B1* variants in Macedonians and Albanians compared to different ethnic populations.

Ethnic Group	*n*[a]	c.388A>G	c.521T>C	c.571T>C	c.597C>T	c.1086C>T	c.1463G>C	c.*439T>G	*p* Value[b]	*p* Value[c]	Refs.
American (African)	22	0.75	0.023	0.045	–	–	0.09	–	<0.00001	<0.00001	12
(European)	49	0.30	0.14	0.53	–	–	0.0	–	0.812508	0.675274	12
(Native)	64	0.63	0.24	0.33	0.28	0.01	0.005	0.041	0.000507	0.003258	11
European	151	0.41	0.18	0.61	0.42	0.0	0.0	0.30	0.118756	0.149653	11
(Caucasian)	236	0.41	0.17	–	–	–	–	–	0.466510	0.328042	38
Sub-Saharan African	105	0.79	0.019	0.13	0.50	0.07	0.03	0.76	<0.00001	<0.00001	11
Oceanian	28	0.66	0.0	0.48	0.52	0.036	0.0	0.30	0.017744	0.055677	11
Algerian	29	0.64	0.17	0.21	0.59	0.017	0.0	0.72	0.004001	0.017564	11
Ugandan	115	0.78	0.039	0.061	0.0	–	0.02	–	<0.00001	<0.00001	11
Indian (Asian)	35	0.60	0.071	–	–	0.0	–	–	0.078203	0.167511	24
North Indian	100	0.57	0.065	0.44	0.22	0.0	–	–	0.000010	0.009301	25
Brazilian	97	–	0.057	–	–	–	–	–			28
(African)	332	–	0.15	–	–	–	–	–			28
(Mulatto)	603	–	0.15	–	–	–	–	–			28
(Caucasian)	182	–	0.28	–	–	–	–	–			28
(Amerindian) Brazilian	143	0.26	0.14	–	–	–	–	–	0.164989	0.136491	19
Chinese	178	0.73	0.11	0.27	0.42	0.0	0.0		<0.00001	<0.00001	11
	100	0.80	0.13	0.26	0.50	0.0	–	0.27	<0.00001	<0.00001	25
	35	0.67	0.086	–	–	–		–	0.124778	0.264367	24
	140	0.71	0.11	–	–	–			0.014801	0.153581	39
Han Chinese	111	0.73	0.14	–	–	–	–	–	0.079881	0.339359	29
Uyghur (Chinese)	731	0.62	0.10	–	–	–	–	–	0.000925	0.119961	40
Finnish	468	0.46	0.20	0.53	0.46	–	–	0.49	0.038427	0.13401	13
(Caucasian)	193	–	–	–	–	0.0	0.0	–			
Dutch	74	–	0.18	–	–	–	–	–			33
German (Caucasian)	300	0.37	0.15	0.35	0.38	–	0.0	–	0.002137	0.078291	36
Israeli	133	0.46	0.20	0.56	0.45	0.0	0.0	0.55	0.322641	0.571025	11
Japanese	120	0.63	0.16	0.36	0.43	–	–	–	<0.00001	0.005088	34
	267	0.64	0.11	–	–	–	–	–	0.011239	0.195709	41
	27	0.74	0.19	0.26	0.26	0.0	0.0	0.19	0.001111	0.003401	11
Korean	24	0.75	0.25	–	–	–	–	–	0.775142	1.00000	35
Malaysian	100	0.87	0.11	0.24	0.50	0.0	–	–	<0.00001	<0.00001	25
	35	0.83	0.13	–	–	–	–	–	0.186616	0.403354	24
Pakistani	192	0.47	0.09	0.56	0.26	0.0	0.005	0.59	0.008402	0.81518	11
Tanzanian	366	0.87	0.06	–	–	–	–	–	<0.00001	0.289424	38
Turkish	94	0.46	0.12	0.0.38	0.36	–	0.0	–	0.89754	0.289424	36
Macedonian	266	0.41	0.14	0.65	0.40	0.0	0.0	0.47	0.928464[d]		this study
Albanian	94	0.42	0.12	0.62	0.45	0.0	0.0	0.50	0.928464		this study
Greek	403	0.43	0.16	–	–	–	–	–	0.811389	0.595368	27
Caucasian	423	0.37	0.15	–	–	–	–	–	0.472334	0.333625	32

[a] *n*: number of patients.
[b] The *p* value of differences in allele frequencies between Macedonians and different ethnic groups.
[c] The *p* value of differences in allele frequencies between Albanians and different ethnic groups.
[d] The *p* value of differences in allele frequencies between Albanians and Macedonians.

