Hellenic Journal of Cardiology (2017) xx, 1–5

Available online at www.sciencedirect.com

ScienceDirect

journal homepage: http://www.journals.elsevier.com/hellenic-journal-of-cardiology/

LETTER TO THE EDITOR

The *ABCB1* 2677G > T/A polymorphism is associated with baseline blood HDL-cholesterol levels in newly diagnosed hyperlipidemic patients

KEYWORDS
ABCB1;
hyperlipidemics;
blood lipids;
cholesterol

ABCB1, also known as P-glycoprotein (P-gp), is the most well studied member of the ATP-binding cassette (ABC) family of transporters. ABCB1 is embedded in the lumen-facing epithelial cell membranes of many biological barriers of organisms and is an efficient ATP-dependent efflux pump that is mainly responsible for the removal of numerous xenobiotics.[1] However, ABCB1 may play a role in additional, more intrinsic functions. A small number of studies have examined the association between common *ABCB1* gene polymorphisms and blood lipids.[2-6] Although all of these studies detected an association, the specifics differed in each study. The T allele of *ABCB1* 3435C>T (rs1045642) was associated with increased levels of total cholesterol (TC),[2,6] low-density lipoprotein cholesterol (LDL-C),[2,3,6] apolipoprotein A1 (ApoA1)[4] or high-density lipoprotein cholesterol (HDL-C).[5] In addition, *ABCB1* polymorphisms affect the efficacy of statin therapies.[7]

In a recently published report co-authored by some of the authors of this study, the distribution of the *ABCB1* 3435C>T and 2677G>T/A (rs2032582) genotypes in hyperlipidemic patients deviated from the corresponding distributions in non-selected health professionals, who served as controls.[8] In this study, we examine the association between rs1045642 and rs2032582 and blood lipids in the same group of hyperlipidemic patients and normolipidemic controls in an effort to further our understanding of the apparently complex but potentially clinically significant relationship between lipid homeostasis and ABCB1.

1. Methods

The participants were all Greek nationals and residents of northern Greece. The hyperlipidemic group consisted of 168 consecutive patients newly diagnosed with hyperlipidemia [total cholesterol (TC) > 240 mg/dL and/ or triglycerides (TG) > 200 mg/dL, LDL-C > 160 mg/dL] in the outpatient clinics of the 1st Propedeutic Department of Internal Medicine, AHEPA Hospital, Thessaloniki and the General Hospital of Goumenissa, Greece, from November 2011 to February 2013. The exclusion criteria were as follows: the use of lipid-lowering drugs or drugs that affect the blood lipid profile; a recent episode of infection or myocardial infarction; and a history or current diagnosis of hypothyroidism, hyperthyroidism, kidney or liver disease. The normolipidemic group consisted of 84 normolipidemic individuals of a similar age and sex distribution, mainly representing the staff of the above hospitals and of the Departments of Pharmacology, Faculty of Medicine, Aristotle University of Thessaloniki. The demographic and clinical characteristics as well as blood lipid levels at presentation are presented in Table 1. The study was approved by the ethics committee of the Aristotle University of Thessaloniki Medical School. Peripheral blood collection for the determination of lipid parameters and DNA isolation was performed following patient informed consent. Blood samples were collected from patients after 12 hrs of fasting. Plasma lipids (TC, TG, HDL-C) were determined by conventional enzymatic methods using the same type of instrument (Hitachi 912 chemistry analyzer, Roche Diagnostics, Indianapolis, USA). The Friedewald equation ([LDL-C] = [TC] − [HDL-C] − [TG]/5) was used to calculate plasma LDL-C concentrations, with the exception of six patients who presented with extreme TG values (> 400 mg/dL).

Genomic DNA was isolated from venous blood using a commercially available kit (Ron's Blood DNA minikit, Bioron

Peer review under responsibility of Hellenic Cardiological Society.

http://dx.doi.org/10.1016/j.hjc.2017.01.023

Please cite this article in press as: Agapakis D, et al., The *ABCB1* 2677G>T/A polymorphism is associated with baseline blood HDL-cholesterol levels in newly diagnosed hyperlipidemic patients, Hellenic Journal of Cardiology (2017), http://dx.doi.org/10.1016/j.hjc.2017.01.023

Table 1 Demographic, clinical, biochemical and genetic characteristics of the participants in the study.

