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ACE *I* allele is associated with more severe portal hypertension in patients with liver cirrhosis: A pilot study

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ABSTRACT

Background: In liver cirrhosis, the renin-angiotensin-aldosterone system is involved in the pathogenesis of portal hypertension. Its effector, angiotensin II, is generated by angiotensin-converting enzyme (ACE). Serum ACE levels are affected by I/D polymorphism of its gene, with alleles I and D being associated, respectively, with lesser and greater activity of the enzyme. In cirrhotic patients carrying the ACE I allele, an increased risk for gastro-oesophageal varices was observed.
Aim: The aim of our study was to evaluate whether ACE I/D polymorphism influenced portal pressure.
Methods: Fifty-one consecutive cirrhotic patients were divided based on ACE genotype (DD, ID, and II). Kidney and liver function tests, upper endoscopy, and hepatic venous pressure gradient measurement (HVPG) were performed in all patients.
Results: The presence of the ACE I allele was associated with a higher HVPG value (18.7 ± 6.4 vs 10.3 ± 6.3 mmHg; $P < .001$), higher frequency of large gastrooesophageal varices (59.3% vs 25.0%; $P < .05$), and higher frequency of variceal bleeding (63.0% vs 29.2%; $P < .05$). No significant differences were found between patients with and those without the ACE I allele regarding Child-Pugh score, MELD score, ascites, and hepatic encephalopathy.
Conclusion: ACE I/D polymorphism seems to influence the severity of portal hypertension and the risk of variceal bleeding in liver cirrhosis, regardless of the severity of liver disease.

1. Introduction

In liver cirrhosis, a major cause of morbidity and mortality is portal hypertension [1], a clinical syndrome caused by the rise in the pressure gradient between the portal vein and the inferior vena cava (normal values of 1–5 mmHg) that occurs because of increased intrahepatic vascular resistances and portal blood flow. It is associated with the formation of collaterals that divert blood flow from the portal to the systemic venous circulation [2]. The most relevant portosystemic collaterals are gastro-oesophageal varices because of the risk of bleeding [2].

The renin-angiotensin-aldosterone system (RAAS) plays a central role in the pathogenesis of portal hypertension through its major physiological effector angiotensin II [3], a potent vasoconstrictor that binds to the angiotensin II type 1 receptor (AT1R), and reduces the bioavailability of nitric oxide (NO), resulting in the contraction of stellate cells of the liver and increasing the resistance level of the hepatic sinusoids [4].

A major component of the RAAS is angiotensin-converting enzyme (ACE), a zinc metallopeptidase that is widely distributed on the surface of endothelial cells and epithelial cells. ACE catalyses the conversion of angiotensin I to angiotensin II and is responsible for regulating short-term and long-term blood pressure and plasma volume. The gene encoding ACE is located on the long arm of chromosome 17 (17q23.3) and consists of 26 exons spanning 21 kb. Serum and tissue ACE levels are substantially stable in the

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same individual, but they have large interindividual differences. Over half of this interindividual variability is a consequence of a biallelic polymorphism that consists of the presence (insertion I) or absence (deletion D) of a 287-bp alu sequence at the level of intron 16 of the gene. The *I* allele is associated with lower enzyme activity compared with the *D* allele [5]. In the study of Coto et al. [6], an increased risk for large gastro-oesophageal varices was observed in patients with alcoholic liver cirrhosis carrying the ACE *I* allele. The authors hypothesized that the lower ACE levels associated with the *I* allele could have a vasodilatory effect in the splanchnic vascular bed favouring the development of portosystemic collaterals.

As far as we know, the relationship between ACE I/D polymorphism and portal pressure has not been previously evaluated. The gold standard for measuring portal pressure is the determination of hepatic venous pressure gradient (HVPG), the difference between the pressure measured by a wedged catheter and that measured by a free catheter inserted into a hepatic vein. Portal hypertension is defined as an HVPG above the upper normal value of 5 mmHg. Clinically significant portal hypertension is defined as an HVPG ≥10 mmHg; values beyond this cut-off are powerful predictors of decompensation in liver cirrhosis [7].

The main aim of the present study was to assess the association between ACE I/D polymorphism and HVPG in patients with liver cirrhosis. The secondary target was to assess the association between ACE I/D polymorphism and the presence of gastro-oesophageal varices, their grade, and previous bleeding.