Table 5. Alignment and frequencies of the *SLCO1B1* haplotypes in 266 Macedonian subjects.

***SLCO1B1* Haplotype**	**c.388 A>G**	**c.521 T>C**	**c.571 T>C**	**c.597 C>T**	**c.1086 C>T**	**c.1463 G>C**	**c.*439 T>G**	**Haplotypes Found**		
Reference	**A**	**T**	**T**	**C**	**C**	**G**	**T**	*n*	%	95% CI
**1J/*1K/*1L*[a]			**C**					148	39.1	0.747-1.338
**18*[b]/New[c]	**G**		**C**	**T**			**G**	44	11.6	0.641-1.559
15*[a]/16*[d]/**17*[c]	**G**	**C**		**T**			**G**	32	8.6	0.602-1.662
**1J/*1K/*1L*[a]			**C**				**G**	32	8.4	0.599-1.668
**1G/*1H*[a]/New[f]/New[g]	**G**			**T**			**G**	29	7.7	0.587-1.705
**1A*[a]*/New*[f]*/*1E*[h]								22	5.9	0.548-1.25
**1B/*1F*[a]/New[f]	**G**						**G**	11	3.1	0.441-2.265
**20*[i]*/*21*[j]/New[f]	**G**			**T**				11	3.0	0.432-2.315
New				**T**			**G**	8	2.1	0.375-2.667
**5*[a]/New[f]		**C**					**G**	7	2.0	0.360-2.779
New	**G**		**C**					7	1.9	0.353-2.836
**18*[k]/New[l]	**G**		**C**	**T**				6	1.7	0.330-3.034
New	**G**	**C**		**T**				5	1.5	0.308-3.250
New			**C**	**T**			**G**	4	1.1	0.249-4.011
New[m]	**G**		**C**				**G**	2	0.6	0.167-6.006
New							**G**	1	0.5	0.130-7.712
New	**G**		**C**				**G**	1	0.3	0.082-12.232
New				**T**				1	0.3	0.073-13.647
New		**C**		**T**				1	0.3	0.068-14.662
New	**G**	**C**					**G**	1	0.3	0.064-15.593

95% CI: 95% confidence interval.

[a] The name includes the presented sequence of the SNPs investigated in this study and referent alleles of the additional SNPs investigated in the study by Pasanen *et al.* [13] (at positions g.-11187, g.-11110, g.-10499, c.411, c.463 and c.1929).
[b,d,e,h,i,j,k] The haplotype name includes a sequence of the SNPs investigated in this study and referent alleles in other SNPs investigated in the cited study [13], except at the following positions **b:** c.411 and c.463; **d:** g.-10499; **e:** g.-11187; **h:** g.-11110; **i:** c.1929; **j:** g.-11187 and c.1929, and **k:** c.411 and c.463, where the variant alleles exist.
[c,f,g,l] The haplotype is assigned as new by Pasanen *et al.* [13], having the same sequence of the SNPs investigated in this study and referent alleles at other SNPs investigated in the cited study [13], except at the following positions **c:** g.-11110, c.411 and c.463; **f:** g.-11187; **g:** g.-10499, and **l:** c.411, c.463 and c.1929, where variant alleles exist.
[m] The haplotype is assigned as new by Pasanen *et al.* [13], having the same sequence of the SNPs investigated in this study and referent alleles in additional SNPs investigated in the cited study.

and c.521T>C, were present in four haplotypes, of which the dominant haplotype **15/*16/*17* had a frequency of 8.6%.

