J Gastroenterol 2007; 42:543–549
DOI 10.1007/s00535-007-2040-1

Journal of
Gastroenterology

Sex-related influence of angiotensin-converting enzyme polymorphisms on fibrosis progression due to recurrent hepatitis C after liver transplantation

Carlo Fabris[1], Pierluigi Toniutto[1], Davide Bitetto[1], Rosalba Minisini[2], Ezio Fornasiere[1], Carlo Smirne[2], and Mario Pirisi[2]

[1]Department of Pathology and Medicine Experimental and Clinical, Internal Medicine, Medical Liver Transplant Unit, University of Udine, P. zale S.M. della Misericordia 1, 33100 Udine, Italy
[2]Department of Clinical and Experimental Medicine, University of Eastern Piedmont "A. Avogadro", Novara, Italy

Background. Experimental evidence and clinical studies suggest that the renin–angiotensin system and its inhibitors may play a role in regulating the mechanisms of liver fibrosis development. The present study aimed to verify whether carriage of specific angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) allelic variants, modulating angiotensin II generation, could affect the outcome of recurrent hepatitis C after liver transplantation, via several metabolic pathways. ***Methods.*** Forty-five (29 men) recipients, with a median histological follow-up of 60 months after orthotopic liver transplantation (OLT), were studied. ACE gene I/D polymorphism was assessed by means of a polymerase chain reaction procedure. Fibrosis progression was evaluated annually during the follow-up. ***Results.*** Weight gain 1 year post-OLT (defined as an increase in body mass index, BMI, of >0.5 kg/m^2) was significantly more common among D/* carriers (22/22 vs. 16/23, $P < 0.005$); patients who 1 year after OLT had an increase in their BMI value of >0.5 kg/m^2 more frequently had a triglycerides/cholesterol ratio of ≤ 0.7 (16/22 vs. 8/23, χ-squared test $P < 0.02$). This association was stronger in men. Female D/D homozygotes had the highest probability of showing significant liver fibrosis (7/10) in comparison with men (11/29) and I/* women (1/6) ($P < 0.01$). ***Conclusions.*** In patients with recurrent hepatitis C, carriers of the D allele appeared to gain more weight after liver transplantation, and in male liver recipients, the D allele was associated with a peculiar lipid profile that was associated with a slower rate of allograft fibrosis progression. Among female recipients, carriage of the D allele may favor more severe allograft fibrosis.

Key words: ACE genotypes, body mass index, hepatitis C, liver fibrosis, liver transplantation

Received: October 9, 2006 / Accepted: March 1, 2007
Reprint requests to: P. Toniutto

Introduction

Recurrence of hepatitis C virus (HCV) infection after orthotopic liver transplantation (OLT) is almost universal.[1] In the majority of patients, recurrent HCV infection is a mild liver disease, progressing to fibrosis at a slow rate;[2] however, in up to 30% of patients with recurrent hepatitis C, fibrosis progresses at a fast rate, leading to allograft cirrhosis within 5 years after OLT.[3] Donor age has emerged as the major risk factor of severe fibrosis progression after OLT;[4,5] however, the exact mechanisms responsible for this association remain unclear, and additional factors are likely to play a role. One example is sex: among female recipients, recurrent hepatitis C has a worse outcome.[6] Recently, our group has provided data suggesting that two other factors may modulate the speed of fibrosis progression among patients with recurrent hepatitis C: (1) body mass index (BMI) changes post-OLT, since a low BMI post-OLT appears to predispose to more severe liver fibrosis progression,[7] and (2) post-OLT lipid profile, assessed as the triglycerides to cholesterol ratio (T/C ratio), where a lower ratio seems to favor milder disease.[8]

In the last 15 years, angiotensin-converting enzyme (ACE) insertion (I)/deletion (D) polymorphisms have been studied in relationship to a possible association with cardiovascular diseases, with controversial results. More consistently, ACE I/D polymorphisms have been found to be associated with being overweight and obesity in men.[9] This association has recently been confirmed in the liver transplantation setting; OLT recipients carrying the ACE D allele are more likely to become overweight 1 year post-OLT than those possessing the ACE I allele.[10] Moreover, a substantial (>2 kg/m^2) post-OLT BMI increase has been found to be independently associated with the carriage of at least one ACE D allele.