1.1. Patients

This was a cross-sectional study, evaluating for enrolment all consecutive patients referred to our institution for liver cirrhosis between January 2010 and December 2011. Clinical, haematochemical, haematological, endoscopic, and radiological data were prospectively recorded. Blood samples used for ACE I/D polymorphism determination were stored to be analysed only after a sample was obtained from the last enrolled patient.

Exclusion criteria were alcohol consumption or the use of any drug affecting splanchnic haemodynamic or portal pressure in the previous 3 months, splenic or portal vein thrombosis, liver neoplasms, previous liver resection, previous liver transplantation, and patient refusal of the HVPG measurement or of the storage and use in research of their biological samples. Liver cirrhosis was diagnosed based on clinical laboratory and radiologic findings in all patients.

1.2. Oesophagogastric varices detection and classification

The presence of gastro-oesophageal varices was assessed with the use of upper gastrointestinal endoscopy. Following the classification of the Italian Liver Cirrhosis Project, the gastroesophageal varices were graded from F0 to F3, with F1 indicating small varices and F2 and F3 indicating large varices [8]. Thirty healthy controls were genotyped for the ACE I/D polymorphism. Informed consent was obtained from all patients and controls. The study protocol was also approved by the ethical committee of our institution. The study was performed in compliance with the Declaration of Helsinki ethical principles.

1.3. HVPG measurement

Measurement of HVPG was performed under local anaesthesia. A venous introducer was inserted via the Seldinger approach in the right internal jugular vein. Under fluoroscopic guidance, a radiopaque balloon catheter was inserted and advanced until a hepatic vein (usually the right hepatic vein) was reached. Intravascular pressures were recorded with a high sensibility transducer. The free hepatic venous pressure (FHVP) was measured with the catheter tip lying freely in the hepatic vein at 2–3 cm from the confluence of the hepatic vein and inferior vena cava. The wedged hepatic venous pressure (WHVP) was measured after the distal balloon was inflated, achieving the occlusion of the hepatic vein, and a contrast agent was injected in order to rule out venovenous communication. The HVPG was calculated as the difference of the two measurements (HVPG = WHVP − FHVP).

1.4. ACE genotyping

Genomic DNA was isolated from peripheral blood samples, collected via venipuncture, by using High Pure PCR Template Preparation kits (ROCHE Diagnostic, Indianapolis, USA). Extracted DNA samples were quantified with spectrophotometry at 260 nm and stored at −20 °C until processed. DNA integrity was electrophoretically tested. The ACE I/D polymorphism was analysed based on polymerase chain reaction (PCR) amplification of a fragment from intron 16 of the *ACE* gene, as previously described [9]. PCR products, a 490-bp fragment (*I* allele) and a 190-bp fragment (*D* allele), were separated with agarose gel electrophoresis. Each DNA sample revealed one of three possible patterns: a 490-bp band (genotype *II*), a 190-bp band (genotype *DD*), or both 490- and 190-bp bands (genotype *ID*). In order to avoid misclassification of *ID* genotypes into *DD* genotypes, we confirmed each *DD* genotype through an additional PCR with the insertion-specific primer, as previously described [10]. This PCR resulted in amplification of a 408-bp fragment only in the presence of the *I* allele.

1.5. Statistical analysis

Categorical variables are presented as frequencies (percentage), and numerical variables are given as mean ± SD (range). The two groups identified by a different ACE genotype (*DD* vs *ID/II*) were compared by using the χ^2 test for categorical variables and the non-parametric Mann–Whitney test for continuous variables. Statistical significance was defined as a two-sided $P < 0.05$ for all analyses, which were carried out using the Stata software package (Release 12, StataCorp, College Station, TX, USA).

2. Results

Fifty-one consecutive cirrhotic patients satisfying the selection criteria were enrolled into the study. Their baseline characteristics are reported in Table 1. Patients (35 males, 68.6%; age 54.9 ± 13.1

Table 1
Characteristics of 51 cirrhotic patients enrolled in the study.

