ORIGINAL PAPER

DOI: http://dx.doi.org/10.15403/jgld-168

SERPINA1 and *HSD17B13* Gene Variants in Patients with Liver Fibrosis and Cirrhosis

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Received: 10.06.2019
Accepted: 31.07.2019

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ABSTRACT

Background & Aims: Two single nucleotide polymorphisms (SNPs) in *SERPINA1* (Pi*Z rs28929474 and Pi*S rs17580) are risk factors for developing liver cirrhosis. A recent study identified a common SNP in *HSD17B13* (rs72613567) that conferred protection from chronic liver disease. The aim of the present study was to test these associations in a cohort of Lithuanian patients with liver fibrosis or cirrhosis.
Methods: The study included 302 patients with cirrhosis, 127 patients with liver fibrosis (METAVIR stages I-III) and 548 controls, all from Lithuania. SNPs were genotyped by quantitative PCR, using TaqMan allelic discrimination assays. Adjusted p value of ≤ 0.016 was considered significant.
Results: Genotype distributions of *SERPINA1* and *HSD17B13* SNPs were in Hardy-Weinberg equilibrium. *SERPINA1* Pi*Z was not associated with liver fibrosis or cirrhosis. *HSD17B13* rs10433937 (in high linkage disequilibrium with rs72613567; r^2=0.96) also showed no overall association with liver disease, but the GG-genotype was associated with reduced risk of liver fibrosis (aOR 0.37, p=0.03). *SERPINA1* Pi*S was associated with higher risk of developing hepatic fibrosis (aOR 3.42, p=0.001) and cirrhosis (aOR 2.59, p=0.02).
Conclusions: We found that *SERPINA1* Pi*S variant conferred an increased risk of developing liver fibrosis, while *SERPINA1* Pi*Z and *HSD17B13* rs10433937 were not associated with liver fibrosis or cirrhosis of different aetiology.

Key words: *SERPINA1* – *HSD17B13* – liver cirrhosis – liver fibrosis.

Abbreviations: A1AT: alpha1-antitrypsin; ALT: alanine aminotransferase; GWAS: genome-wide association studies; HSD17B13: 17-beta-hydroxysteroid dehydrogenase 13; NAFLD: non-alcoholic liver disease; SNP: single nucleotide polymorphisms; HBV: hepatitis B virus; HCV: hepatitis C virus.

INTRODUCTION

Chronic liver disease is one of the leading causes of illness worldwide and is associated with high mortality rates [1]. The most common causes of chronic liver injury are viral hepatitis C (HCV) and B (HBV) infections, alcohol consumption, non-alcoholic fatty liver disease (NAFLD), autoimmune hepatitis and other conditions [1–4]. However, the disease course differs between individuals and this variability at least in part is attributed to genetic risk factors [5–7]. In recent years, the role of genetic factors in progression of chronic liver disease has become one of the major research areas in the field of hepatology [8–11].

Genome-wide association studies (GWAS) have identified the relationship between *PNPLA3*, *TM6SF2*, *MBOAT7* single nucleotide polymorphisms (SNPs) and increased risk for chronic liver disease [12]. In previous studies, we found a significant association between *PNPLA3* rs738409 and chronic liver injury in our cohort, although no significant associations between *TM6SF2* and *MBOAT7* variants were observed [13, 14].

GWAS represent a powerful tool to identify common genetic variants involved in the pathogenesis of polygenic diseases [15]. *SERPINA1* gene encodes alpha1-antitrypsin (A1AT). Single nucleotide polymorphisms in *SERPINA1* gene lead to decreased serum A1AT levels and systemic disease that affects mostly lungs and liver [16]. Homozygous carriage and severe alpha1-antitrypsin deficiency disease (A1ATD) course is well studied, but the significance of heterozygous carriage (Pi*Z and Pi*S variants) was only recently related with liver

cirrhosis caused by NAFLD and alcohol in Western European population [17].

A recent large-scale study used the exome sequencing method in four independent cohorts to identify associations between 17-beta-hydroxysteroid dehydrogenase 13 (*HSD17B13*) variants and chronic liver injury [18]. The clear mechanisms of action of this gene and its variants is not yet known, but the results showed that *HSD17B13* rs72613567 was associated with a reduced risk of alcoholic and non-alcoholic liver diseases, including cirrhosis and steatosis progressing to steatohepatitis [18].