Experimental evidence indicates that the renin–angiotensin system (RAS) and its inhibitors play a role

in regulating the mechanisms of liver fibrosis development,[11–16] though this issue has been scarcely addressed by clinical studies. On the basis of the reported observations, it can be hypothesized that carriage of ACE allelic variants may affect the outcome of recurrent hepatitis C. The present study aimed to verify this hypothesis.

Patients and methods

Patients

A consecutive series of 45 patients who underwent their first cadaveric OLT at our institution for HCV-related liver disease were studied. All had a minimum follow-up period of 2 years after the transplant operation, and had been subjected to at least one liver biopsy, obtained not earlier than 1 year after OLT. Table 1 shows the demographic and clinical characteristics of the studied population. All patients were maintained on an immunosuppressive regimen that was either cyclosporine- or tacrolimus-based, in conjunction with, in the first few months, corticosteroids. Cyclosporine dosage was calculated to obtain serum levels (measured 2 h after drug administration) ranging from 800 to 1200 µg/l in the first 6 weeks after the transplant and from 600 to 800 µg/l thereafter. Tacrolimus dosage was calculated to obtain serum levels ranging from 10 to 15 µg/l in the first 6 weeks after the transplant and from 5 to 10 µg/l thereafter. Corticosteroid tapering was completed within 90 days after the transplant operation in 28 patients and within 1 year after OLT in 12 patients, while in the remaining three patients corticosteroid treatment, at the dosage of 7.5 mg/daily, was utilized indefinitely owing to the presence of Addison's disease, which occurred after OLT. HCV recurrence was defined as detectable serum HCV-RNA, serum alanine aminotransferase (ALT) levels above the upper normal limit, Ishak grading score ≥ 2, and no evidence of rejection. Twenty-seven (60.0%) of the 45 patients received antiviral treatment with interferon α 2b (Intron-A, Shering Plough, Kenilworth, NJ, USA) at the dosage of 3 MU three times weekly subcutaneously plus ribavirin (Rebetol 200 mg tablets, Schering Plough) at the dosage of 600–800 mg daily orally. The scheduled combination therapy, if tolerated, was maintained for 12 months. The interferon dose was reduced to 50% for leukocyte count $<1.50 \times 10^9$/l, neutrophil count $<0.75 \times 10^9$/l, platelet count $<50 \times 10^9$/l, or hemoglobin level <100 g/l. Interferon was discontinued for leukocyte count $<1.0 \times 10^9$/l, neutrophil count $<0.50 \times 10^9$/l, platelet count $<25 \times 10^9$/l, or hemoglobin level <85 g/l. The ribavirin dose was reduced to 400–600 mg/day if hemoglobin levels decreased below 100 g/l and was discontinued if the hemoglobin levels decreased below 85 g/l. Antiviral treatment was started at a median time of 14.0 months (range, 0.7–75.9) after OLT and was completed in 15 (55.6%) patients. In the remaining 12 (44.4%) patients, antiviral therapy had to be stopped prematurely owing to adverse effects. Three patients achieved a sustained viral response defined as undetectable serum HCV-RNA 6 months after the end of treatment. Body weight was measured to the nearest 0.1 kg and height to the nearest 1 cm, with study participants wearing only underwear and no shoes. BMI was calculated as weight in kilograms divided by the square of the height in meters; it was measured pretransplant and 1 and 2 years after transplantation. In patients with ascites, pre-OLT BMI was calculated after subtracting from the body weight the amount of ascitic fluid calculated on the basis of an ultrasound evaluation. This study was reviewed by the local ethics committee and was performed in accordance with the 2000 Declaration of Helsinki. All patients gave their informed consent prior to their inclusion in the study.

