IMPACT OF GENETIC VARIATION IN THE VASOPRESSIN 1A RECEPTOR ON THE DEVELOPMENT OF ORGAN FAILURE IN PATIENTS ADMITTED FOR ACUTE DECOMPENSATION OF LIVER CIRRHOSIS

Short title: AVP1aR SNPs in liver cirrhosis

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List of abbreviations: ACLF, acute-on-chronic liver failure; AD, acute decompensation; APTT, activated partial thromboplastin time; AVP, arginine vasopressin; CANONIC, chronic liver failure (CLIF) Acute-on-Chronic Liver Failure in Cirrhosis; CRP, C-reactive protein; DBP, diastolic blood pressure; HBV, hepatitis B virus; HCV, hepatitis C virus; HR, hazard ratio; INR, international normalized ratio; NAFLD, non-alcoholic fatty liver disease; PBC, primary biliary cholangitis; PT, prothrombin time; SNP, single nucleotide polymorphism; V1aR, vasopressin 1a receptor; WBC, white blood cell count.

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ABSTRACT

Background: Vasopressin receptor mediated vasoconstriction is thought to be involved in the pathogenesis of organ failure in acute-on-chronic liver failure (ACLF).

Methods: We studied the association between six single nucleotide polymorphisms (SNPs) of the vasopressin 1a receptor gene and the development of organ failure in 826 patients admitted for acute decompensation of liver cirrhosis (AD, n=641) or ACLF (n=185).

Results: No associations were found for SNPs with presence of circulatory or renal failure. A C>T mutation in SNP rs7308855 and a T>A mutation in SNP rs7298346, showed an association with the presence of coagulation failure in the whole population (n=61, p=0.024 and p=0.060, respectively) and in the subgroup of patients with ACLF (n=44, p=0.081 and p=0.056, respectively).

Conclusion: Genetic variation in the vasopressin 1a receptor was found not to be associated with circulatory or renal failure, but with the presence of coagulation failure in patients with AD and ACLF.

Key-words: arginine vasopressin 1a receptor; single nucleotide polymorphisms; cirrhosis; acute-on-chronic liver failure

INTRODUCTION

Acute decompensation of liver cirrhosis (AD) is defined as the acute development of one or more complications of the underlying liver disease. Acute-on-chronic liver failure (ACLF) is a distinct syndrome from AD, as it is associated with the presence of organ failure, high short-term mortality rates, age and precipitating events [1]. Systemic inflammation seems to play a key role in the development of ACLF. Also systemic hemodynamic dysfunction and the activation of endogenous vasoconstrictor systems are thought to be involved in the pathogenesis [2]. A decreased systemic vascular resistance leads to the activation of compensatory vasoconstrictor systems and the non-osmotic release of arginine vasopressin (AVP) [3, 4]. AVP is a neurohypophyseal hormone, which plays a prominent role in the cardiovascular system and mediates vascular smooth muscle contraction via the V1a receptor (AVP1aR) [5]. A previous study has found an association between single nucleotide polymorphisms (SNPs) in the promotor region of AVP1aR and presence of essential hypertension in non-obese Japanese subjects [6]. Considering the important role of AVP1aR in regulating vascular tone and baroreceptor sensitivity [7], we hypothesized that heterogeneity in AVP1aR may affect the risk of developing renal and circulatory failure in cirrhotic patients. This may be relevant information in clinical practice, as patients with certain genotypes of AVP1aR may need more intensive surveillance and treatment. Aim of this study was to investigate whether genetic variation of AVP1aR is associated with the presence of circulatory failure, renal failure and outcome in cirrhotic patients with AD and ACLF.