In the present study, we evaluated the association between *SERPINA1* rs28929474 (Pi*Z), rs17580 (Pi*S), and *HSD17B13* rs10433937 (which is in high linkage disequilibrium with the *HSD17B13* rs72613567 (r^2=0.96), studied by Abul-Husn et al. [18]) and the risk of liver fibrosis or cirrhosis of different aetiology in an Eastern European patient cohort. To our knowledge, this is the first study in an Eastern European population that replicated the findings of a large case-control study of Western European population [17] and exome sequencing analysis [18] in liver diseases.

METHODS

Patients

In this study, 977 patients (548 controls, 302 patients with liver cirrhosis and 127 patients with liver fibrosis) were recruited during the period 2012 – 2019 at the Department of Gastroenterology in the Lithuanian University of Health Sciences. The diagnosis of cirrhosis was based on clinical features, laboratory tests and ultrasound findings. The liver fibrosis group included patients with stage I-III METAVIR confirmed by histology [19]. Control samples included 548 voluntary, unrelated Lithuanian blood donors, from our previous genotyping study on the prevalence of *HFE* mutations in the Lithuanian population [20].

The study was in accordance with the 1975 Declaration of Helsinki (6th revision, 2008) and was approved by the Regional Kaunas Ethics Committee (Protocol No. BE-10-2, approval date: 08 March 2011). All subjects gave an informed consent to participate in this study.

Genotyping

The genotyping was performed at the Institute for Digestive Research at the Lithuanian University of Health Sciences Hospital. Genomic DNA from samples was isolated from whole blood mononuclear cells using a salting-out method and stored at –20^{0}C until analysis as described previously [21]. *SERPINA1* rs28929474, rs17580 and *HSD17B13* rs10433937 SNPs were genotyped by real-time PCR (RT-PCR), using TaqMan® allelic discrimination assays with a 7500TM Fast real-time PCR system (Life Technologies, Carlsbad, California, USA).

Statistical analysis

Analysis of the data were carried out using PLINK software version 1.07 [22]. Alleles and genotypes frequencies between groups were compared using Pearson's goodness-of-fit χ^2. Associations between control and cases groups with SNP alleles and genotypes were calculated using logistic regression analysis and adjustment for age and gender was performed. Age difference between groups was analysed using ANOVA. Gender distributions were compared using the χ^2 test. Adjusted p value of ≤ 0.016 was considered to be statistically significant.

RESULTS

Characteristics of study participants

Demographic and clinical characteristics of our cohort are represented in Table I. Cirrhotic patients were significantly (p<0.001) older than those with liver fibrosis or controls. Gender distribution between groups was similar, with no significant differences. The major causes of liver cirrhosis were alcohol and chronic HCV infection. Most of the patients in liver fibrosis group had chronic HCV infection. To eliminate the potential bias of differences in age and gender distribution among the groups, these parameters were included as covariates in the logistic regression analysis. The frequencies of alleles and genotypes were in Hardy-Weinberg equilibrium (p>0.05).

Table I. Demographic and clinical characteristics of the patients

	Liver cirrhosis (n=302)	Liver fibrosis (n=127)	Controls (n=548)	p value
Age (mean ± SD), years	50.3 ± 12.7	47.5 ± 12.1	47.2 ± 8.9	<0.001
Gender, n (%)				
Male	150 (49.7)	78 (61.4)	278 (50.7)	0.06
Female	152 (50.3)	49 (38.6)	270 (49.3)	
Aetiology of liver disease, n (%)				
Alcohol	180 (56.3)			
HCV infection	122 (43.7)	116 (91.3)		
Autoimmune		4 (3.1)		
Cryptogenic		4 (3.1)		
Steatohepatitis		3 (2.5)		

HCV: hepatitis C virus

SERPINA1 Pi*Z and Pi*S variants and risk of liver cirrhosis and fibrosis

We found similar *SERPINA1* Pi*Z variant distribution among all the groups of the study (Tables II, III and IV). In the control group, the risk allele accounted for 1.09%, in the liver fibrosis group for 1.57%, in the cirrhosis group for 1.32%, in the combined fibrosis and cirrhosis group for 1.4%, in the alcohol-induced cirrhosis group for 1.67% and in the HCV-induced cirrhosis group for 0.82%. Likewise, no significant differences among the different study groups and genotypes were observed. No patient had homozygous Pi*ZZ genotype.