Table 1. Clinical and demographic characteristics of the studied population

Recipient male sex, no. (%)	29 (64.4)
Donor male sex, no. (%)	22 (48.9)
Recipient age at transplantation in years, median (range)	56 (23–66)
Donor age in years, median (range)	40 (17–77)
CMV infection, no. (%)	14 (31.1)
Immunosuppressive regimen, no. (%)	
Tacrolimus-based	33 (73.3)
Cyclosporine-based	12 (26.7)
Corticosteroid withdrawal within 90 days, no. (%)	28 (62.2)
Antiviral therapy, no. (%)	27 (60.0)
Patients with ≥1 treated rejection episodes, no. (%)[a]	10 (22.2)

Categorical variables are expressed as frequency (%); continuous variables as median (range)
CMV, cytomegalovirus; HCV, hepatitis C virus
[a] Seven patients had one and three patients had two rejection episodes that required therapy with methylprednisolone boluses

Genotyping of the ACE gene I/D polymorphism

Genomic DNA was extracted from 200 µl of whole blood with a QIAamp Blood Mini Kit (Qiagen, Tokyo, Japan). The I/D polymorphism of the ACE gene was determined according to previously published methods,[17,18] with slight modifications. Briefly, the sequences of the sense and antisense primers used were 5′-CTG GAG ACC ACT CCC ATC CTT TCT-3′ and 5′-GAT GTG GCC ATC ACA TTC GTC AGA T-3′, respectively. Polymerase chain reaction (PCR) was performed in a final volume of 20 µl that contained 1.5 mmol/l $MgCl_2$, 5% dimethyl sulfoxide (DMSO), and 1 U *Taq-Gold* DNA polymerase (Applied Biosystems, Foster City, CA, USA). Amplification was performed with a Gene Amp PCR System 2400 (Applied Biosystems). Samples were denatured for 3 min at 96°C and then cycled 35 times through the following steps: 45 s at 96°C, 1 min at 62°C, and 1 min at 72°C. Amplicons were resolved in 1.5% agarose gel with ethidium bromide staining, being visualized as a 490-bp band, corresponding to the insertion allele (I), and a 190-bp band, corresponding to the deletion allele (D). Whenever, according to the results of the above PCR, a D/D type was identified, a second, independent PCR amplification was performed with insertion-specific sequence primers (5′-TGG GAC CAC AGC GCC CGC CAC TAC-3′); (5′-TCG CCA GCC CTC CCA TGC CCA TAA-3′). In this second reaction, PCR conditions were unchanged, except for an annealing temperature of 67°C and 5% DMSO was not used.

Liver histology

In follow-up liver biopsies, grading and staging were scored according to the method of Ishak et al.[19] Fibrosis progression was evaluated annually during the follow-up after OLT. It was based on the staging score at the corresponding per-protocol liver biopsy, or, when unavailable, on the closest on-demand liver biopsy. The speed of fibrosis progression (SFP) expressed in fibrosis units per month (FU/mo) was calculated for each 1-year time interval after OLT. It was obtained by dividing the change observed in the fibrosis score at the end of the pertinent interval of time, with respect to either the transplant operation (for the first posttransplant year) or the previous liver biopsy (for the following years), by the number of months elapsed. One hundred fifty-six determinations of SFP were available: 45 pertained to the first year post-OLT, 42 to the second, 38 to the third, and 31 to the fourth year after OLT.

Statistical analysis

Statistical analysis of data was performed using the BMDP dynamic statistical software package 7.0 (Statistical Solutions, Cork, Ireland). Continuous variables are presented as median (range) and categorical variables as frequency (%). Associations between categorical variables were performed by means of the χ-squared test and, when appropriate, the χ-squared test for linear trend. The χ-squared goodness-of-fit test was used to assess whether the observed frequencies of ACE I/D alleles significantly differed from those expected according to the Hardy-Weinberg equation. Time-to-event analysis was used to verify the association between the histological outcome of recurrent hepatitis C (time necessary to reach an Ishak staging score >2, indicating "significant" fibrosis) and the recipient's lipid profile or ACE allelic variants. To take into account possible sex-related differences, data were dichotomized according to recipient's sex.

Results

Histology

The patients underwent a total of 295 liver biopsies (range, 2–13 each), of which 124 (range, 0–6 each) were per-protocol. The median histological follow-up was 60 months, range, 12–96 months. At the end of follow-up, the majority of patients (26/45, 57.8%) had a staging score ≤2, while 19/45 (42.2%) had significant fibrosis (Ishak staging score >2), and six (13.3%) had cirrhosis. The percentage of patients showing a SFP above zero was highest in the first year post-OLT (37/45) and declined during the second (20/42), third (11/38), and fourth (7/31) years after OLT with a significant linear trend ($P < 0.0001$).

