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.

Table 1. Background of 64 Adult-to-adult Living Donor Liver Transplantation Patients

Demographics	Total
Preoperative factors	
Gender (male/female)	41 (64.1%)/23 (35.9%)
Age (mean ± SD)	52.2 ± 11.4
Child-Pugh (A/B/C)	12 (18.8%)/21 (32.8%)/31 (48.4%)
MELD score (mean ± SD)	16.5 ± 7.5
ABO compatibility (Identical/ Compatible/incompatible)	51 (79.7%)/12 (18.8%)/1 (1.6%)
Hypertension (HTN)	23 (35.9%)
Diabetes mellitus (DM)	19 (29.7%)
HCV antibody positive	21 (32.8%)
Creatinine (mg/dl)	0.88 ± 0.37
eGFR (mL/min/1.73m^2)	69.8 ± 27.1
ACE genotype (I/I, I/D, D/D)	19 (29.7%)/38 (59.4%)/7 (10.9%)
Operative factors	
CIT (min)	113.6 ± 89.3
WIT (min)	46.5 ± 16.6
Blood loss (ml)	15170 ± 15709
GRWR	1.01 ± 0.20
GV/SLV (%)	53.38 ± 9.64
Postoperative factors	
Immunosuppression (CNI) (tacrolimus/cyclosporine)	52 (81.3%)/12 (18.7%)
LRD (negative/positive)	40 (62.5%)/24 (37.5%)

Abbreviations: MELD, model for end-stage liver disease; HCV, hepatitis C virus; ACE, angiotensin converting enzyme; CYP23A5, cytochrome P490 3A5; CIT, cold ischemia time; WIT, warm ischemia time; GRWR, graft-to-recipient weight ratio; GV/SLV, graft volume/standard liver volume; CNI, calcineurin inhibitor.

Comparing preoperative factors between the two groups (I/I, I/D and D/D), age and the incidences of HCV-positive cases were significantly higher in the D/D group than those in the I/I, I/D group. The other preoperative factors such as HTN, DM, and operative factors did not show any significant difference between the two groups. Although preoperative creatinine levels did not differ between I/I, I/D and D/D groups, preoperative eGFR was significantly lower in the D/D group (53.9 ± 36.3) than in the I/I, I/D group (74.8 ± 30.0) ($P = .046$). The incidence of LRD was significantly higher in the D/D group than in the I/I, I/D group at 85.7% (6/7) versus 31.6% (18/57) ($P = .010$) (Table 2).

When the changes of eGFR levels were compared in the I/I, I/D group and the D/D group, eGFR levels were significantly lower in the D/D group than in the I/I, I/D group at preoperative, 2, 3, and 4 years after LDLT (Fig 1). There was no significant difference in survival rates between I/I, I/D and D/D type (Fig 2).

Risk Factor Analysis for LRD

Comparing the categorical variables between 24 patients with LRD (LRD group) and 40 without LRD (non-LRD group), age and the incidences of HTN, HCV-positive, and ACE D/D type, preoperative renal dysfunction was significantly higher in the LRD group (Table 3). Preoperative eGFR was significantly lower in the LRD group (52.5 ± 22.6

Table 2. Results of Univariate Analysis Between I/I, I/D and D/D Groups

	I/I, I/D (n = 57)	D/D (n = 7)	*P* Value
Preoperative factors			
Gender (male/female)	38/19	4/3	.617
Age	50.4 ± 11.4	60.2 ± 10.7	**.041**
Child-Pugh (A/B/C)	11/17/29	1/3/3	.778
MELD score	17.1 ± 7.5	15.6 ± 6.3	.787
Emergency/elective	8/49	0/7	.299
ABO compatibility (identical/ compatible/ incompatible)	46/10/1	5/2/0	.743
Hypertension (HTN)	19	4	.215
Diabetes mellitus (DM)	16	2	.978
HCV antibody positive	16/41	5/2	**.021**
Creatinine (mg/dl)	0.84 ± 0.29	1.21 ± 0.68	.108
eGFR (mL/min/ 1.73m^2)	74.8 ± 30.0	53.9 ± 36.3	**.046**
Operative factors			
CIT (min)	108 ± 77	190 ± 148 0	.171
WIT (min)	45 ± 15	46 ± 10	.741
Blood loss (ml)	13,214 ± 11,544	17,691 ± 13,494	.130
GRWR	1.02 ± 0.20	1.15 ± 0.27	.971
GV/SLV (%)	51.8 ± 8.4	59.5 ± 9.7	.530
Postoperative factors			
Immunosuppression (CNI) (tacrolimus/ cyclosporine)	47/10	5/2	.481
LRD (negative/ positive)	39/18 (31.6%)	1/6 (85.7%)	**.010**