SERPINA1 Pi*S risk allele was associated with a higher risk of liver fibrosis (aOR 3.42, p=0.01) and showed a tendency towards the likelihood of developing liver cirrhosis overall (aOR 2.59, p=0.02). This tendency was also observed in the alcohol induced cirrhosis group (aOR 2.59, p=0.04), but not in the HCV cirrhosis group (aOR 2.67, p=0.07). Moreover, *SERPINA1* Pi*S heterozygous genotype was associated with liver fibrosis and cirrhosis risk (aOR 2.77, p=0.004) and showed a trend towards increased alcohol induced liver cirrhosis risk (aOR 2.57, p=0.03). No patients had the homozygous Pi*SS genotype.

Table II. Distribution of the *SERPINA1* and *HSD17B13* SNP alleles and genotypes in controls, liver fibrosis and liver cirrhosis groups

Allele/Genotype	Controls (n=548)	Liver fibrosis (n=127)			Cirrhosis (n=302)		
	n (%)	n (%)	aOR (95% CI)	p	n (%)	aOR (95% CI)	p
HSD17B13 rs10433937							
G	147 (26.82)	29 (22.83)	0.81 (0.60-1.10)	0.18	82 (27.15)	0.99 (0.81-1.22)	0.95
T	401 (73.18)	98 (77.17)			220 (72.85)		
GG	55 (10.04)	5 (3.94)	0.37 (0.14-0.96)	**0.03**	38 (12.58)	1.22 (0.78-1.920	0.39
GT	182 (33.21)	46 (36.22)	1.03 (0.69-1.56)	0.87	88 (29.14)	0.85 (0.62-1.17)	0.33
TT	311 (56.75)	76 (59.84)	1 (Reference)		176 (58.28)	1 (Reference)	
SERPINA1 Pi*Z variant (rs28929474)							
T	6 (1.09)	2 (1.57)	1.07 (0.30-3.87)	0.92	4 (1.32)	1.13 (0.45-2.81)	0.80
C	542 (98.91)	125 (98.43)			298 (98.68)		
TT	0 (0)	0 (0)	NA	NA	0 (0)	NA	NA
TC	12 (2.19)	3 (2.36)	1.08 (0.30-3.89)	0.91	7(2.32)	1.06 (0.41-2.72)	0.90
CC	536 (97.81)	124 (97.64)	1 (Reference)		295 (97.68)	1 (Reference)	
SERPINA1 Pi*S variant (rs17580)							
A	6 (1.10)	4 (3.15)	3.42 (1.34-8.76)	**0.01**	7 (2.32)	2.59 (1.16-5.77)	**0.02**
T	542 (98.90)	123 (96.85)			295 (97.68)		
AA	0 (0)	0 (0)	NA	NA	0 (0)	NA	NA
AT	11 (2.01)	8 (6.30)	3.28 (1.29-8.34)	**0.008**	15 (4.97)	2.55 (1.16-5.63)	**0.02**
TT	537 (97.99)	119 (93.70)	1 (Reference)		287 (95.03)	1 (Reference)	

aOR: adjusted odds ratio; CI: confidence interval; NA: not available

Table III. Distribution of the *SERPINA1* and *HSD17B13* SNP alleles and genotypes in the different etiology of liver cirrhosis groups