METHODS

Patients

This study is an ancillary study of the prospective, observational CANONIC study [1]. In that study, 1343 patients hospitalized for AD of cirrhosis were included between February and September 2011. The HCB-IDIBAPS Biobank in Barcelona (Spain) manages the CANONIC database and storage of biomaterials. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki (6^{th} revision, 2008). Initially, we performed a pilot study including 188 patients from the CANONIC database without (n=93) and with ACLF (n=95). These samples were centrally randomly selected as stratified groups by the HCB-IDIBAPS Biobank personnel, who were not involved in this study. Based on these preliminary results, the study population was extended involving all 826 CANONIC patients who gave informed consent for isolation and storage of genomic DNA for future research. ACLF and individual organ failures were defined using the CLIF-Organ Failure score [8]. This scoring system is a simplification of the CLIF-sequential Organ Failure Assessment (SOFA) scale, which was developed by the CANONIC study for defining and diagnosing organ failure in cirrhotic patients. The CLIF-Organ Failure score involves a total of 6 organ systems (i.e. liver, kidney, brain, coagulation, circulation and respiration). For each system, 3 subscores have been defined: subscore 1= normal or moderate organ dysfunction, subscore 2= marked organ dysfunction, subscore 3= organ failure. According to the CLIC-Organ Failure score, the following criteria are defined for individual organ failures: liver failure= bilirubin ≥12 mg/dl; kidney failure= creatinine ≥2 mg/dl and <3.5 mg/dl (subscore 2) or creatinine ≥3.5 mg/dl or renal replacement (subscore 3); cerebral failure= West-Haven grade 3-4; coagulation failure= INR ≥2.5; circulatory failure= use of vasopressors; respiratory failure= PaO2/FiO2 ratio ≤200 or SpO2/FiO2 ratio ≤214. Patient characteristics and clinical data were retrieved from the CANONIC database.

Genotyping

For genetic testing, DNA was isolated from 10 mL EDTA blood of each patient with consent for genetic testing. DNA samples were stored at -80°C. Genotyping was performed in the Leiden University Medical Centre, Leiden, the Netherlands. Six SNPs of AVP1aR with potential clinical relevance were identified from preliminary studies [6, 9]. The genotype of rs7298346 was identified by polymerase chain reaction (PCR) with allele-specific amplification primers. Genotypes of the other 5 variants were identified by PCR followed by restriction fragment length polymorphism. PCR was performed in a 25 µl reaction volume containing 50 ng DNA, ReddyMix (Thermo Scientific, Waltham, MA, USA) and 0.24 µM of each primer. Restriction enzymes (New England BioLabs, Ipswich, MA, USA) used to determine the genotypes were BfaI, MLuCI, PstI, Tsp45I and Sau3AI for rs113481894, rs11174817, rs7308855, rs1042615 and rs10747983 respectively. The DNA fragments were separated by electrophoresis on 2,5% agarose gel, and visualised by staining with ethidium bromide. The investigators were blinded for clinical outcomes during determination of genotypes of the AVP1a receptor gene.

Statistical analysis

For all SNPs, deviation from Hardy-Weinberg equilibrium was calculated using Pearson's chi –square test. The association between SNPs and presence of ACLF, individual organ failures and levels of relevant laboratory values were evaluated using Fisher's exact test. A Cox proportional hazard regression analysis was performed in order to assess the relation of SNPs with overall survival in all patients and in the subgroup of patients with ACLF.

RESULTS

In the pilot study (n=188), an association for a T>A mutation in rs7298346 and, to a lesser extent, for a C>T mutation in rs7308855 with the presence of renal failure at time of hospital admission was found in patients with ACLF (n=64, p=0.025 and p=0.103, respectively). The same mutations showed significant associations with lower 90-day survival in all patients (HR=1.81, 95%CI=1.02-3.23, p=0.044 and HR=2.17, 95%CI=1.17-4.01, p=0.013, respectively). No association was found between SNPs and the presence of circulatory failure.

Patient characteristics of the whole cohort study at time of hospital admission for AD of cirrhosis (n=641) or ACLF (n=185) are shown in table 1. All SNPs were in Hardy-Weinberg Equilibrium, except for rs10747983 (P<0.05). In contrast to the results of the pilot study, no association between the studied SNPs and the presence of renal failure or 90-day survival was found. Moreover, no association between SNPs and the presence of ACLF (table 1) or single circulatory, liver, cerebral or respiratory failure was found. When comparing patients with CLIF-Organ Failure subscore 1 (normal or moderate organ dysfunction) vs. 2 (marked organ dysfunction) or 3 (organ failure), no associations between SNPs and these organ functions were found either.

Instead, a C>T mutation in SNP rs7308855 showed a significant association with the presence of 'coagulation failure' (defined as INR ≥2.5 according to CLIF-Organ Failure score) in cirrhotic patients admitted with AD or ACLF (table 2) and showed a clear trend towards the presence of coagulation failure in the subgroup of patients with ACLF (n=44, p=0.081). A trend was also found for a T>A mutation in SNP rs7298346 to be associated with the presence of coagulation failure in the whole study population (table 2) and in the subgroup of patients with ACLF (p=0.056). When comparing patients with CLIF-Organ Failure subscore 1 (n=643) vs. 2 and 3 (n=170), the same mutations in these SNPs were more frequently present in patients with subscore 2 or 3 as compared to patients with subscore 1 (p=0.050 and p=0.055, respectively). Despite of the association found for a mutation in SNP rs7308855 and rs7298346 with coagulation failure, median values of markers of

coagulation function (INR, prothrombin time, activated partial thromboplastin time and platelet count) did not significantly differ between patients with or without a mutation in these SNPs.

