incidence of hepatic artery complications, probably thanks to microvascular surgical technique. This technique should be woven into the fabric of living donor liver transplantation procedure. The use of left or lateral grafts with double or triple arteries yielded the same survival outcomes as observed in grafts with a single artery. However, the use of right grafts with multi-arteries was discouraging in the current study.

MATERIALS AND METHODS

Patients

Four hundred forty-six cases of LDLT (437 cases of primary LDLT and 9 cases of reLDLT) performed in Kyushu University hospital between October 1996 and October 2012 after obtaining approval in each transplant from the Ethics and Indications Committees for LDLT were retrospectively analyzed. The patients were divided into the following three groups according to the number of arteries on the graft: the single group (n=331), the double group (n=108), and the triple group (n=7). The representative hepatic artery reconstructions in each group are depicted in Figure 1. The patients were managed and treated as described previously (*3, 18*). Neither antiplatelet agents nor anticoagulants for the purpose of preventing hepatic artery thrombosis were used. Our criteria for selecting the type of hepatic graft are as follows. A lateral graft or a left graft was selected according to the capacity of each recipient's abdominal cavity in pediatric patients. A left graft was preferentially selected when the estimated volume of the left graft exceeded 35% of the standard liver volume (*19*) of the recipient in adult patients (*11, 12, 20*). A right graft was selected only when the estimated remnant liver volume of the donor was no less than 35% of the total liver volume (*11, 12*).

Hepatic Artery Reconstruction

Hepatic arteries were reconstructed as described previously (*4, 21*). Almost all hepatic artery reconstructions were performed under a microscope. A representative schematic diagrams of hepatic artery reconstruction are depicted in Figure 3. The more arteries a liver graft had, the more complex hepatic artery reconstruction was needed. Figure 4 shows representative Doppler sonography after hepatic artery reconstruction. Reconstructing only the left hepatic artery resulted in poor pulsatile arterial flow in segment 4. After the middle hepatic artery was reconstructed, the arterial flow in the segment 4 became strong. Our policy on how to reconstruct hepatic arteries in grafts with two or more arteries was that when technically feasible, all arteries were reconstructed to maximize hepatic arterial inflow. Primary candidates of recipients' inflow arteries were the left, middle, and the right hepatic arteries. When these hepatic arteries could not be used, extra-anatomic inflow arteries (e.g., the right gastroepiploic artery, the left gastric artery, the gastroduodenal artery) were used (*22*). Only when extensive dissection of candidate inflow artery was needed, the second or the third hepatic artery reconstruction was abandoned. For example, in a case of a graft with double arteries, that is, the small middle hepatic artery and the relatively large left hepatic artery, we reconstructed the graft left hepatic artery first. When only the recipient splenic artery was a candidate inflow artery for reconstructing the small middle hepatic artery of the graft, we abandoned the second reconstruction.

Outer diameters of the graft arteries were recorded after hepatic artery reconstruction. Daily Doppler ultrasound confirmed intact pulsatile arterial flows in the grafts for 1 week postoperatively. When Doppler ultrasound could not detect pulsatile arterial flows in the grafts and any artery-related complications were suspected, contrast-enhanced CT was perfumed. The contrast-enhanced CT also suggested an artery-related complication; conventional angiography was performed to confirm what type of artery-related complication had occurred.

Statistical Analyses

Student *t* test or one-way ANOVA was used for comparing variables. Proportions were compared using χ^2 test. Survival statistics were calculated by a Kaplan-Meier analysis, and patient survival curves were compared using a log-rank test. The censor date was set at November 30, 2012. Statistical significance was defined as having a *P* value of less than 0.05. All statistical analyses were performed using the NCSS 2007 software package (Hintze JL, Keysville, UT).

