Influence of Angiotensin-converting Enzyme Genetic Polymorphism on Late Renal Dysfunction After Adult-to-adult Living-donor Liver Transplantation

T. Matsui[a], M. Usui[a,*], K. Fujinaga[a], K. Nakatani[b], Y. Iizawa[a], H. Kato[a], A. Tanemura[a], Y. Murata[a], Y. Azumi[a], N. Kuriyama[a], M. Kishiwada[a], S. Mizuno[a], H. Sakurai[a], and S. Isaji[a]

[a]Department of Hepatobiliary Pancreatic and Transplant Surgery, Mie University Graduate School of Medicine, Mie, Japan; and the [b]Department of Clinical Laboratory, Mie University Graduate School of Medicine, Mie, Japan

ABSTRACT

Background. Late renal dysfunction (LRD) is known to be one of the most important complications to affect long-term outcome after living-donor liver transplantation (LDLT). The relationship between angiotensin-converting enzyme insertion (I)/deletion (D) gene polymorphism and renal function after LDLT are still unknown. The aim of this study was to elucidate the risk factors for LRD after LDLT, focusing on ACE gene polymorphism.

Materials and Methods. Among the 94 recipients who underwent adult-to-adult LDLT between March 2002 and September 2009, the total number of subjects who survived more than 1 year after LDLT and in whom angiotensin-converting enzyme genotype could be measured was 64. LRD was defined as estimated glomerular filtration rate level less than 60 mL/min/1.73 m^2 at any point after 1 year from undergoing LDLT.

Results. LRD was found in 24 patients (37.5%). The incidence of LRD was significantly higher in D/D type than in I/I or I/D type: 85.7% (6/7) vs. 42.1% (8/19), 35.7% (10/38) ($P = .010$). Preoperative estimated glomerular filtration rate was significantly lower in D/D type than in I/I, I/D types, and postoperatively they were significantly lower in D/D type at 2, 3, and 4 years after LDLT. By multivariate analysis, age and hypertension were the independent risk factors for LRD. The 10-year survival rate was much lower in the recipients with LRD than in those without LRD at 66.7% versus 87.5%, respectively ($P = .053$).

Conclusion. In conclusion, age and hypertension were determined as significant independent risk factors for LRD after adult-to-adult LDLT, and the recipients with D/D genotype should be strictly cared for the development of LRD.

LATE renal dysfunction (LRD) after the living-donor liver transplantation (LDLT) is one of the important complications that may affect the long-term outcome. According to the previous reports, causes of LRD after LDLT are multifactorial, including recipient age, preoperative RD, diabetes mellitus (DM), hyperlipidemia, calcineurin inhibitor (CNI), and acute renal dysfunction [1–4]. Previously, we reported that hypertension (HTN) and hepatitis C virus (HCV) infection were determined as independent risk factors for LRD after LDLT including pediatric cases [5].

On the other hand, importance of the genetic polymorphism in the organ transplantation was reported, and we reported that genetic polymorphism of CYP3A5 had an influence on the blood level of CNI [6]. It was reported that genetic polymorphism was present in angiotensin-

*Address correspondence to Masanobu Usui, Department of Hepatobiliary Pancreatic and Transplant Surgery, Mie University, Tsu, Mie 514-8507, Japan. E-mail: m-usui@clin.medic.mie-u.ac.jp

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converting enzyme (ACE), and that it has an influence on the renal failure after transplantation [7].

ACE insertion (I)/deletion (D) gene polymorphisms are classified into three genotypes, insertion/insertion (I/I) genotype, insertion/deletion (I/D) genotype, and deletion/deletion (D/D) genotype. In patients with ACE D/D genotype, ACE activity increases as compared with other genotypes [8] and ACE D/D genotype is known as a risk factor to exacerbate various circulatory disease and RD [9,10].

The aim of this study is to evaluate the incidence of LRD after adult-to-adult LDLT, focusing on ACE genotype, and the independent risk factors for LRD after adult-to-adult LDLT.

MATERIALS AND METHODS

Among the 94 recipients who underwent adult-to-adult LDLT between March 2002 and September 2009, the total number of subjects who survived more than 1 year after LDLT and in whom ACE genotype could be measured was 64. The present study was approved by the Ethical Committee of Mie University Hospital in accordance with the ethical standards established in the Declaration of Helsinki (No.587).

The primary disease was HCV in 21 patients (liver cirrhosis [LC] in 8, hepatocellular carcinoma [HCC] in 13), hepatitis B virus in 13 (fulminant hepatitis in 2, LC in 2, HCC in 9), alcoholic liver disease in 8 (acute liver failure in 1, LC in 3, HCC in 4), primary biliary cirrhosis in 8, non–hepatitis B virus/HCV in 7 (acute liver failure in 2, LC in 3, HCC in 1, glycogen storage disease in 1), fulminant hepatitis in 5, biliary atresia in 1, and primary sclerosing cholangitis in 1.