Finally, no association between the studied SNPs and survival after 28 days and 3, 6 and 12 months of follow-up was found.

DISCUSSION

The results of the present study suggest that there is a weak association between two of the studied SNPs of AVP1aR with an INR ≥2.5 in patients admitted for AD of cirrhosis or ACLF. No associations with SNPs were found with the presence of other types of organ failure.

AVP1aR is widely expressed and is involved in diverse functions including vascular smooth muscle contraction [10]. The presence of peripheral vasodilation contributes to the development of portal hypertension in cirrhosis. The subsequent activation of endogenous vasoconstrictor systems, such as AVP, plays a role in the development of ascites, hyponatremia and hepatorenal syndrome [1,3]. In ACLF, activation of these vasoconstrictor systems is thought to contribute to the pathogenesis [2]. Because of its prominent role in the cardiovascular system, we hypothesized that genetic heterogeneity in AVP1aR might be involved in the development of organ failure in cirrhosis, especially in circulatory and renal failure. The present study is the first to investigate the implication of AVP1aR SNPs in recognizing cirrhotic patients with AD who are at risk of developing (multi-)organ failure.

We did not find an association with AVP1aR SNPs and the presence of ACLF, the majority of individual organ failures (i.e., renal, liver, circulatory, respiratory and cerebral failure) and outcome in the whole study cohort. Instead, an association was found between mutations in rs7308855 and rs7298346 and the presence of coagulation failure, which was defined as an INR≥2.5. Our observation of discrepancy between the results of the hypothesis-driven pilot study and the full

cohort study once more underlines that results obtained in such a relatively small sample size pilot study, using stratified groups of patients, does not allow to draw firm conclusions, in our case on possible associations and trends between SNPs in AVP1aR and the development of renal failure and 90-day survival.

AVP1aR is expressed on the platelet membrane and is involved in the coagulation cascade [11]. Stimulation of AVP1aR activates the phosphatidyl-inositol-cascade leading to an increase in cytoplasmatic calcium and stimulation of platelet formation and aggregation [12, 13]. It has previously been shown that there is significant heterogeneity in the aggregation response of normal human platelets to AVP. The authors of that study hypothesized that this variability in aggregation response might be related to a SNP in AVP1aR [14]. A more recent study investigated the association between four SNPs in the promotor region of AVP1aR and platelet vasopressin responsiveness [15]. No significant associations were found in that study. There are no data available regarding the effect of heterogeneity of the thrombocyte aggregation response in cirrhosis. Coagulopathy is a major concern in chronic liver failure. Cirrhotic patients are at an increased risk of bleeding, due to portal hypertension and synthetic dysfunction of the liver. Increased bleeding tendency in cirrhosis is associated with an increased risk of morbidity and mortality in patients undergoing invasive procedures. In cirrhotic patients with sepsis, a common feature in ACLF, haemostasis seems to be even further impaired [16]. Therefore, identifying cirrhotic patients who are at an increased risk of bleeding might be beneficial for developing treatment and prevention strategies for these patients. However, further research in even larger cohorts of cirrhotic patients is needed in order to validate the results and to explore the pathophysiological mechanisms. The fact that markers of coagulation function were not different in patients with or without a mutation in rs7308855 and rs7298346, suggests that associations with coagulation failure found in the current study are rather indirect and not functionally reflected.

It is also important to consider that the definition of coagulation failure used in this study (INR≥2.5) does only represent the extrinsic pathway of the coagulation cascade. Furthermore,

changes in INR are multifactorial. A more specific definition considering the function of the complete coagulation system should be applied in future studies.

We conclude that 6 SNPs of AVP1aR may not be useful as genetic markers to identify cirrhotic patients with AD who are at an increased risk of developing ACLF. However, an association of two genotypes (rs7308855 and rs7298346) with coagulation failure in patients with AD of cirrhosis or ACLF was found, which needs further functional evaluation.