In the Albanian population, 10 haplotypes occurred at a frequency equal or greater than 3.0% (Table 6). The most common haplotype was the same as in the Macedonian ethnic group, **1J/*1K/*1L,* with a frequency of 26.6%. The variant allele C at position c.571 existed in nine haplotypes with a frequency between 1.4 and 26.6%. The variant allele G at position c.388 existed in 10 haplotypes, while c.597C>T SNP in 11 haplotypes, both occurring at frequencies between 1.0 and 12.4%. The c.*439T>G occurred in 10 haplotypes, with a frequency between 0.6 and 12.4%, and the c.521T>C SNP existed in seven haplotypes, with a frequency between 0.6 and 3.7%. Three of the haplotypes contained the variant alleles of the c.388A>G and c.521 T>C SNPs with a frequency ≥1.0%, with the major haplotype **15/*16/*17* having a frequency of 2.4%.

DISCUSSION

It is clearly evident that the mutations in the *SLCO1B1* gene and their clinical significance for a large number of endogenous and xenobiotic substrates

Table 6. Alignment and frequencies of the *SLCO1B1* haplotypes in 94 Albanian subjects.

***SLCO1B1* Haplotype**	**c.388 A>G**	**c.521 T>C**	**c.571 T>C**	**c.597 C>T**	**c.1086 C>T**	**c.1463 G>C**	**c.*439 T>G**	**Haplotypes Found**		
Reference	**A**	**T**	**T**	**C**	**C**	**G**	**T**	*n*	%	95% CI
**1J/*1K/*1L*[a]			**C**					45	26.6	0.618-1.618
**18*[b]/New[c]	**G**		**C**	**T**			**G**	21	12.4	0.525-1.905
**1G/*1H*[a]/New[f]/New[g]	**G**			**T**			**G**	19	11.5	0.514-1.947
**1J/*1K/*1L*[a]			**C**				**G**	11	6.7	0.424-3.344
**1A*[a]*/*1E*[h]/New[f]								8	5.1	0.380-2.630
*1B/*1F[a]/New[f]	**G**						**G**	6	3.8	0.330-0.301
*5[a]/New[f]		**C**		**T**			**G**	6	3.7	0.322-3.103
New			**C**	**T**			**G**	6	3.5	0.316-3.162
New							**G**	5	3.2	0.297-3.370
New	**G**		**C**					5	3.0	0.286-3.497
New		**C**	**C**	**T**				4	2.9	0.280-3.573
**20*[i]*/*21*[j]/New[f]	**G**			**T**				4	2.7	0.268-3.734
*1B/*1F	**G**							4	2.5	0.259-3.865
**15*[a]*/*16*[k]*/*17*[l]	**G**	**C**		**T**			**G**	4	2.4	0.251-3.990
New				**T**				3	2.2	0.236-4.236
New[m]	**G**		**C**				**G**	3	1.9	0.212-4.716
New			**C**	**T**				3	1.9	0.210-4.755
New	**G**	**C**	**C**	**T**				2	1.4	0.168-5.938
New	**G**	**C**		**T**				1	1.0	0.114-8.806
New		**C**						1	0.7	0.082-12.179
New[m]		**C**					**G**	1	0.6	0.065-15.451

95% CI: 95% confidence interval.

[a] The name includes the presented sequence of the SNPs investigated in this study and referent alleles of the additional SNPs investigated in the study by Pasanen *et al.* [13].

[b,h,i,j,k,l] The haplotype name includes a sequence of the SNPs investigated in this study and referent alleles in other SNPs investigated in the cited study [13], except at the following positions **b**: c.411 and c.463; **h**: g.-11110; **i**: c.1929; **j**: g.-11187 and c.1929; **k**: g.-10499; and **l**: g.-11187, where variant alleles exist.

[c,f,g] The haplotype is assigned as new by Pasanen *et al.* [13], having the same sequence of the SNPs investigated in this study and referent alleles in other SNPs investigated in the cited study, except at the following positions **c**: g.-11110, c.411 and c.463; **f**: g.-11187; and **g**: g.-10499, where variant alleles exist.