Post-transplantation data were collected up to 5 years for estimated glomerular filtration rate (eGFR) levels and up to 13 years for survival. The median follow-up period after liver transplantation (LT) was 102 months (range: 60 months to 150 months). LRD was defined as when the eGFR level showed less than 60 mL/min/1.73 m^2 at any point after 1 year from undergoing LDLT, according to the chronic kidney disease (CKD) definition from the Kidney Disease Outcomes Quality Initiative Guidelines from the National Kidney Foundation in 2002 [11].

Determination of ACE Genotypes

The ACE gene is present on chromosome 17q and consists of 26 exons and 25 introns. The gene in which Alu repeated sequence is inserted in intron 16 is called the insertion allele and the gene in which it is not inserted is called the deletion allele. By the combination of insertion and deletion alleles, the ACE gene can be classified into the three types: insertion/insertion (I/I) type, insertion/deletion (I/D) type, and deletion/deletion (D/D) type.

Genotyping of the patients was performed by polymerase chain reaction (PCR) and fragment analysis. PCR was performed using 20 ng of genomic DNA and 200 μmol/L of primers. The thermocycling procedure (PTC 100, MJ research, Waltham, Massachusetts, United States) consisted of initial denaturation at 95 °C for 10 minutes followed by 35 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minutes, and a final extension of 72°C for 7 minutes. We used the following primers to detect deletion of intron16 (forward primer in exon16, 5′GAGAGAGACTCAAGCACGCC3′; forward primer in intron 16, 5′CATTCTCCTGCCTCAGCCT3′; reverse primer in exon 17, 5′CCATCACATTCGTCAGATCTG3′). The PCR product was visualized by ultraviolet transillumination in a 2% agarose gel containing ethidium bromide. In the PCR products generated by the three primers, the I allele was detected as 565-bp and 315-bp fragments and the D allele was detected as a 276-bp fragment.

Immunosuppression

The immunosuppression protocol consisted of tacrolimus and low-dose steroids. The target whole-blood trough level for tacrolimus was 10 ng/mL to 12 ng/mL during the first 2 weeks, approximately 10 ng/mL thereafter, and 5 ng/mL to 10 ng/mL from the second month after LDLT. Methylprednisolone (1 mg/kg/d, intravenously) was administered on postoperative days (PODs) 1 to 3, followed by 0.5 mg/kg/d on PODs 4 to 6. Steroid administration was then switched to oral prednisolone (0.3 mg/kg/d) on POD 7, and the dose was reduced to 0.1 mg/kg/d at 1 month after LDLT. If the patients' liver functions were stable, recipients were weaned off steroids at 3 to 6 months after LDLT. When the side effect of tacrolimus developed, we changed the immunosuppressive drug to cyclosporine.

Statistical Analysis

Continuous variables were presented as mean ± SD. Categorical variables were expressed as numbers and percentages. The χ^2 test was used to compare categorical variables and the Mann-Whitney U test was used to compare continuous variables. Multivariate analysis was performed after univariate analysis to identify the independent risk factors for LRD. Survival was calculated using the Kaplan-Meier method and was compared between the groups using log-rank test. A P value $< .05$ was considered statistically significant, and all analyses were performed using the IBM SPSS statistics version 22 (IBM Corp., Armonk, NY, USA).

RESULTS

The backgrounds for the 64 recipients are summarized in Table 1. Pretransplantation coexisting illnesses associated with potential risks of RD were DM in 19 patients (29.7%), HTN in 23 (35.9%), and HCV infection in 21 (32.8%). We classified these 64 recipients into the three groups (I/I, I/D, D/D type), according to ACE genetic polymorphism. ACE genotype was I/I in 19, I/D in 38, and D/D in 7. The frequency of D allele in ACE gene in study cohort was determined as 40.6% (52/126).

The Incidence of LRD and Long-term Outcomes According to ACE Genotype

LRD was found in 24 patients (37.5%). Comparing background factors among the three ACE genotypes, the incidence of development of LRD in D/D type was significantly higher than those in I/I and I/D type (I/I vs. D/D type: 42.1% (8/19) vs. 85.7% (6/7), $P = .048$; I/D vs. D/D type: 26.3% (10/38) vs. 85.7% (6/7), $P = .003$), and there was no significant difference between I/I and I/D type (I/I vs. I/D type: 42.1% (8/19) vs. 26.3% (10/38), $P = .227$). Therefore, we reclassified the three groups into the following two groups (I/I, I/D group: 57 patients; D/D group: 7 patients), to clarify the clinical characteristics of the patients of the D/D group.