Variables	
Age, y	54.9 ± 13.1 (20–80)
Males	35 (68.6)
Females	16 (31.4)
Aetiology	
Viral Hepatitis	32 (62.7)
Alcohol	12 (23.5)
Nonalcoholic steatohepatitis	7 (13.7)
Serum bilirubin (mg/dL)	2.0 ± 2.4 (0.5–13.9)
INR	1.4 ± 0.3 (1.0–2.4)
Serum albumin (g/dL)	3.2 ± 0.5 (2.1–4.5)
Plasma creatinine (mg/dL)	1.0 ± 0.3 (0.6–2.3)
Child-Turcotte-Pugh score	7.3 ± 2.0 (5–13)
MELD score	12.6 ± 5.0 (6–25)
Platelet count ($\times 10^9/mm^3$)	76.7 ± 38.6 (11.0–170.0)
Gastro-esophageal varices	44 (86.3)
Previous variceal bleeding	24 (47.1)

Qualitative variables are expressed as *n* (%) and quantitative as mean ± SD (range). INR, international normalized ratio; MELD, Model for End-stage Liver Disease.

Table 2
Characteristics of cirrhotic patients according to the ACE genotype.

Variables	ACE genotypes		*P* value
	DD (24)	ID/II (27)	
Age, y	57.4 ± 10.9 (29–75)	52.6 ± 14.6 (20–80)	0.192
Males	18 (75.0)	17 (63.0)	0.355
Plasma creatinine (mg/dL)	0.9 ± 0.2 (0.6–1.5)	1.0 ± 0.4 (0.7–2.3)	0.374
Child-Turcotte-Pugh score	7.7 ± 2.3 (5–13)	6.8 ± 1.7 (5–12)	0.104
MELD score	12.8 ± 4.9 (6–25)	12.3 ± 5.1 (7–25)	0.609
Platelet count ($\times 10^9/mm^3$)	77.9 ± 37.0 (11–146)	75.6 ± 40.9 (33–170)	0.527
HVPG (mmHg)	10.3 ± 6.3 (1–23)	18.7 ± 6.4 (4–34)	**<0.001**
Gastro-esophageal varices	20 (83.3)	24 (88.9)	0.565
Large gastro-esophageal varices	6 (25.0)	16 (59.3)	**<0.05**
Previous variceal bleeding	7 (29.2)	17 (63.0)	**<0.05**
Ascites	14 (58.3)	13 (48.1)	0.467
Hepatic encephalopathy	6 (25.0)	4 (14.8)	0.361

Qualitative variables are expressed as n (%) and quantitative as mean ± SD (range).
MELD, Model for End-stage Liver Disease; HVPG, hepatic venous pressure gradient.
Statistically significant p value is highlighted in bold.

years; 16 females, 31.4%, age 57.1 ± 13.5 years) were divided based on ACE genotype: 24 have *DD* (47.1%), 20 have *ID* genotype (39.2%), and 7 have *II* genotype (13.7%). Allele frequency was 33.3% for allele *I* and 66.7% for allele *D*. In controls (13 males, 43.3%; age 49.1 ± 12.4 years; 17 females, 56.6%, age 45.8 ± 14.8;), frequencies of *DD, ID*, and *II* genotypes were 46.7%, 40.0%, and 13.3%, respectively; frequencies of *I* and *D* allele were 33.3% and 66.7%, respectively. Allele and genotype frequencies were not significantly different between cirrhotic patients and controls. The genotype distribution was in line with the Hardy–Weinberg equilibrium among cases (P = .40) and controls (P = .58). Characteristics of cirrhotic patients were divided according to the ACE genotype, as summarized in Table 2. According to ACE genotype, significant differences of HVPG mean value, variceal grade, and previous bleeding were found. The presence of ACE *I* allele was associated with a higher HVPG. The mean HVPG value of patients carrying the *ID* and *II* genotypes was significantly higher than that of patients carrying the *DD* genotype (18.7 ± 6.4 vs 10.3 ± 6.3 mmHg; P < .001). Although the mean HVPG of patients carrying the *II* genotype was higher than that of patients carrying the *ID* genotype, the difference was not significant (21.4 ± 7.2 vs 17.7 ± 6.0 mmHg; P = .188). When patients were divided based on the HVPG cutoff value for clinically significant portal hypertension, 37 patients had an HVPG ≥10 mmHg and 14 had an HVPG <10 mmHg. The ACE *I* allele distribution was significantly different between these two groups: it was present in 26 (70.3%) patients with HVPG >10 mmHg and in only one (7.1%) patient with HVPG <10 mmHg (P < .001). The prevalence of gastro-oesophageal varices was similar between patients with *DD* genotype and those with *ID* and *II* genotypes (83.3 vs 88.9%: P = .565). However, patients carrying the ACE *I* allele (*ID* and *II*) had a higher prevalence of large gastro-oesophageal varices in comparison with patients with *DD* genotype (59.3% vs 25.0%; P < .05). Moreover, patients carrying the ACE *I* allele more frequently experienced variceal haemorrhage (63.0% vs 29.2%; P < .05). No significant differences were found regarding MELD score, Child-Turcotte-Pugh score, ascites, and hepatic encephalopathy among patients with different ACE genotypes.