Significant values are shown in bold.

Abbreviations: LRD, late renal dysfunction; MELD, model for end-stage liver disease; HCV, hepatitis C virus; ACE, angiotensin converting enzyme; CYP23A5, cytochrome P490 3A5; CIT, cold ischemia time; WIT, warm ischemia time; GRWR, graft-to-recipient weight ratio; GV/SLV, graft volume/standard liver volume; CNI, calcineurin inhibitor.

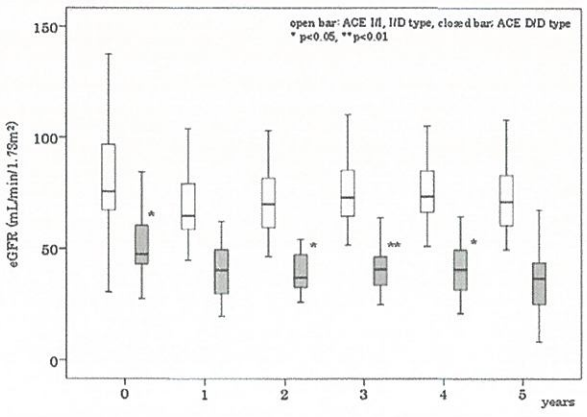

Fig 1. Chronological changes of estimated glomerular filtration levels in angiotensin-converter enzymes (ACE) I/I, I/D, and D/D type before and after living-donor liver transplantation. Abbreviations: I, insertion; D, deletion.

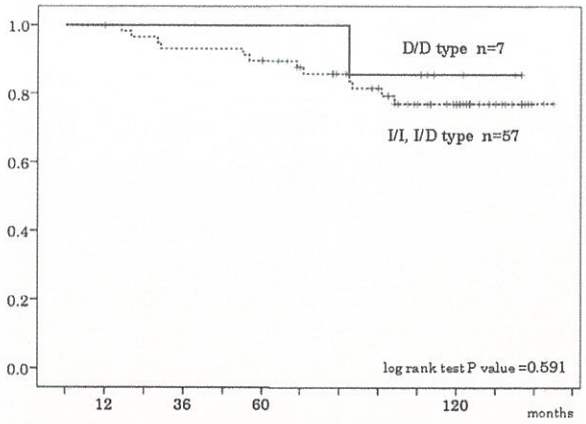

Fig 2. Kaplan-Meier actuarial survival curves in angiotensin-converter enzyme (ACE) I/I, I/D and D/D type. Abbreviations: I, insertion; D, deletion.

mL/min/1.73 m^2) than in non-LRD group (84.4 ± 29.7 mL/min/1.73 m^2) ($P < .01$), and preoperative creatinine level was significantly higher in the LRD group (1.12 ± 0.46 mL/min/1.73 m^2) than in the non-LRD group (0.74 ± 0.19 mL/min/1.73 m^2) ($P < .01$). By multivariate analysis of the factors influencing LRD, recipient's age and HTN were determined to be independent risk factors (Table 4).

When we compared eGFR levels between the LRD and the non-LRD group until 5 years after LDLT, they had been significantly lower in the LRD group than in the non-LRD group (Fig 3). However, in the LRD group, eGFR levels did not decrease significantly as compared with the levels at 1 year after LDLT. Finally, even in the LRD group, hemodialysis was needed in only 2 (8.3%) of 24 patients. The 10-year survival rate was much lower in the LRD group than in the non-LRD group at 66.7% versus 87.5% ($P = .053$; Fig 4), showing nearly significant difference. Among the 24 recipients in the LRD group, 8 died, including 2 from HCC recurrence, 2 from liver failure due to HCV recurrence, 1 from pancreatic tail cancer, 1 from post-transplantation lymphoproliferative disorder, 1 from a diabetic coma, and 1 from sepsis.

