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Original research article

Common *ABCB1* polymorphisms in Greek patients with chronic hepatitis C infection: A comparison with hyperlipidemic patients and the general population

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ABSTRACT

Background: Hepatitis C virus infectivity and replication efficiency appears to be dependent on the lipid content and organization of the plasma membrane of the host cell, as well as of the intracellular membranous web. As there is increasing awareness of a role played by the efflux pump ABCB1 (p-glycoprotein, P-gp) in lipid homeostasis, its function could be a determinant of chronic HCV infection. The aim of the present study was to examine and compare the distribution of common *ABCB1* genotypes in patients with chronic HCV infection ($n = 168$), hyperlipidemic patients ($n = 168$) and a control group ($n = 173$), all from Greece.

Methods: Participants were genotyped for the *ABCB1*2677G>T/A and 3435C>T polymorphisms with previously reported PCR-RFLP methods. Genotype and allele frequency distributions were compared between the three groups with the χ^2 test of independence.

Results: The *ABCB1* 2677GG (ancestral) genotypes were significantly over-represented in patients with chronic hepatitis C compared to controls (39.3% *vs.* 26.6%, $p = 0.015$ according to the dominant model). A similar result was obtained when hyperlipidemic patients were compared to controls (45.2% *vs.* 26.6%, $p < 0.001$ according to the dominant model). Comparison of *ABCB1* 3435C>T genotype and allele distributions provided similar but not as significant differences. Genotype and allele distributions for both *ABCB1*2677G>T/A and 3435C>T were very similar between HCV patients and hyperlipidemic patients.

Conclusion: Our findings imply an influence of *ABCB1* polymorphisms on HCV infectivity, possibly through an effect on lipid homeostasis.

Introduction

Chronic hepatitis C virus (HCV) infection is a major health problem which affects an estimated 2–3% of the world's population and a leading cause of liver cirrhosis and hepatocellular carcinoma [1]. Although HCV appears to infect persons of all ages, genders and

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races worldwide, published prevalence data indicate a regional differentiation, with African and Central Asian countries bearing most of the burden [2]. While health policy, environmental and life-style factors could conceivably explain some of that differentiation, genetic factors – of the pathogen as well as of the host – could be involved as well; spontaneous clearance is reported to occur much less frequently in persons of African ancestry compared to patients of non-African ancestry [3]. So far very few studies have examined the association between host gene polymorphisms and susceptibility to HCV infection, with mostly negative results [4,5].

Both infection and propagation of HCV in the host liver cell are tightly linked to lipoprotein homeostasis; following an initial attachment to heparin sulfate proteoglycans (HSPGs), viral particles enter the cell by interacting with lipoprotein receptors (LDLR, SR-B1) among others, such as the tetraspanin CD81, and the tight junction proteins claudin-1 (CLDN1) and occludin (OCLN), while transferrin receptor 1 and the Niemann–Pick C1-like 1 cholesterol receptor (NPC1L1) were also assigned accessory but essential roles [6]. In addition, high density lipoprotein cholesterol (HDL-C) serum levels were recently reported as the most significant determinant of fasting lipoviral particle (LVP) load, and the LDLR-degrading proprotein convertase subtilisin kexin type 9 (PCSK9) levels as the strongest negative predictor of LVP ratio (LVP/all forms of infectious particles) in genotype 3-infected patients [7]. HCV assembly takes place in an endoplasmic reticulum-derived membranous web, in a process which hijacks enzymes, apolipoproteins and lipids that are part of host lipoprotein synthesis, and eventually leads to the formation of infectious lipoviral particles [8]. Silencing of miR-122, a liver-specific micro RNA which facilitates HCV replication in infected cells, was shown to down regulate the expression of cholesterol biosynthesis and transport genes [9]. Moreover, the cholesterol and sphingomyelin (SM) content of the target membranes was shown to affect the membrane fusion and cell entry of HCV and other flaviviruses [10], whereas HCV infectivity appears to be also related to the lipid content and structure of lipoviral particles [11].

ABCB1 (also known as P-glycoprotein, P-gp) – the product of the *ABCB1/MDR1* gene – is a well-studied cytoplasmic membrane efflux pump, normally expressed in various biological barriers, including the apical surface of hepatocytes lining bile canaliculi and small biliary ductules [12,13]. Recent and older evidence suggest that ABCB1 is involved in an intracellular trafficking system for cholesterol and cholesterol liposomes [14,15]. As cholesterol enrichment of not only the target membranes but also of the HCV particles appears to be critical for their infectivity [10,11], the latter could conceivably be influenced by factors affecting ABCB1 function, including *ABCB1* gene polymorphisms.