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ORIGINAL ARTICLE

Sarcopenia Is a Prognostic Factor in Living Donor Liver Transplantation

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The aims of this study were to investigate sarcopenia as a novel predictor of mortality and sepsis after living donor liver transplantation (LDLT) and to evaluate the effects of early enteral nutrition on patients with sarcopenia. Two hundred four patients undergoing preoperative computed tomography within the month before LDLT were retrospectively evaluated. The lengths of the major and minor axes of the psoas muscle were simply measured at the caudal end of the third lumbar vertebra, and the area of the psoas muscle was calculated. A psoas muscle area lower than the 5th percentile for healthy donors of each sex was defined as sarcopenia. Ninety-six of the 204 patients (47.1%), including 58.3% (60/103) of the male patients and 35.6% (36/101) of the female patients, were diagnosed with sarcopenia. Sarcopenia was independently and significantly associated with overall survival: there was an approximately 2-fold higher risk of death for patients with sarcopenia versus patients without sarcopenia (hazard ratio = 2.06, $P = 0.047$). Sarcopenia was an independent predictor of postoperative sepsis (hazard ratio = 5.31, $P = 0.009$). Other independent predictors were a younger recipient age ($P < 0.001$) and a higher body mass index ($P = 0.02$). Early enteral nutrition within the first 48 hours after LDLT was performed for 24.2% in 2003-2007 and for 100% in 2008-2011, and the incidence of postoperative sepsis for patients with sarcopenia (n = 96) was 28.2% (11/39) in 2003-2007 and 10.5% (6/57) in 2008-2011 ($P = 0.03$). In conclusion, sarcopenia is an independent predictor of mortality and sepsis after LDLT. The incidence of postoperative sepsis was reduced even in patients with sarcopenia after the routine application of early enteral nutrition. ***Liver Transpl 20:401-407, 2014.*** **© 2013 AASLD.**

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Sarcopenia is a term used to describe skeletal muscle loss with aging.[1,2] Sarcopenia can occur in patients with a variety of chronic illnesses, such as cancer, cardiovascular disease, bone fractures, chronic liver disease, and malnutrition.[3] More than 40% of patients with liver cirrhosis reportedly have concomitant sarcopenia.[4]

An evaluation of muscle loss in patients with liver cirrhosis was recently reported to be an important and novel predictor of survival, although its mechanisms are not fully understood. Montano-Loza et al.[4] showed that sarcopenia was associated with mortality in patients with cirrhosis, but it did not correlate with the degree of liver dysfunction as evaluated with a conventional scoring system. A few reports regarding mortality after liver transplantation and sarcopenia have been recently published. Englesbe et al.[5] reported that central sarcopenia strongly correlated with mortality after deceased donor liver transplantation (DDLT). Kaido et al.[6] reported that patients with sarcopenia had worse survival after living donor liver transplantation (LDLT). Our first hypothesis is that sarcopenia is associated with outcomes and the rate of sepsis after LDLT.

Abbreviations: *a*, radius of the major axis; *b*, radius of the minor axis; BCAA, branched-chain amino acid; BMI, body mass index; DDLT, deceased donor liver transplantation; GV/SLV, graft volume/standard liver volume; LDLT, living donor liver transplantation; MELD, Model for End-Stage Liver Disease.

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For the evaluation of central muscle loss, preoperative computed tomography scans were used to measure the psoas muscle area, which is a valid part of a sarcopenia evaluation. In many reports, this has been complicated by the fact that specific area-tracing software or manual tracing was needed to calculate the psoas muscle area. In the current study, to simplify the measurements, the lengths of the major and minor axes of the psoas muscle were measured. The area of the psoas muscle was simply approximated with the radii of the major and minor axes.

It has been reported that enteral nutrition prevents intestinal mucosal atrophy and preserves intestinal structure and functions.[7] Previously, we have reported the beneficial impact of early enteral nutrition within the first 48 hours after LDLT in reducing postoperative sepsis.[8] However, the actual impact of early enteral nutrition on patients with sarcopenia is not known. Our second hypothesis is that there are some differences in the impact of early enteral nutrition on patients with sarcopenia and patients without sarcopenia.

The aims of this study were (1) to investigate sarcopenia as a novel predictor of mortality and sepsis after LDLT and (2) to evaluate the effects of early enteral nutrition on patients with sarcopenia.