DISCUSSION

LRD after LT is known as an important factor affecting long-term prognosis. The incidence of LRD was reported as 7.3% to 18.1% at 5 years after LT [12,13]. According to the previous reports, causes of LRD after LT are multifactorial, including recipient age, preoperative RD, DM, hyperlipidemia, CNI, and acute RD [1–4]. Previously we reported that HTN and HCV infection were independent risk factors of LRD in after LDLT including pediatric cases [5]. The difference between our previous report and the present study is the definition of LRD and the study cohort. Because our previous report included 15 pediatric cases, we did not use eGFR data to difine LRD; therefore LRD was defined as when the serum creatinine level showed 1.5 mg/mL or more

Table 3. Results of Univariate Analysis Between Non LRD and LRD Groups

	non-LRD (n = 40)	LRD (n = 24)	*P* value
Preoperative factors			
Gender (male/female)	27/13	9/15	.878
Age	48.4 ± 11.7	56.4 ± 9.9	**.003**
Child-Pugh (A/B/C)	9/11/21	3/10/11	.422
MELD score	15.2 ± 6.4	18.9 ± 8.2	.780
Emergency/elective	35/5	21/4	.659
ABO compatibility (identical/ compatible/ incompatible)	33/6/1	18/6/0	.471
Hypertension (HTN)	9	14	**.005**
Diabetes mellitus (DM)	11	7	.912
HCV antibody positive	8	13	**.006**
Creatinine (mg/dL)	0.74 ± 0.19	1.12 ± 0.46	**<.01**
eGFR (mL/min/ 1.73m^2)	84.4 ± 29.7	52.5 ± 22.6	**<.01**
Preoperative RD (negative/positive)	40/0	21/3	**.022**
ACE genotype (I/I, I/D vs. D/D)	39/1	18/6	**.006**
Operative factors			
CIT (min)	117 ± 84	113 ± 109	.701
WIT (min)	48 ± 16	37 ± 14	.111
Blood loss (ml)	14,462 ± 13,342	11,821 ± 8,471	.094
GRWR	1.07 ± 0.24	0.97 ± 0.13	.053
GV/SLV (%)	54.5 ± 9.8	49.9 ± 6.1	.052
Postoperative factors			
Immunosuppression (CNI) (tacrolimus/ cyclosporine)	34/6	18/6	.492

Significant values are shown in bold.

Abbreviations: LRD, late renal dysfunction; MELD, model for end-stage liver disease; HCV, hepatitis C virus; ACE, angiotensin converting enzyme; CYP23A5, cytochrome P490 3A5; CIT, cold ischemia time; WIT, warm ischemia time; GRWR, graft-to-recipient weight ratio; GV/SLV, graft volume/standard liver volume; CNI, calcineurin inhibitor.

at any point after 1 year from undergoing LDLT, which is different from the international criteria for CKD [11]. By excluding pediatric cases and using the international criteria for CKD in the present study, we aimed to clarify more reliable risk factors for LRD in adult-to-adult LDLT, focusing on the ACE gene polymorphism.

According to the ACE gene polymorphism, frequency of the D allele in the 237 cases in Japan (133 hypertensive and 104 normotensive subjects) was reported as 41.6% [14], which is similar to 40.6% in the present study. However, the incidence of D/D type in their population was 20.3%, which is higher than the 10.9% in our study. It is known that serum

Table 4. Multivariate Analysis of Risk Factors for LRD

	Odds Ratio	95% CI	*P* Value
Age	1.079	1.001–1.163	.048
Hypertension (HTN)	3.774	1.058–13.468	.041

Abbreviation: LRD, late renal dysfunction.