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303 **Table 1.** Baseline characteristics and distributions of 6 variants of vasopressin 1a receptor genotypes
304 and allele frequencies in the study population

Variable	All patients (n=826)	No ACLF (n=641)	ACLF (n=185)	p-value
Age (y)	57.6±11.8	57.7±12.1	57.4±11.0	0.752
Male gender, n (%)	525 (63.6)	405 (63.2)	120 (64.9)	0.675
Etiology of cirrhosis, n (%)				
Alcohol	490 (60.0)	363 (57.3)	127 (69.4)	0.003
HBV	39 (5.0)	33 (5.5)	6 (3.4)	0.266
HCV	253 (32.4)	203 (33.7)	50 (28.3)	0.176
NAFLD	39 (5.0)	28 (4.7)	11 (6.3)	0.389
PBC	22 (2.8)	18 (3.0)	4 (2.3)	0.628
Cryptogenic	50 (6.4)	42 (7.0)	8 (4.6)	0.247
Other	52 (6.7)	44 (7.3)	8 (4.6)	0.202
Organ failures at baseline, n (%)				
Liver	116 (14.0)	42 (6.6)	74 (40.0)	<0.001
Kidney	109 (13.2)	-	109 (58.9)	-
Cerebral	49 (5.9)	15 (2.3)	34 (18.4)	<0.001
Coagulation	61 (7.4)	17 (2.7)	44 (23.8)	<0.001
Respiration	18 (2.2)	4 (0.6)	14 (7.6)	<0.001
Circulation	34 (4.1)	4 (0.6)	30 (16.2)	<0.001
Laboratory data				
INR	1.5 (1.3-1.8)	1.5 (1.3-1.7)	1.8 (1.4-2.4)	<0.001
PT (s)	19 (16-26)	18 (16-25)	23 (17-32)	0.016
APTT (s)	1.5 (1.2-31)	1.4 (1.2-30)	1.9 (1.3-37)	0.002
Platelet count ($x10^9$/L)	86 (55-137)	89 (56-139)	75 (51-121)	0.019
Bilirubin (mg/dL)	3.0 (1.6-6.9)	2.8 (1.5-5.5)	6.7 (2.0-16.7)	<0.001
Creatinine (mg/dL)	1.0 (0.7-1.4)	0.9 (0.7-1.2)	2.2 (1.0-3.1)	<0.001
Sodium (mmol/L)	135±6	135±6	134±7	0.009
CRP (mg/L)	18 (7-40)	15 (6-35)	27 (12-53)	<0.001
WBC ($x10^9$/L)	6.0 (4.1-9.2)	5.7 (4.0-8.3)	7.7 (5.3-12.3)	<0.001
Genetic variants of AVP1aR, n (%)				
Rs113481894 CC CT/ TT	697 (82.5) 144 (17.5)	528 (82.8) 110 (17.2)	151 (81.6) 34 (18.4)	0.720
Rs7298346 TT TA/ AA	635 (77.0) 175 (21.2)	497 (77.7) 143 (22.3)	138 (74.6) 47 (25.4)	0.384
Rs11174817 AA AG/ GG	223 (27.1) 601 (72.9)	167 (26.1) 472 (73.9)	56 (30.3) 129 (69.7)	0.265
Rs1042615 AA AG/ GG	129 (15.6) 696 (84.4)	99 (15.5) 541 (84.5)	30 (16.2) 155 (83.8)	0.805
Rs10747983 GG GC/ CC	136 (72.3) 52 (27.7)	69 (74.2) 24 (25.8)	67 (70.5) 28 (29.5)	0.574
Rs7308855 CC CT/ TT	692 (84.0) 132 (16.0)	541 (84.7) 98 (15.3)	151 (81.6) 34 (18.4)	0.321

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Results are described as numbers (percentage), mean ± standard deviation or median (interquartile range)

APTT, activated partial thromboplastin time; AVP1aR, vasopressin 1a receptor; CRP, C-reactive protein; HBV, hepatitis B virus; HCV, hepatitis C virus; INR, international normalized ratio; NAFLD, non-alcoholic fatty liver disease; PBC, primary biliary cholangitis; PT, prothrombin time; WBC, white blood cell count

Table 2. The association of a mutation in two single nucleotide polymorphisms in the vasopressin 1a receptor gene with the presence of coagulation failure (INR≥2.5) in cirrhotic patients admitted for acute decompensation and acute-on-chronic liver failure.

Variants	**No coagulation failure (n= 765)**	**Coagulation failure (n= 61)**	**p-value**
rs7308855, n (%) CC CT/ TT	 647 (84.8) 116 (15.2)	 45 (73.8) 16 (26.2)	0.024
rs7298346, n (%) TT TA/ AA	 594 (77.8) 170 (22.5)	 41 (67.2) 20 (32.8)	0.060