PATIENTS AND METHODS

Patients

Two hundred twenty-eight recipients of LDLT performed at Kyushu University Hospital between November 2003 and December 2011 were retrospectively investigated. Twenty-three patients with acute hepatic failure and 1 patient who died from operative blood loss were excluded from this study. Psoas muscle measurements from computed tomography were available for 204 recipients. Written informed consent was obtained from all patients. The institutional review board approved this study.

Assessment of the Area of the Psoas Muscle

All study patients underwent preoperative computed tomography within the month before LDLT. Instead of using any area-measuring software, we simply measured the lengths of the major and minor axes of the psoas muscle at the caudal end of the third lumbar vertebra. The area of the psoas muscle was calculated with the following formula:

$$\text{Area} = a \times b \times \pi \quad (1)$$

where a and b are the radii of the major and minor axes, respectively.

In this study, for the definition of sarcopenia, we consulted our previous study of the cross-sectional area of the psoas muscle at the caudal end of the third lumbar vertebra of healthy donors.[9] An area of the psoas muscle lower than the 5th percentile for each sex was defined as sarcopenia. The cutoff levels were defined as 800 cm^2 for men and 380 cm^2 for women.[9]

Evaluation of the Prognostic Factors After LDLT

Predictors of sarcopenia were evaluated only with preoperative values. The following were used as preoperative factors: recipient age, donor age, recipient sex, recipient status, preoperative renal failure, body mass index (BMI), Child-Pugh class, Model for End-Stage Liver Disease (MELD) score, and graft volume/standard liver volume (GV/SLV) ratio. Prognostic factors were investigated with the foregoing preoperative values and sarcopenia.

Evaluation of the Correlation Between Sarcopenia and Postoperative Sepsis

Postoperative sepsis was defined as the isolation of bacteria other than common skin contaminants from a single blood culture within the first 3 months after transplantation along with clinical symptoms.[8,10] Risk factors for postoperative sepsis were investigated with the preoperative factors.

We introduced early enteral nutrition after LDLT in 2003. Initially, the adoption of enteral nutrition was determined on a case-by-case basis. Since 2008, early enteral nutrition via a nasojejunal tube has been routinely applied for all recipients within the first 24 hours after LDLT.[8] In order to evaluate the effects of early enteral nutrition on postoperative sepsis in patients with sarcopenia, the postoperative sepsis rates for patients with sarcopenia and patients without sarcopenia before and since 2008 were investigated.

Statistical Analysis

All values are expressed as means and standard deviations. Univariate analyses were performed with the chi-square test or Fisher's exact probability test for categorical values and with the Mann-Whitney U test for continuous variables. Overall survival rates were calculated and compared with the Kaplan-Meier method and the log-rank test or Cox regression. Multivariate analyses were performed with the Cox proportional hazards regression model for overall survival. Differences with a P value < 0.05 were considered to be significant. All statistical analyses were performed with StatView 5.0 (SAS Institute, Cary, NC).

RESULTS

Definition of Sarcopenia

The median calculated area of the psoas muscle was 530.6 cm^2 for all patients (range = 122.5-1667.5 cm^2), 760.9 cm^2 for male patients (range = 192.7-1667.5 cm^2), and 423.1 cm^2 for female patients (range = 122.5-1195.6 cm^2). Histograms of the area of the psoas muscle for all patients (Fig. 1A), male patients (Fig. 1B), and female patients (Fig. 1C) are shown. The histograms of all populations were normally distributed.