Allele/Genotype	Controls (n=548)	Alcohol induced cirrhosis (n=180)			HCV induced cirrhosis (n=122)		
	n (%)	n (%)	aOR (95% CI)	p	n (%)	aOR (95% CI)	p
HSD17B13 rs10433937							
G	147 (26.82)	44 (24.44)	0.89 (0.69-1.15)	0.37	38 (31.15)	1.13 (0.85-1.50)	0.41
T	401 (73.18)	136 (75.56)			84 (68.85)		
GG	55 (10.04)	21 (11.66)	0.99 (0.57-1.71)	0.96	17 (13.93)	1.53 (0.83-2.80)	0.17
GT	182 (33.21)	46 (25.56)	0.70 (0.48-1.04)	0.08	42 (34.43)	1.14 (0.74-1.75)	0.55
TT	311 (56.75)	113 (62.78)	1 (Reference)		63 (51.64)	1 (Reference)	
SERPINA1 Pi*Z variant (rs28929474)							
T	6 (1.09)	3 (1.67)	1.47 (0.54-4.00)	0.45	1 (0.82)	0.62 (0.13-2.93)	0.55
C	542 (98.91)	177 (98.33)			121 (99.18)		
TT	0 (0)	0 (0)	NA	NA	0 (0)	NA	NA
TC	12 (2.19)	6 (3.33)	1.54 (0.57-4.17)	0.39	1 (0.82)	0.37 (0.05-2.87)	0.32
CC	536 (97.81)	174 (96.67)	1 (Reference)		121 (99.18)	1 (Reference)	
SERPINA1 Pi*S variant (rs17580)							
A	6 (1.10)	5 (2.78)	2.59 (1.05-6.40)	0.04	3 (2.46)	2.67 (0.93-7.65)	0.07
T	542 (98.90)	175 (97.22)			119 (97.54)		
AA	0 (0)	0 (0)	NA	NA	0 (0)	NA	NA
AT	11 (2.01)	9 (5.00)	2.57 (1.05-6.30)	0.03	6 (4.92)	2.53 (0.92-6.97)	0.06
TT	537 (97.99)	171 (95.00)	1 (Reference)		116 (95.08)	1 (Reference)	

aOR: adjusted odds ratio; CI: confidence interval; NA: not available

Table IV. Distribution of the *SERPINA1* and *HSD17B13* SNP alleles and genotypes in controls and combined liver fibrosis and cirrhosis groups

Allele/Genotype	Controls (n=548)	Combined liver fibrosis and cirrhosis (n=429)		
	n (%)	n (%)	aOR (95% CI)	P
HSD17B13 rs10433937				
G	147 (26.82)	111 (25.87)	0.93 (0.75-1.16)	0.51
T	401 (73.18)	318 (74.13)		
GG	55 (10.04)	43 (10.02)	0.97 (0.63-1.49)	0.97
GT	182 (33.21)	134 (31.24)	0.91 (0.69-1.20)	0.91
TT	311 (56.75)	252 (58.74)	1 (Reference)	
SERPINA1 Pi*Z variant (rs28929474)				
T	6 (1.09)	6 (1.40)	0.88 (0.40-2.05)	0.77
C	542 (98.91)	423 (98.60)		
TT	0 (0)	0 (0)	NA	NA
TC	12 (2.19)	10 (2.33)	1.07 (0.46-2.49)	0.88
CC	536 (97.81)	419 (97.67)	1 (Reference)	
SERPINA1 Pi*S variant (rs17580)				
A	6 (1.10)	11 (2.56)	2.61 (0.40-5.55)	0.016
T	542 (98.90)	418 (97.44)		
AA	0 (0)	0 (0)	NA	NA
AT	11 (2.01)	23 (5.36)	2.77 (1.33-5.74)	0.004
TT	537 (97.99)	406 (94.64)	1 (Reference)	

aOR: adjusted odds ratio; CI: confidence interval; NA: not available

***HSD17B12* rs10433937 variant and risk of liver cirrhosis and fibrosis**

HSD17B12 rs10433937 polymorphism allelic distribution among controls and different liver injury aetiologies showed no significant differences (Tables II, III and IV). We found no significant link between *HSD17B12* rs10433937 alleles and the risk to develop liver disease. However, in genotypic analysis, genotype GG showed a tendency to be protective against liver fibrosis (aOR = 0.37, p=0.03).

DISCUSSION

In the present study, we evaluated the role of two *SERPINA1* gene variants and *HSD17B13* rs10433937 variant in our Eastern European cohort of liver fibrosis and cirrhosis of different aetiology. *HSD17B13* rs10433937 and *SERPINA1* Pi*Z variant did not confer an increased risk to develop liver disease in our cohort. *SERPINA1* Pi*S variant was associated with the development of liver fibrosis and cirrhosis. This is the first study that has replicated the findings of a large case-control study [17] and exome sequencing analysis [18] on liver diseases recently reported in a Western European population.