ACE genotypes

Twenty patients (44.4%) were D/D homozygotes, 18 (40.0%) I/D heterozygotes, and seven (15.6%) I/I homozygotes. The frequency distribution of the ACE allelic variant observed was not statistically different from that expected according to the Hardy-Weinberg formula (χ-squared goodness-of-fit test, $P > 0.2$). Table 2 lists the associations observed between ACE I/D polymorphisms and several demographic and clinical characteristics of the patients studied. Carriage of the D allele was found to be statistically correlated with the probability of increasing the BMI values at 1 and 2 years post-OLT.

BMI, lipid profiles, and liver fibrosis progression 1 year post-OLT

The associations between the T/C ratios and the BMI increases or the SFP, 1 year post-OLT, are reported in

Table 2. Association between ACE I/D polymorphisms and several demographic and clinical parameters of the studied population

	ACE D/D $n = 20$	ACE I/D $n = 18$	ACE I/I $n = 7$	P*
Recipient male sex	10	15	4	NS
Donor male sex	9	9	4	NS
Recipient age at transplantation >55 years	12	8	5	NS
Donor age at transplantation >45 years	9	6	3	NS
BMI increase 1 year post OLT >0.5 kg/m^2	11	11	0	<0.005
BMI increase 2 years post OLT >0.6 kg/m^2	10	11	1	<0.05
T/C ratio one year post OLT ≤0.7	9	13	2	NS
CMV infection	6	5	3	NS
Corticosteroid withdrawal within 90 days	13	11	4	NS
Patients with treated rejection episodes	3	4	3	NS
Patients subjected to antiviral therapy	15	8	4	NS
Sustained responders to antiviral therapy	2	1	0	NS
Post-OLT diabetes mellitus	10	4	3	NS

ACE, angiotensin-converting enzyme; I, insertion allele; D, deletion allele; BMI, body mass index; OLT, orthotopic liver transplantation; T/C ratio, triglycerides/cholesterol ratio
* Pearson χ-squared test comparing patients with the I/I versus those with D/* genotype

Table 3. Relationship between the T/C ratio, BMI increase, and speed of allograft fibrosis progression 1 year after OLT

	T/C ratio		
	≤0.7 ($n = 24$)	>0.7 ($n = 21$)	P*
BMI increase 1 year post OLT >0.5 kg/m^2	16	6	<0.02
Speed of fibrosis progression (FU/mo >0.100)	2	12	<0.02

FU/mo, fibrosis units/month
* Pearson χ-squared test

Table 3. The relationships observed were, at least in part, sex related. In fact, men with increased BMI value had the highest frequency of low (≤0.7) T/C ratio (10/13). An intermediate behavior was seen in male patients without a BMI increase and in female patients with an increased BMI value (12/25). Finally, female patients who did not increase their BMI value had the lowest frequency of a low T/C ratio (2/7, $P < 0.05$, χ-squared test for linear trend). Furthermore, male patients with a low T/C ratio had the highest frequency of a low SFP (14/16) in comparison with female patients with a low T/C ratio and male patients with a high T/C ratio (14/21) and to female patients with a high T/C ratio (3/8, $P < 0.02$, χ-squared test for linear trend).

Eleven patients started antiviral therapy owing to recurrent hepatitis C within the first year after OLT (median, 6.9 months). No differences were found between patients who started early antiviral treatment and untreated or later-treated patients in frequencies of BMI increase >0.5 kg/m^2 (5/11 vs. 17/34, P = NS) or of SFP ≤0.100 FU/mo (7/11 vs. 22/34, P = NS).

Time-to-event analysis in reaching significant fibrosis

A significant trend was found in the frequency of patients who reached a fibrosis score >2 when stratified as follows: male patients with a low T/C ratio (4/16), male patients with a high T/C ratio, and female patients with a low T/C ratio (10/21), female patients with a high T/C ratio (5/8, Mantel-Cox test for trend, $P < 0.02$) (Fig. 1). Moreover, female patients carrying the ACE I/* allele polymorphism had the lowest probability of reaching a fibrosis staging score >2 (1/6), while those carrying the ACE D/D alleles had the highest probability of reaching a staging score >2 (7/10). In male patients an intermediate behavior was observed (11/29, Mantel-Cox test for trend, $P < 0.01$) (Fig. 2). This analysis gave similar results when only patients who did not complete the scheduled antiviral treatment for recurrent hepatitis C ($n = 30$) were considered (1/6 vs. 6/17 vs. 5/7, Mantel-Cox test for trend, $P < 0.05$).