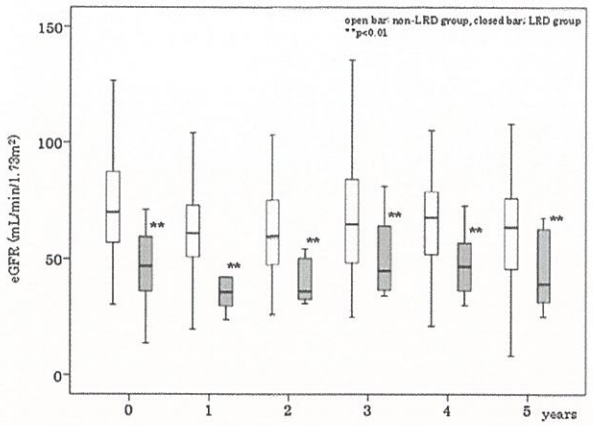

Fig 3. Chronological changes of estimated glomerular filtration rate (eGFR) levels in non-late renal dysfunction (LRD) and LRD groups before and after living-donor liver transplantation.

concentrations of ACE in the D/D type [8] are increased as compared with the other genotypes, and furthermore the incidences of various circulatory diseases and renal failures are significantly higher in the D/D type [9,10]. Therefore, we elucidated the risk factors of LRD after LDLT, focusing on ACE genetic polymorphism in the present study.

Lorenzo et al [7] reported that the ACE gene D/D polymorphism, HCV infection, and CNI (cyclosporine A) were independent risk factors for LRD (defined by serum creatinine 1.5 mg/dL or more) after deceased-donor LT. Based on those results, they concluded that the recipient with D/D type should avoid CNI use as initial immunosuppressive therapy because risk for CNI nephrotoxicity was high for them. To the best of our knowledge, our study is the first report to evaluate the risk factors for LRD (defined by eGFR) after LDLT focusing on ACE genotype. In our analysis, the ACE gene D/D type did not become the independent risk factor by multivariable analysis, although it was determined to be a risk factor by univariate analysis. As

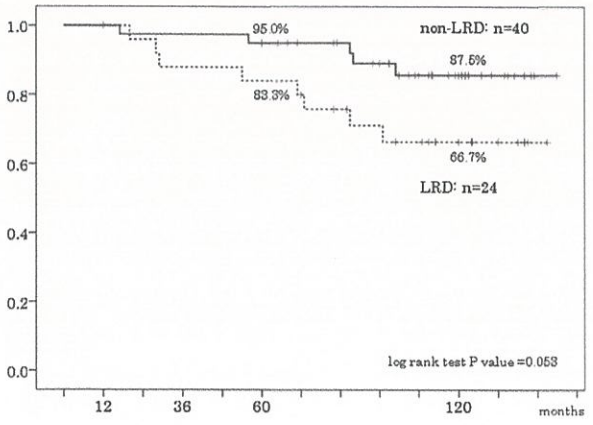

Fig 4. Kaplan-Meier actuarial survival curves in non-late renal dysfunction (LRD) and LRD groups.

a result of our multivariable analysis, the independent risk factors of LRD were age and HTN. Structural and functional change in the kidney occurs with aging [15]. In addition to aging, the recipients with HTN may develop nephrosclerosis, resulting in RD. The difference in the risk factors for LRD between the Lorenzo et al study and ours may be due to the differences in the definitions of LRD and in types of LT between studies.

As for renal function according to ACE genotype, the preoperative eGFR level was significantly lower in the D/D group than in the I/I, I/D group, although preoperative creatinine levels did not differ, demonstrating that eGFR is superior to creatinine in terms of precise assessment of renal function. Postoperatively, eGFR levels were also significantly lower in the D/D group than in the I/I, I/D group at 2, 3, and 4 years after LDLT. These results indicate that the patients with the D/D genotype already have impaired renal function preoperatively; therefore, we should manage these patients very carefully during the pre- and postoperative periods. As for long-term survival, there was no significant difference between the I/I, I/D and D/D types.

In our study, long-term prognosis was poorer in the LRD group than in the non-LRD group, showing nearly significant difference. The causes of death in both groups did not differ, and there were no deaths directly related to LRD.

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

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