Impact of genetic variation in the vasopressin 1a receptor on the development of organ failure in patients admitted for acute decompensation of liver cirrhosis

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

Patients and 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 ($n = 641$) or ACLF ($n = 185$).

Results No associations were found for SNPs with the 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 entire population ($n = 61$, $P = 0.024$ and 0.060, respectively) and in the subgroup of patients with ACLF ($n = 44$, $P = 0.081$ and 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 acute decompensation of liver cirrhosis and ACLF. Eur J Gastroenterol Hepatol 29:535–538

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 believed to be involved in the pathogenesis [2]. A decreased systemic vascular resistance leads to the activation of compensatory vasoconstrictor systems and the nonosmotic release of arginine vasopressin (AVP) [3,4]. AVP is a neurohypophyseal hormone that plays a prominent role in the cardiovascular system and mediates vascular smooth muscle contraction through the V1a receptor (AVP1aR) [5]. A previous study has found an association between single nucleotide polymorphisms (SNPs) in the promotor region of AVP1aR and the presence of essential hypertension in nonobese Japanese patients [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. The 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.

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Patients and 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 (6th revision, 2008). Initially, we carried out 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. On the basis of these preliminary results, the study population was extended involving all 826 CANONIC patients who provided 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 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 six organ systems (i.e. liver, kidney, brain, coagulation, circulation and respiration). For each system, three subscores have been defined: subscore 1 = normal or moderate organ dysfunction, subscore 2 = marked organ dysfunction, and subscore 3 = organ failure. According to the CLIF-Organ Failure score, the following criteria are defined for individual organ failures: liver failure = bilirubin ≥ 12 mg/dl; kidney failure = creatinine ≥ 2 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 = international normalized ratio (INR) ≥ 2.5; circulatory failure = use of vasopressors; and respiratory failure = PaO_2/FiO_2 ratio ≤ 200 or SpO_2/FiO_2 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 PCR with allele-specific amplification primers. Genotypes of the other five 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, Massachusetts, USA) and 0.24 µmol/l of each primer. Restriction enzymes (New England BioLabs, Ipswich, Massachusetts, 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 a 2.5% agarose gel and visualized by staining with ethidium bromide. The investigators were blinded to the clinical outcomes during the determination of genotypes of the AVP1a receptor gene.

Statistical analysis

For all SNPs, deviation from Hardy–Weinberg equilibrium was calculated using Pearson's χ^2-test. The association between SNPs and the 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 carried out 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 the time of hospital admission was found in patients with ACLF ($n=64$, $P=0.025$ and 0.103, respectively). The same mutations showed significant associations with lower 90-day survival in all patients (hazard ratio = 1.81, 95% confidence interval = 1.02–3.23, $P=0.044$ and hazard ratio = 2.17, 95% confidence interval = 1.17–4.01, $P=0.013$, respectively). No association was found between SNPs and the presence of circulatory failure.

Patient characteristics of the entire 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 was found between the studied SNPs and the presence of renal failure or 90-day survival. Moreover, no association was found between SNPs and the presence of ACLF (Table 1) or single circulatory, liver, cerebral or respiratory failure. When comparing patients with CLIF-Organ Failure subscore 1 (normal or moderate organ dysfunction) versus 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 the 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 entire study population (Table 2) and in the subgroup of patients with ACLF ($P=0.056$). When comparing patients with CLIF-Organ Failure subscore 1 ($n=643$) versus 2 and 3 ($n=170$), the same mutations in these SNPs were more frequently present in patients with subscore 2 or 3 compared with patients with subscore 1 ($P=0.050$ and 0.055, respectively). Despite the association found for a mutation in SNP rs7308855 and rs7298346 with coagulation failure, the median values of markers of coagulation function (INR, prothrombin time, activated partial thromboplastin time and platelet count) did not differ significantly between patients with or without a mutation in these SNPs.

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

Table 1. Baseline characteristics and distributions of six variants of vasopressin 1a receptor genotypes and allele frequencies in the study population

Variables	*n* (%) All patients (*n* = 826)	No ACLF (*n* = 641)	ACLF (*n* = 185)	*P*-value
Age (years)	57.6 ± 11.8	57.7 ± 12.1	57.4 ± 11.0	0.752
Male sex	525 (63.6)	405 (63.2)	120 (64.9)	0.675
Aetiology of cirrhosis				
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				
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 ($\times 10^9$/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 ($\times 10^9$/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				
Rs113481894				
CC	697 (82.5)	528 (82.8)	151 (81.6)	0.720
CT/TT	144 (17.5)	110 (17.2)	34 (18.4)	
Rs7298346				
TT	635 (77.0)	497 (77.7)	138 (74.6)	0.384
TA/AA	175 (21.2)	143 (22.3)	47 (25.4)	
Rs11174817				
AA	223 (27.1)	167 (26.1)	56 (30.3)	0.265
AG/GG	601 (72.9)	472 (73.9)	129 (69.7)	
Rs1042615				
AA	129 (15.6)	99 (15.5)	30 (16.2)	0.805
AG/GG	696 (84.4)	541 (84.5)	155 (83.8)	
Rs10747983				
GG	136 (72.3)	69 (74.2)	67 (70.5)	0.574
GC/CC	52 (27.7)	24 (25.8)	28 (29.5)	
Rs7308855				
CC	692 (84.0)	541 (84.7)	151 (81.6)	0.321
CT/TT	132 (16.0)	98 (15.3)	34 (18.4)	