[m] The haplotype is assigned as new by Pasanen *et al.* [13], having the same sequence of the SNPs investigated in this study and referent alleles at additional SNPs investigated in the cited study (at positions g.-11187, g.-11110, g.-10499, c.411, c.463 and c.1929).

transported by OATP1B1 is a persistent motivation for scientific research. To the best of our knowledge, this is the first study in which polymorphisms contained in the *SLCO1B1* gene were studied in the populations living in the Western Balkan Peninsula. For this reason, commonly seen mutations (c.388A>G, c.521T>C, c.571T>C, c.597C>T, c.*439 T>G) as well as coding region SNPs that were not identified in the Caucasian (European) population (c.1086C>T, c.1463G>C) were selected for genotyping. Our data confirmed that *SLCO1B1* is highly polymorphic and that several variants appear at a high frequency, both in the Macedonian and Albanian populations. The SNPs c.388A>G (Asn130Asp), c.571T>C (Leu-191Leu), c.597C>T (Phe199 Phe) and c.*439T>G, all occurred with an allelic frequency between 40.0 and 65.0%. The non synonymous c.521T>C SNP, which has been constantly associated with a reduced OATP1B1 activity, was found with an allele frequency of approximately 14.0 and 12.0% in the Macedonian and Albanian population, respectively, which is nearly equal to that reported for Caucasians (15.0%) [32], slightly lower than that reported previously for Dutch (18.0%) [33], Finish (20.0%) [13],

	c.388A>G	c.521T>C	c.571T>C	c.597C>T	c.1086C>T	c.1463G>C	c.*439T>G
c.388A>G		0.698	0.487	0.740	0.000	0.000	0.613
c.521T>C	0.113		0.919	0.872	0.000	0.000	0.645
c.571T>C	0.187	0.233		0.520	0.000	0.000	0.426
c.597C>T	0.531	0.178	0.218		0.000	0.000	0.699
c.1086C>T	0.000	0.000	0.000	0.000		0.000	0.000
c.1463G>C	0.000	0.000	0.000	0.000	0.000		0.000
c.*439T>G	0.289	0.072	0.115	0.373	0.000	0.000	

D'

***r*²**

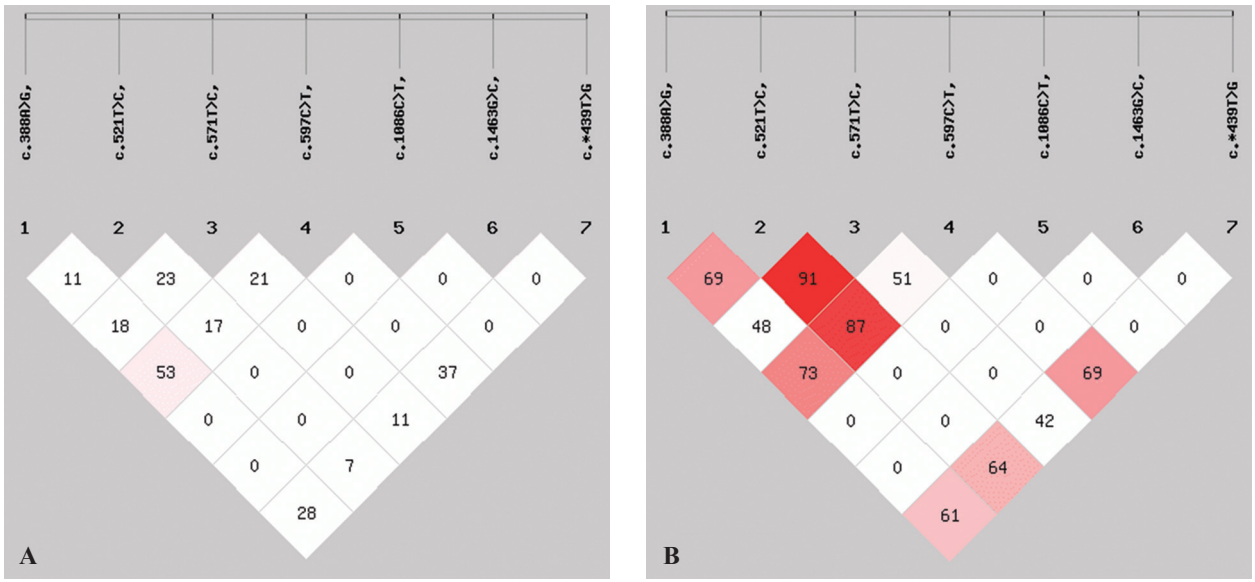

Figure 1. Pairwise LD profiles for *SLCO1B1* SNPs in Macedonians (n = 266); r2 cells (below the diagonal, A) and D' (cells above the diagonal, B) values for each pair of the seven SNPs are presented.