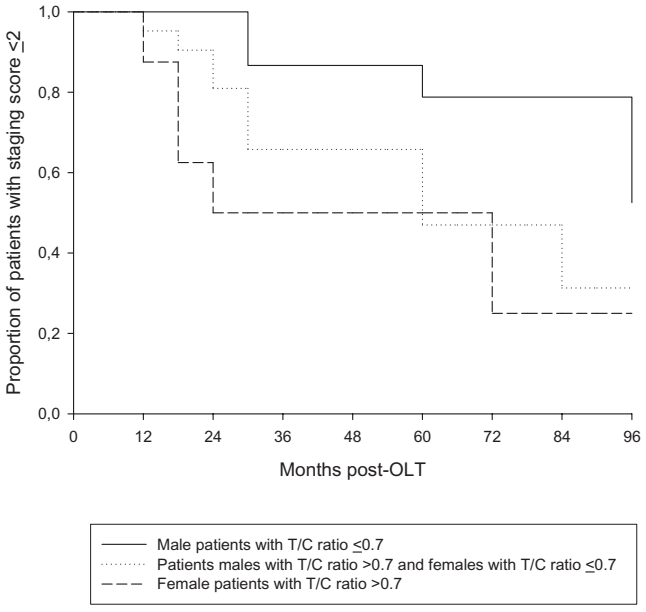

Fig. 1. Time-to-event analysis in reaching an Ishak staging score >2 in relationship to sex and to the 1-year post-orthotopic liver transplantation (*OLT*) lipid profile. *T/C ratio*, triglycerides to cholesterol ratio. There was a significant increasing trend in the rate of fibrosis progression from male patients with T/C ratio <0.7 to female patients with T/C ratio >0.7 (Mantel-Cox test for trend, $P < 0.02$)

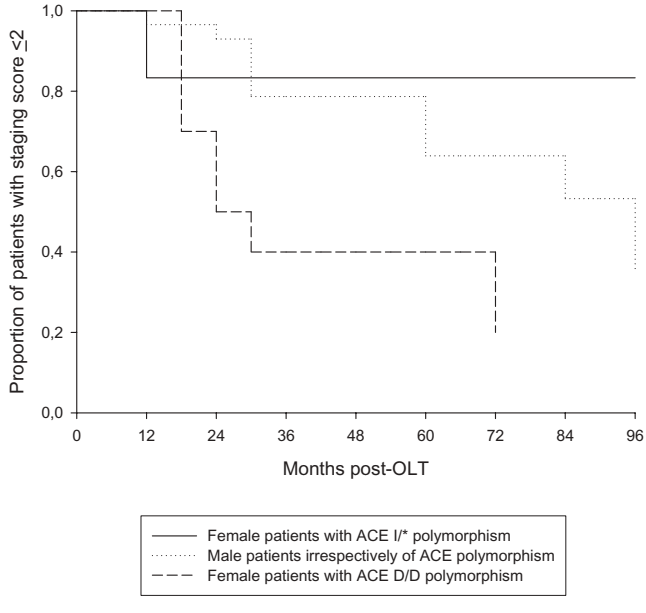

Fig. 2. Time-to-event analysis in reaching an Ishak staging score >2 in relationship to sex and angiotensin-converting enzyme (*ACE*) insertion/deletion (I/D) polymorphism. There was a significant increasing trend in the rate of fibrosis progression from female patients with ACE I/* to female patients with ACE D/D polymorphisms (Mantel-Cox test for trend $P < 0.01$)

Discussion

ACE is a key component of the RAS, converting angiotensin I to angiotensin II; it also inactivates the vasodilator bradykinin.[20] Both peptides play central roles in blood pressure regulation and are believed to be important in the pathogenesis of cardiovascular diseases. ACE levels in plasma and tissues are under genetic control.[21–23] There is a common I/D polymorphism in the ACE gene characterized by the presence or absence of a 287-bp *Alu* repeat. Subjects with the D/D genotype have higher plasma ACE activity compared with those with I/D or I/I genotypes. This finding predicts that carriers of the DD genotype may have increased blood pressure and a higher prevalence of cardiovascular diseases.[24] Besides the direct relationship between the ACE genotype and hypertension, evidence is growing in the literature that the ACE D/D polymorphism may favor the development of atherosclerosis through the influence exerted on other atherogenic factors such as lipids, plasma glucose, and BMI values.[9,25,26]