Homozygosity for Pi*Z allele is a well-established risk factor for the development of liver damage in patients with A1ATD. Mutations in *SERPINA1* gene result in accumulation of misfolded A1AT in endoplasmic reticulum of hepatocytes and toxicity [23]. Heterozygosity for Pi*Z variant was recently confirmed to be a risk factor for NAFLD and alcohol-induced liver cirrhosis in a large cohort of patients, although the clear mechanism how heterozygous AAT variants predispose to liver damage is not known [17]. Previously published studies also confirmed Pi*Z variant association with liver injury. Schaefer et al. [24] found that Pi*Z variant in cirrhosis was associated with more advanced disease and decompensation [24]. The study by Regev et al. [25] confirmed that heterozygous Pi*Z variant carriage worsens already existing liver disease. However, the study by Al-Jameil et al. [26] could not establish an association between heterozygous A1AT genotypes and liver cirrhosis. Valenti et al. [27] found no link between liver injury in NAFLD and heterozygosity for *SERPINA1* variants, but reported that A1AT mutations increased hyperferritinemia and the risk for sinusoidal iron accumulation.

The role of Pi*S variant remains controversial, because this variant does not cause abnormal A1AT structure and is not associated with an increased liver disease risk [28]. Few studies investigated the link between heterozygosity for Pi*S variant and liver injury risk [17, 27, 29], without confirming strong associations. We found that Pi*S variant was associated with a greater risk to develop liver fibrosis and cirrhosis than Pi*Z variant. These findings suggest that heterozygosity for Pi*S variant is relevant in developing liver injury, specifically when environmental factors, such as alcohol or chronic viral hepatitis are present. These conditions may lead to some changes in the A1AT structure and serum level. Moreover, Pi*S variant seemed to increase the risk of cirrhosis of any aetiology.

HSD17B13 is a member of 17-beta-hydroxysteroid dehydrogenases that mostly are involved in sex hormone metabolism [30]. The role of *HSD17B13* variants in liver fibrosis and cirrhosis is not yet fully understood. Previous studies demonstrated an association between this gene and its variants with increased lipogenesis in mouse liver and cultured hepatocytes [31] and with histological features of NAFLD [32]. However, another study found that these gene variants were protective against NAFLD [33]. A novel association of *HSD17B13* rs7261356 with reduced liver disease risk and decreased serum alanine aminotransferase (ALT) levels was reported by Abul-Husn et al. [18]. Recently, a Danish population study showed that carriage of *HSD17B13* rs72613567 variant was associated with lower ALT levels and found that this ALT-lowering effect was amplified by a genetic risk of fatty liver disease and alcohol consumption [34]. Yang et al. [35] confirmed that *HSD17B13* rs72613567 had a protective effect against alcoholic liver disease and NAFLD. Whitfield et al. [36] found potentially protective trait of another *HSD17B13* variant (rs10433879) against alcohol-induced liver injury. We evaluated *HSD17B13* rs10433879, which is in a close proxy to *HSD17B13* rs7261356 (r^2=0.958), association with liver injury. We found no link between *HSD17B13* rs10433879 and liver injury. However, our results showed that genotype GG might be protective against liver fibrosis and a larger cohort is required to confirm this tendency.

CONCLUSIONS

Our data revealed that *SERPINA1* Pi*S variant confers an increased risk of developing liver fibrosis and cirrhosis, while *SERPINA1* Pi*Z variant and *HSD17B13* rs10433937 are not associated with liver fibrosis or cirrhosis of different aetiology.

Conflict of interests: None to declare.

Authors' contributions: J.Sk., J.K. conception and design of the study; V.B.-B., J.Sk., I.V., J.S., V.P., J.K. D.P. data acquisition; J.Sk., V.B.-B. statistical analysis; V.B.-B., J. Sk. DNA extraction and genotyping; V.B.-B., J.Sk., J.K., F.L. interpreted the results; V.B.-B., J.K. analyzed the data and drafted the manuscript. All authors critically revised the manuscript, approved the final version to be published, and agreed to be accountable for all aspects of the work.

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