Algerian (17.0%) [11], Israeli (20.0%) [11], Japanese (16.0-19.0%) [11,34] and Korean (25.0%) [35] ethnic groups, and much higher than that reported for African Americans (2.3%) [12] and Sub-Saharan Africans (1.9%) [11] (Table 4). So far, literature data point to equal allele frequency for this SNP in Macedonians and European Americans [12] and Han Chinese [29], although a lower number of subjects in the last two groups was included in the study. The same was observed in Albanian and Turkish subjects, with an equal number of subjects in the study [36]. Compared to studies with Native Americans, Caucasian Europeans, Sub-Saharan Africans, Japanese and Israeli subjects [11], the variant alleles found in the Macedonian and Albanian subjects were lower for c.388A>G (41.0-42.0% *vs.* 46.0-79.0%), higher for c.571 T>C (62.0-65.0% *vs.* 13.0-61.0%) and nearly equal for c.597C>T (40.0-45.0% *vs.* 42.0-50.0%), with the exception of Native Americans in which a much lower allele frequency was observed (28.0%). The allele frequency for SNP c.*439T>G was lower in comparison with Sub-Saharan Africans (47.0-50.0% *vs*. 76.0%), higher than that of Caucasian Europeans (30.0%) and almost equal to the frequency of other ethnic groups, where a variant G allele existed with a frequency between 41.0% (Native Americans) and 55.0% (Israeli) (Table 4). No variant alleles were found for c.1086C>T and c.1463G>C SNPs in the Macedonian and Albanian populations and the same was observed in German, Finish, Japanese, Israeli and Turkish subjects [11,36], while in the studied Native American and Sub-Saharan African, Ugandan and Pakistani ethnic groups, a low frequency of variant alleles was observed, between 1.0 and 7.0% for vari-

	c.388A>G	c.521T>C	c.571T>C	c.597C>T	c.1086C>T	c.1463G>C	c.*439T>G
c.388A>G		0.219	0.370	0.498	0.000	0.000	0.477
c.521T>C	0.009		0.635	0.746	0.000	0.000	0.238
c.571T>C	0.119	0.091		0.206	0.000	0.000	0.137
c.597C>T	0.221	0.097	0.031		0.000	0.000	0.505
c.1086C>T	0.000	0.000	0.000	0.000		0.000	0.000
c.1463G>C	0.000	0.000	0.000	0.000	0.000		0.000
c.*439T>G	0.169	0.008	0.011	0.214	0.000	0.000	

D'