In this study concerning exclusively patients transplanted for hepatitis C virus-related end-stage liver disease, we confirmed our previously published results obtained in transplanted patients with liver diseases caused by any etiology.[10] Carrying at least one ACE D allele was strongly associated with the possibility of an increased BMI over pre-OLT levels at both 1 and 2 years post-OLT, although the exact mechanisms responsible for ACE-related BMI increases remain largely speculative. At the same time points and in association with BMI changes, modifications of the serum lipid profile, exemplified by a simple index such as the T/C ratio, occur. The relationship between changes in BMI and blood lipid profile was sex-related, as previously reported in both liver transplanted patients[8] and immune-competent subjects.[27] Men who increased their BMI 1 year post-OLT showed the highest frequency of a low T/C ratio (i.e., more cholesterol than triglycerides), whereas in contrast, female patients who did not gain body weight after OLT presented the highest frequency of a high T/C ratio. Approximately one in four OLT recipients in the present study underwent early antiviral therapy for recurrent hepatitis C; conceivably, BMI and fibrosis progression could have been different in treated patients. However, these effects were not observed. A likely explanation is that in the majority of patients who started early antiviral treatment, the time elapsed since the initiation of therapy to the completion of the first year after OLT was too short to allow metabolic consequences.

Why might BMI variations associated with a peculiar lipid profile influence liver fibrosis progression due to hepatitis C virus reinfection after liver transplantation? This process is not believed to progress linearly but to

reach a large magnitude early during the first year post-OLT and to subside thereafter.[7,28] This was confirmed in the present series, emphasizing the importance of the relationship between host modifications post-OLT and early allograft fibrosis progression. In fact, patients with a low T/C ratio 1 year post-OLT, rarely at that time point displayed fast fibrosis progression. At least two aspects of HCV infection are connected with lipid metabolism and lipid profile modifications: HCV circulates in blood in association with several different lipoproteins, and entry of HCV virions into hepatocytes may be mediated by the low density lipoprotein (LDL) receptor.[29] Virions are released by liver cells and may infect other liver cells via LDL receptors; free beta lipoproteins may regulate the rate of infection of liver cells by competing with the virus.[30] Thus, one might hypothesize that a particular lipid profile might be associated with different viral kinetics and, in turn, with lack of early fibrosis progression in the graft.

A number of studies have provided experimental evidence that RAS contributes to the pathogenesis of chronic liver disease. There is a marked upregulation of intrahepatic RAS components in animal models of liver injury and in cell cultures of activated human hepatic stellate cells.[13,16] The angiotensin II type I receptor has been found to be a major regulator of liver fibrosis development in animal and in vitro studies.[11,14,15] Accordingly, ACE inhibition using captopril is associated with a lower progression of hepatic fibrosis in the rat.[12] Despite the strong experimental evidence of the involvement of the RAS in inducing liver fibrosis, on the clinical side, the role of angiotensin II in modulating the liver fibrosis process in humans has not been clearly demonstrated. A preliminary study has raised the possibility that an angiotensin II antagonist may be therapeutically efficacious for nonalcoholic steatohepatitis.[31] As far as chronic hepatitis C is concerned, a preliminary report suggested that angiotensin II antagonist may reduce the development of hepatic fibrosis in the early stages of the disease,[32] and a retrospective study has demonstrated a beneficial effect of angiotensin-blocking agents on graft fibrosis in hepatitis C recurrence after liver transplantation.[33] Nevertheless, in a large population of patients with chronic hepatitis C no difference in the distribution of the ACE gene polymorphisms was found in relationship to the severity of liver fibrosis.[34]

In the present paper, ACE gene polymorphisms were found to be associated with the development of liver fibrosis in patients with recurrent hepatitis C. The association between ACE polymorphisms and fibrosis progression was found to occur preferentially in female recipients; conversely, lipid modifications due to weight gain after OLT were more evident in male than in female recipients. Accordingly, female homozygotes for the D allele reached an Ishak staging score >2 more frequently than those carrying I/* alleles; among male patients, no association between ACE polymorphisms and fibrosis development was observed. We speculate that in men the profibrogenic effects of the ACE D/D alleles may be blunted by the more frequent occurrence in these patients, early post-OLT, of significant weight gain. The latter would be associated with a favorable lipid profile, able to modulate the early effects of HCV infection in the graft. These observations may at least in part account for the discrepancies existing in the literature on the role of RAS in liver fibrosis progression and for the difficulty in transposing results derived from well-defined experimental models into the clinical setting.