Results are described as *n* (%), mean ± SD or median (interquartile range).
ACLF, acute-on-chronic liver failure; 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, nonalcoholic 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 (international normalized ratio ≥ 2.5) in cirrhotic patients admitted for acute decompensation and acute-on-chronic liver failure

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

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 of at least 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 expressed widely and is involved in diverse functions including vascular smooth muscle contraction [10]. The presence of peripheral vasodilation contributes towards 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, hyponatraemia and hepatorenal syndrome [1,3]. In ACLF, activation of these vasoconstrictor systems is considered to contribute towards 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 entire 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 of at least 2.5. Our observation of discrepancy between the results of the hypothesis-driven pilot study and the full cohort study once more underlines that the results obtained in such a relatively small sample size pilot study, using stratified groups of patients, do 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 been shown previously 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 on 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 because of 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, identification of 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 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 $\geq$2.5) only represents 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 six 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 requires further functional evaluation.

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Conflicts of interest

There are no conflicts of interest.

References

1 Moreau R, Jalan R, Gines P, Pavesi M, Angeli P, Cordoba J, *et al*. Acute-on-chronic liver failure is a distinct syndrome that develops in patients with acute decompensation of cirrhosis. *Gastroenterology* 2013; 144:1426–1437.
2 Bernardi M, Moreau R, Angeli P, Schnabl B, Arroyo V. Mechanisms of decompensation and organ failure in cirrhosis: from peripheral arterial vasodilation to systemic inflammation hypothesis. *J Hepatol* 2015; 63:1272–1284.
3 Schrier RW, Arroyo V, Bernardi M, Epstein M, Henriksen JH, Rodés J. Peripheral arterial vasodilatation hypothesis: a proposal for the initiation of renal sodium and water retention in cirrhosis. *Hepatology* 1988; 8:1151–1157.
4 Bosch J, Arroyo V, Betriu A, Mas A, Carrilho F, Rivera F, *et al*. Hepatic hemodynamics and the renin–angiotensin–aldosterone system in cirrhosis. *Gastroenterology* 1980; 78:92–99.
5 Robertson GL. Antidiuretic hormone. Normal and disordered function. *Endocrinol Metab Clin North Am* 2001; 30:671–694.
6 Hasan KN, Shoji M, Sugimoto K, Tsutaya S, Matsuda E, Kudo R, *et al*. Association of novel promotor single nucleotide polymorphisms in vasopressin V1a receptor gene with essential hypertension in non-obese Japanese. *J Hum Hypertens* 2007; 21:825–827.
7 Koshimizu TA, Nasa Y, Tanoue A, Oikawa R, Kawahara Y, Kiyono Y, *et al*. V1a vasopressin receptors maintain normal blood pressure by regulating circulating blood volume and baroreflex sensitivity. *Proc Natl Acad Sci USA* 2006; 103:7807–7812.
8 Jalan R, Saliba F, Pavesi M, Amoros A, Moreau R, Ginès P, *et al*. Development and validation of a prognostic score to predict mortality in patients with acute-on-chronic liver failure. *J Hepatol* 2014; 61:1038–1047.
9 Enhörning S, Leosdottir M, Wallström P, Gullberg B, Berglund G, Wirfält E, *et al*. Relation between human vasopressin 1a gene variance, fat intake and diabetes. *Am J Clin Nutr* 2009; 89:400–406.
10 Share L. Role of vasopressin in cardiovascular regulation. *Physiol Rev* 1988; 68:1248–1284.
11 Haslam RJ, Rosson GM. Aggregation of human blood platelets by vasopressin. *Am J Physiol* 1972; 223:958–967.
12 Filep J, Rosenkranz B. Mechanism of vasopressin induced platelet aggregation. *Thromb Res* 1987; 45:7–15.
13 Jin J, Kunapuli SP. Coactivation of two different G protein-coupled receptors is essential for ADP-induced platelet aggregation. *Proc Natl Acad Sci USA* 1998; 95:8070–8074.
14 Lachant NA, Smith MR, Xie ZJ, Romani WR. Heterogeneity of the aggregation response of human platelets to arginine vasopressin. *Am J Hematol* 1995; 49:56–66.
15 Hasan KN, Shoji M, Tsutaya S, Kudo R, Matsuda E, Saito J, *et al*. Study of V1a vasopressin receptor gene single nucleotide polymorphisms in platelet vasopressin responsiveness. *J Clin Lab Anal* 2006; 20: 87–92.
16 Plessier A, Denninger MH, Consigny Y, Pessione F, Francos C, Durand F, *et al*. Coagulation disorders in patients with cirrhosis and severe sepsis. *Liver Int* 2003; 23:440–448.