r^2

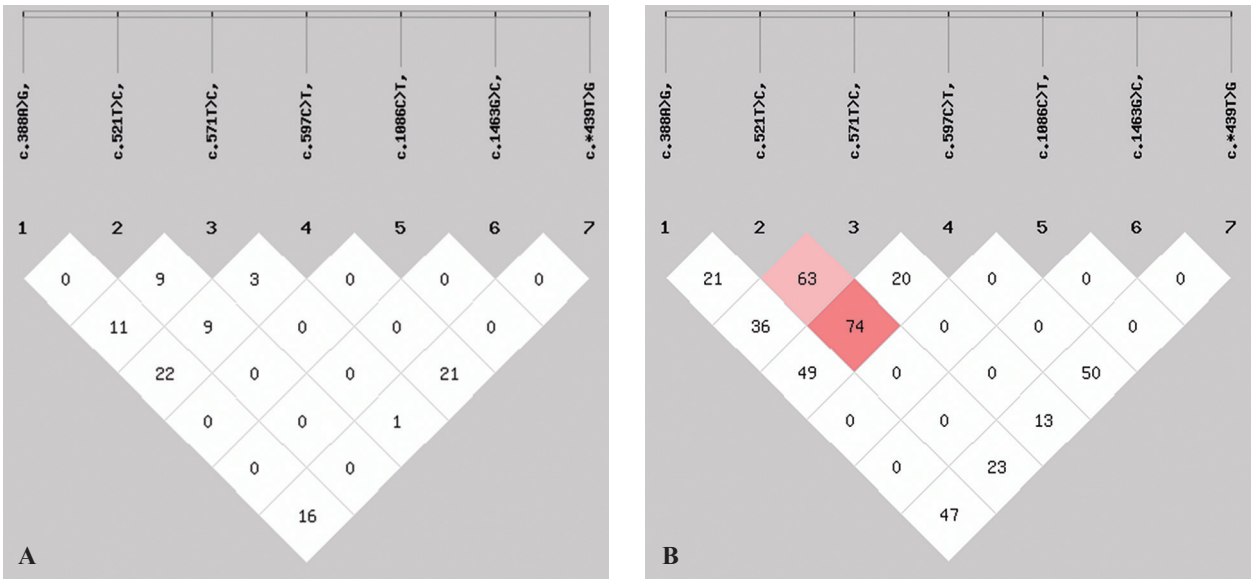

Figure 2. Pairwise LD profiles for *SLCO1B1* SNPs in Albanians (n = 94); r2 cells (below the diagonal, A) and D' (cells above the diagonal, B) values for each pair of the seven SNPs are presented.

ant T in c.1086C>T and 0.5 and 3.0% for variant C in c.1463G>C [11].

For all SNPs, the distributions of the genotypes did not differ significantly (p >0.05) between healthy subjects and patients and between male and female subjects. These data are partly in accordance with the results obtained in the study of Hubacek *et al.* [37], in which no difference for genotype distributions of rs4149056 variant between male and female subjects was observed. However, the results of the same study pointed to possible gender-dependent effects of this SNP within the *SLCO1B1* gene on statin treatment efficacy.

It is increasingly evident that the most relevant variants, the SNPs 388A>G and 521C>T, have a major effect on OATP1B1 activity. However, their association and other SNPs in LD with these functionalities may modify the respective phenotype and explain the discrepant effects of some SNPs on OATP1B1 activity *in vivo*. Most of the literature data point to a strong association between this SNP pair and its effect on drug response [20,29]. In the actual study, the association between c.388A>G and c.521 T>C was relatively weaker compared to other SNP pairs, especially those in the Albanian population. These data and generally, the differences between the two populations in LD data, are probably explained by the significantly smaller number of Albanian subjects included in the study and random sampling variation. The c.521T>C SNP showed the strongest correlation with the c.597C>T in both populations and the similar results have been obtained in the study of Pasanen *et al.* [13], in which a large sample (468) of Caucasian subjects was included.

Compared with the analysis performed with single SNPs, haplotypes often better predict the associated phenotype. In the present study, the most common SLCO1B1 haplotype, **IJ/*IK/*IL,* contained the synonymous c.571 T>C SNP as compared with the reference sequence. It occurred at a frequency (35.6%) similar to that reported in the study of Pasanen *et al.* [13]. The c.521T>C SNP existed in two (**5* and **15/*16/*17*) major haplotypes in the Macedonian and Albanian populations and one new, identified in the Albanian population only. Both common haplotypes, with a frequency of 2.0 and 3.7% (for *5) and 2.4 and 8.6% (for **15/*16/*17*) in the Macedonian and Albanian subjects, respectively, contained the c.597C>T and c.439T>G SNPs. In the new haplotype identified in the Albanian population, instead of variant G allele in the c.439T>G SNP, variant C allele of c.571T>C existed, with referent alleles in other SNPs. Considering the significantly smaller number of subjects in the Albanian population, as potential limitation of this study, this result should be confirmed in a study in which a larger number of Albanian subjects would be included. The frequencies of the major haplotype **15/*16/*17* containing the variant alleles of the functionally most significant SNP pair c.388A>G/ c.521T>C (8.6 and 2.4% for Macedonians and Albanians, respectively) were lower than the frequency of haplotype **15* reported for Chinese (14.0%) and Japanese (15.8%), higher for Macedonians and comparable for Albanians with that of Caucasians (2.4%) and significantly higher than the one of African Americans (0.0%) [13,29].

In conclusion, this study presents an extensive analysis of *SLCO1B1* variant genotype and haplotype distribution in selected populations living in the Western Balkan Peninsula, Macedonians and Albanians for the first time. No significant differences ($p > 0.05$) in allelic frequencies and genotype distributions of the analyzed SNPs were observed between the two ethnic groups and the data are similar to those for Caucasians. About 8.6 and 2.4% of the Macedonians and Albanians, respectively, carrying the *SLCO1B1*15* or SLCO1B1**16* or SLCO1B1**17* variant may exhibit altered/impaired transport activity of OATP1B1.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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