3. Discussion

Portal hypertension is a common complication of liver cirrhosis. In the early stage, portal hypertension is caused by the increase in intrahepatic vascular resistances resulting from a fixed component due to the angioarchitectural distortion by fibrotic scar tissue and regenerative nodules and from a dynamic component due to an altered intrahepatic vasoreactivity. At a more advanced stage, the increase in portal venous blood inflow from splanchnic vasodilation together with renal sodium and water retention contributes to the persistence and worsening of portal hypertension [2].

As a consequence of increased portal pressure, portosystemic collaterals may develop, causing potentially life-threatening events such as bleeding from gastro-oesophageal varices. It has long been known that the activation of systemic RAAS is implicated in portal hypertension and its complications [11]. As a result of the activation of the RAAS, angiotensin II levels increase in liver cirrhosis and correlate with the severity of portal hypertension [11,12]. Angiotensin II induces both an increase in intrahepatic vascular resistance by stimulating activated stellate cells contraction [13] and fibrous tissue deposition [14] and an increase in the portal venous blood inflow by stimulating the synthesis of aldosterone involved in the sodium and water retention [15]. On the other hand, the vasoconstrictor effect of angiotensin II on the splanchnic vascular bed is less than expected due to the splanchnic hyporesponsiveness to angiotensin II [16,17] and, despite elevated angiotensin II levels, a marked and persistent splanchnic vasodilation is present, leading to increased portal venous blood inflow [18].

On a clinical context, in contrast with the known effects of RAAS activation in liver cirrhosis, reduced angiotensin II and ACE levels have been associated with increased risk of developing large oesophageal varices [6].

Our results confirm this observation. Patients carrying the *I* allele, those with lower ACE activity, showed higher HVPG values, a higher prevalence of large gastro-oesophageal varices, and a higher frequency of previous variceal bleeding.

To resolve this conundrum, we can speculate that in advanced liver cirrhosis, when reduced angiotensin II levels do not significantly influence intrahepatic vascular resistances that are mainly determined by structural distortion of the liver, the effect of lower angiotensin II levels could become prevalent in the splanchnic vascular bed and in portosystemic collaterals, exacerbating the vasodilation and further increasing portal venous blood inflow and facilitating the development of large gastro-oesophageal varices.

Intriguingly, we did not identify any significant association between ACE genotype and other complications of portal hypertension such as the presence of ascites or hepatic encephalopathy. Furthermore, the prevalence of oesophageal varices seems to be unaffected by the *I* allele. As in the study of Coto et al. [6], we did not find any significant difference in the degree of liver dysfunction between patients with and without ACE *I* allele, indicating that in our patients, higher HVPG values were not determined by more severe liver disease.

Our work has some limitations, such as the limited number of patients analysed and the fact that the levels of angiotensin II and aldosterone were not measured. Most importantly, this study

did not evaluate the effect of ACE genotype on the natural history of portal hypertension in cirrhosis. Even if our data suggest the independent role of the *I* allele on portal pressure and on variceal degree, other results, lacking significant associations with the presence of ascites and hepatic encephalopathy and especially with the prevalence of varices, require further explanation. In order to clarify these issues and to establish if ACE genotyping may help to identify patients requiring closer follow-up, long-term prospective studies with hard clinical endpoints (e.g., appearance and progression of varices and ascites, variceal bleeding) are needed.

In conclusion, our study showed that patients carrying ACE *I* allele have a higher HVPG value, a higher prevalence of large gastro-oesophageal varices, and a higher prevalence of previous variceal bleeding. In liver cirrhosis, ACE I/D polymorphism might play a role in determining the severity of portal hypertension and the risk of variceal bleeding, regardless of the severity of liver disease. Prospective studies focused on a broader cohort of patients will be needed to confirm these data.

Conflict of interest

None declared.

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