In conclusion, in patients with recurrent hepatitis C, carriage of ACE allelic variants may exert important and antithetic effects. Carriers of the D allele appear to gain more weight after liver transplantation, while in male recipients the D allele is associated with a peculiar lipid profile that favors a slower pace of allograft fibrosis progression. On the other hand, among female recipients, carriage of the D allele, may favor early and more severe allograft fibrosis.

Acknowledgment. This work was supported in part by restricted grants from the Fondazione Cassa di Risparmio di Udine e Pordenone, Udine, and from Regione Piemonte, Italy.

References

1. Berenguer M, Lopez-Labrador FX, Wright TL. Hepatitis C and liver transplantation. J Hepatol 2001;35:666–78.
2. Berenguer M. Natural history of recurrent hepatitis C. Liver Transpl 2002;8:S14–8.
3. Berenguer M. Outcome of posttransplantation hepatitis C virus disease—is it the host, the virus, or how we modify the host and/or the virus? Liver Transpl 2002;8:889–91.
4. Wali M, Harrison RF, Gow PJ, Mutimer D. Advancing donor liver age and rapid fibrosis progression following transplantation for hepatitis C. Gut 2002;51:248–52.
5. Machicao VI, Bonatti H, Krishna M, Aqel BA, Lukens FJ, Nguyen JH, et al. Donor age affects fibrosis progression and graft survival after liver transplantation for hepatitis C. Transplantation 2004;77:84–92.
6. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. Gastroenterology 2002;122:889–96.
7. Toniutto P, Fabris C, Avellini C, Minisini R, Bitetto D, Rossi E, et al. Excess body weight, liver steatosis, and early fibrosis progression due to hepatitis C recurrence after liver transplantation. World J Gastroenterol 2005;11:5944–50.
8. Fabris C, Toniutto P, Bitetto D, Minisini R, Smirne C, Caldato M, et al. Low fibrosis progression of recurrent hepatitis C in apolipoprotein E epsilon4 carriers: relationship with the blood lipid profile. Liver Int 2005;25:1128–35.
9. Strazzullo P, Iacone R, Iacoviello L, Russo O, Barba G, Russo P, et al. Genetic variation in the renin–angiotensin system and abdominal adiposity in men: the Olivetti Prospective Heart Study. Ann Intern Med 2003;138:17–23.