In this study we examine the effect of two common, genetically linked, *ABCB1* gene polymorphisms, 2677G>T/A (rs2032582) and 3435C>T (rs1045642), on the risk of chronic HCV infection, by comparing their respective genotype and allele frequencies in Greek patients chronically infected with HCV and control individuals with no indication of HCV infection. Given the importance of lipids for HCV propagation, we have also included a group of hyperlipidemic patients in our study.

Materials and methods

Study subjects

The study involved a cohort of 168 unrelated Greek patients with chronic hepatitis C, undergoing treatment with pegylated interferon-α and ribavarin or interferon-α, ribavarin and telaprevir, in Hepatology Departments of Thessaloniki and Athens, Greece (Table 1). Diagnosis of chronic hepatitis C was based on the detection of both HCV antibodies and HCV RNA in the presence of signs of chronic hepatitis, either by elevated aminotransferases or by histological changes of chronic hepatitis C. One hundred and seventy two apparently healthy, unrelated Greek adults (age (±SD): 45.1 ± 13.79; 52.7% males, 47.3% females), with no symptoms or positive diagnosis of chronic HCV or other viral infection, recruited mainly from the medical and paramedical staff of the aforementioned centers, served as the control group. A third group included 168 Greek adult, hyperlipidemic patients (age (±SD): 57.3 ± 10.96; 55.4% males, 44.6% females), newly diagnosed with hypercholesterolemia, hypertriglyceridemia or both (total cholesterol (TC) > 240 mg/dL and/or triglycerides (TG) > 200 mg/dL, low density lipoprotein cholesterol (LDL-C) > 160 mg/dL) in the outpatient clinics of the 1st Propedeutic Department of Internal Medicine, AHEPA Hospital, Thessaloniki, Greece. Informed consent was obtained from each participant in the study. The study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki, as revised in 1983, as reflected in a priori approval by the Ethical Committee of the Aristotle University of Thessaloniki Medical School.

Table 1
Comparison of characteristics of patients stratified according to geographical origin.

	Athens (n = 116)	Thessaloniki (n = 52)	p
Age (years ± SD)	48.4 ± 15.13	47.6 ± 13.57	0.799
Sex (% male:female)	60.6:39.4	70.0:30.0	0.341
HCV genotype (% 1/4:2/3)	76.5:23.5	88.5:11.5	0.060
TC (mg/dL ± SD)	163.7 ± 41.93	169.5 ± 44.94	0.717
TG (mg/dL ± SD)	123.7 ± 63.00	127.9 ± 80.36	0.864
LDL-C (mg/dL ± SD)	94.4 ± 35.70	102.4 ± 43.93	0.560
HDL-C (mg/dL ± SD)	54.4 ± 16.01	46.5 ± 19.87	0.324
ABCB1 2677 G>T/A[a] (%)	GG: 37.9	GG: 42.3	0.525
	GT: 47.4	GT: 38.5	
	TT: 14.7	TT: 19.2	
ABCB1 3435 C>T (%)	CC: 27.6	CC: 30.8	0.887
	CT: 46.6	CT: 46.2	
	TT: 25.9	TT: 23.1	
Haplotype 2677–3435 (%)	G-C: 49.8	G-C: 49.8	0.270
	G-T: 11.7	G-T: 11.3	
	T-C: 4.0	T-C: 0.6	
	T-T: 34.4	T-T: 38.3	

[a] The A allele was not determined.
SD, standard deviation; TC, total cholesterol; TG, triglycerides; LDL-C, low density lipoprotein cholesterol; HDL-C, high density lipoprotein cholesterol.

Genotyping

Genomic DNA was isolated from peripheral blood using a commercial DNA isolation kit (Ron's Blood DNA minikit, Bioron GmbH, Ludwigshaften, Germany). Genotyping for either *ABCB1* polymorphism was accomplished with a previously published PCR-RFLP method [16], with small modifications. Briefly, the sequence harboring the *ABCB1* 2677G>T/A polymorphism was amplified at an annealing temperature of 60 °C, using the forward primer: TGCAGGCTATAGGTTCCAGG, and the reverse primer: TTTAGTTTGACTCACCTTCCCG (which is mutagenic in that its 3′ G allows to create a *Bsh*NI (*Ban*I) recognition sequence). The amplicon which has a length of 224 bp was then incubated at 37 °C with *Bsh*NI (Thermo Fisher Scientific Inc., Waltham, MA, USA) which leaves it undigested in presence of the T allele, but cuts into a 198 and a 26 bp fragment in presence of the G or the A allele. The latter was not independently determined in our study, given its very low frequency in Caucasian populations and our own preliminary experiments (<2%). Similarly, the *ABCB1* 3435C>T-containing sequence was amplified at an annealing temperature of 52 °C, with the forward primer: CTCACAGTAACTTGGCAG, and the reverse primer: CTTACATTAGGCAGTGAC, and digested at 37 °C with *Mbo*I (Bioron GmbH, Ludwigshaften, Germany) to produce