	Hyperlipidemics	Normolipidemics
n	168	84
Age (years ± SD)	57.3 ± 11.0	53.5 ± 11.0
Females (%)	55.4	47.7
Smokers (%)	14.9	20.9
Alcohol consumption (%)	17.3	ND*
Hypertension (%)	68.5	ND
History of stroke (%)	7.1	ND
History of CAD (%)	13.7	ND
BMI (kg/m^2 ± SD)	30.2 ± 4.8	27.4 ± 4.4
TC (mg/dL ± SD)	245.3 ± 46.1	183.9 ± 29.1
TG (mg/dL ± SD)	236.5 ± 112.5	98.8 ± 35.5
LDL-C (mg/dL ± SD)	157.9 ± 48.4	115.9 ± 24.6
HDL-C (mg/dL ± SD)	41.0 ± 9.8	49.1 ± 12.8
ABCB1 2677G>T/A genotype (n)	168	84
GG (%)	45.2	31.0
GT (%)	40.5	47.6
TT (%)	14.3	21.4
Hardy-Weinberg equilibrium	Yes (p = 0.400)	Yes (p = 0.935)
ABCB1 3435 C>T genotype (n)	165	84
CC (%)	30.3	23.8
CT (%)	48.5	44.0
TT (%)	21.2	32.1
Hardy-Weinberg equilibrium	Yes (p = 0.961)	Yes (p = 0.585)
ABCB1 2677G>T/A-3435 C>T haplotype	r^2 = 0.589	r^2 = 0.512
G-C (%)	53.9	42.7
G-T (%)	11.8	12.7
T-C (%)	0.4	2.5
T-T (%)	33.9	42.1

* not determined.

GmbH, Ludwigshaften, Germany). The rs1045642 and rs2032582 polymorphisms were determined according to previously established polymerase chain reaction (PCR-RFLP) methods.[9] Possible deviations of genotype distributions from Hardy − Weinberg equilibrium were tested with the χ^2 test of goodness-of-fit. Univariate analyses with plasma lipid concentrations as dependent variables and the two *ABCB1* gene polymorphisms as independent variables were performed using type III sums of square statistics. Age, sex, BMI, and smoking served as covariates in both groups. A comparison of the genotype frequencies of the two groups was performed with the χ^2 test of independence. The *ABCB1* haplotype frequencies were calculated with the PHASE program version 2.1. P = 0.05 was used as the limit of statistical significance. The SPSS (v.22) statistical package was used for all calculations, with the exception of haplotypes.

2. Results

The genotype distributions of both polymorphisms were in accordance with Hardy-Weinberg equilibrium (p = 0.907 and 0.400 for rs1045642 and rs2032582, respectively). The two polymorphisms were in linkage disequilibrium in both groups (D' = 0.973 and r^2 = 0.589; D' = 0.881 and r^2 = 0.512 in hyperlipidemics and normolipidemics, respectively).

The results of the univariate analyses of the association between the *ABCB1* polymorphisms and the blood lipid concentrations are presented for hyperlipidemics and normolipidemics in Table 2. In the former, rs2032582 but not rs1045642 was strongly associated with HDL-C, but less so with LDL-C and TC. When the analysis followed the recessive model (TT vs. GG+GT), the association of rs2032582 with HDL-C was strengthened (p = 0.003) and the association with LDL-C and TC became marginally significant (p = 0.033 and p = 0.036, respectively). The direction of the association was consistently the same for all forms of cholesterol, with the TT genotypes displaying the highest values. In the normolipidemics group, no statistically significant associations were detected at first despite a general tendency of the TT genotypes of both polymorphisms to display higher cholesterol values. Application of the recessive model increased the association of rs1045642 (TT vs. CC+CT) with LDL-C to the level of statistical significance (p = 0.038).

Finally, a comparison of the distributions of the two polymorphisms in the two groups revealed a marginally significant over-representation of the ancestral homozygous genotypes (GG; CC) in hyperlipidemic patients (p = 0.029 and 0.058 for rs2032582 and for rs1045642, respectively). Based on our results, homozygotes for the ancestral genotypes were, on average, approximately twice as likely to be hyperlipidemics compared with carriers of

Please cite this article in press as: Agapakis D, et al., The *ABCB1* 2677G>T/A polymorphism is associated with baseline blood HDL-cholesterol levels in newly diagnosed hyperlipidemic patients, Hellenic Journal of Cardiology (2017), http://dx.doi.org/10.1016/j.hjc.2017.01.023