10. Toniutto P, Fabris C, Minisini R, Apollonio L, Fumo E, Caldato M, et al. Weight gain after liver transplantation and the insertion/deletion polymorphism of the angiotensin-converting enzyme gene. Transplantation 2005;79:1338–43.
11. Yoshiji H, Kuriyama S, Yoshii J, Ikenaka Y, Noguchi R, Nakatani T, et al. Angiotensin-II type 1 receptor interaction is a major regulator for liver fibrosis development in rats. Hepatology 2001;34:745–50.
12. Jonsson JR, Clouston AD, Ando Y, Kelemen LI, Horn MJ, Adamson MD, et al. Angiotensin-converting enzyme inhibition attenuates the progression of rat hepatic fibrosis. Gastroenterology 2001;121:148–55.
13. Paizis G, Cooper ME, Schembri JM, Tikellis C, Burrell LM, Angus PW. Up-regulation of components of the renin–angiotensin system in the bile duct-ligated rat liver. Gastroenterology 2002;123:1667–76.
14. Leung PS, Suen PM, Ip SP, Yip CK, Chen G, Lai PB. Expression and localization of AT1 receptors in hepatic Kupffer cells: its potential role in regulating a fibrogenic response. Regul Pept 2003;116:61–9.
15. Kanno K, Tazuma S, Chayama K. AT1A-deficient mice show less severe progression of liver fibrosis induced by CCl(4). Biochem Biophys Res Commun 2003;308:177–83.
16. Bataller R, Sancho-Bru P, Gines P, Lora JM, Al-Garawi A, Sole M, et al. Activated human hepatic stellate cells express the renin–angiotensin system and synthesize angiotensin II. Gastroenterology 2003;125:117–25.
17. Cambien F, Poirier O, Lecerf L, Evans A, Cambou JP, Arveiler D, et al. Deletion polymorphism in the gene for angiotensin-converting enzyme is a potent risk factor for myocardial infarction. Nature 1992;359:641–4.
18. Rigat B, Hubert C, Corvol P, Soubrier F. PCR detection of the insertion/deletion polymorphism of the human angiotensin converting enzyme gene (DCP1) (dipeptidyl carboxypeptidase 1). Nucleic Acids Res 1992;20:1433.
19. Ishak K, Baptista A, Bianchi L, Callea F, De Groote J, Gudat F, et al. Histological grading and staging of chronic hepatitis. J Hepatol 1995;22:696–9.
20. Erdos EG, Skidgel RA. The angiotensin I-converting enzyme. Lab Invest 1987;56:345–8.
21. Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F. An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 1990;86:1343–6.
22. Tiret L, Rigat B, Visvikis S, Breda C, Corvol P, Cambien F, et al. Evidence, from combined segregation and linkage analysis, that a variant of the angiotensin I-converting enzyme (ACE) gene controls plasma ACE levels. Am J Hum Genet 1992;51:197–205.
23. Danser AH, Schalekamp MA, Bax WA, van den Brink AM, Saxena PR, Riegger GA, et al. Angiotensin-converting enzyme in the human heart. Effect of the deletion/insertion polymorphism. Circulation 1995;92:1387–8.
24. Tiret L, Kee F, Poirier O, Nicaud V, Lecerf L, Evans A, et al. Deletion polymorphism in angiotensin-converting enzyme gene associated with parental history of myocardial infarction. Lancet 1993;341:991–2.
25. Nagi DK, Foy CA, Mohamed-Ali V, Yudkin JS, Grant PJ, Knowler WC. Angiotensin-1-converting enzyme (ACE) gene polymorphism, plasma ACE levels, and their association with the metabolic syndrome and electrocardiographic coronary artery disease in Pima Indians. Metabolism 1998;47:622–6.
26. Uemura K, Nakura J, Kohara K, Miki T. Association of ACE I/D polymorphism with cardiovascular risk factors. Hum Genet 2000;107:239–42.
27. Wilsgaard T, Arnesen E. Change in serum lipids and body mass index by age, sex, and smoking status: the Tromso study 1986–1995. Ann Epidemiol 2004;14:265–73.
28. Neumann UP, Berg T, Bahra M, Seehofer D, Langrehr JM, Neuhaus R, et al. Fibrosis progression after liver transplantation in patients with recurrent hepatitis C. J Hepatol 2004;41:830–6.
29. Agnello V, Abel G, Elfahal M, Knight GB, Zhang QX. Hepatitis C virus and other *Flaviviridae* viruses enter cells via low density lipoprotein receptor. Proc Natl Acad Sci U S A 1999;96:12766–71.
30. Monazahian M, Bohme I, Bonk S, Koch A, Scholz C, Grethe S, et al. Low density lipoprotein receptor as a candidate receptor for hepatitis C virus. J Med Virol 1999;57:223–9.
31. Yokohama S, Yoneda M, Haneda M, Okamoto S, Okada M, Aso K, et al. Therapeutic efficacy of an angiotensin II receptor antagonist in patients with nonalcoholic steatohepatitis. Hepatology 2004;40:1222–5.
32. Terui Y, Saito T, Watanabe H, Togashi H, Kawata S, Kamada Y, et al. Effect of angiotensin receptor antagonist on liver fibrosis in early stages of chronic hepatitis C. Hepatology 2002;36:1022.
33. Rimola A, Londono MC, Guevara G, Bruguera M, Navasa M, Forns X, et al. Beneficial effect of angiotensin-blocking agents on graft fibrosis in hepatitis C recurrence after liver transplantation. Transplantation 2004;78:686–91.
34. Forrest EH, Thorburn D, Spence E, Oien KA, Inglis G, Smith CA, et al. Polymorphisms of the renin–angiotensin system and the severity of fibrosis in chronic hepatitis C virus infection. J Viral Hepat 2005;12:519–24.