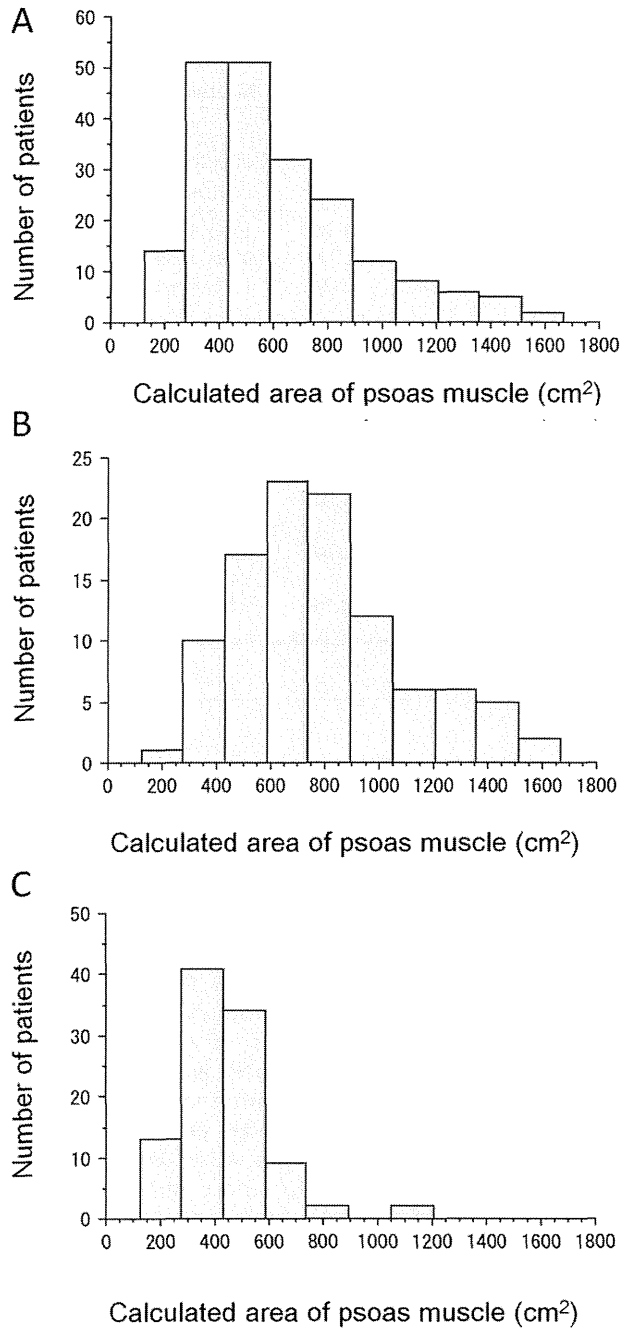

Figure 1. Histograms of the area of the psoas muscle for (A) all patients, (B) male patients, and (C) female patients. The histograms of all populations were normally distributed.

When we defined the cutoff levels as 800 cm^2 for men and 380 cm^2 for women on the basis of our previous data for healthy donors, 96 of the 204 patients (47.1%), including 58.3% (60/103) of the male patients and 35.6% (36/101) of the female patients, were diagnosed with sarcopenia.

Comparisons of the clinical characteristics of patients with sarcopenia and patients without sarcopenia are shown in Table 1. In the univariate analysis, the rates were higher in the sarcopenia group for the following variables: male sex (P = 0.001), hospitalized (P = 0.005), renal failure (P = 0.04), Child-Pugh class C (P = 0.02), and a MELD score $\geq$ 20 (P = 0.01). Patients with sarcopenia had lower BMIs (P = 0.004) than patients without sarcopenia. A logistic regression analysis revealed that a higher recipient age (P = 0.05), male sex (P < 0.001), and a lower recipient BMI (P = 0.002) were associated with sarcopenia.

As for the diagnoses of the recipients, 12 of 26 patients (46.2%) with hepatitis B virus–positive cirrhosis, 45 of 103 patients (43.7%) with hepatitis C virus–positive cirrhosis, 12 of 27 patients (44.4%) with primary biliary cirrhosis, 7 of 10 patients (70.0%) with alcoholic cirrhosis, and 20 of 38 patients (52.6%) with other diagnoses suffered from sarcopenia (P = 0.79).

Prognostic Factors After LDLT

Patients with sarcopenia showed significantly worse overall survival in comparison with patients without sarcopenia (P = 0.02; Fig. 2). The 3- and 5-year overall survival rates were 74.5% and 69.7%, respectively, for patients with sarcopenia and 88.9% and 85.4%, respectively, for patients without sarcopenia (P = 0.02). Twenty-three patients with sarcopenia died during the follow-up period. The causes of death were postoperative sepsis for 26.1% (6/23), recurrence of hepatocellular carcinoma for 21.7% (5/23), postoperative bleeding for 13.0% (3/23), and other causes for 39.1% (9/23).

The univariate analysis showed that patients with a lower overall survival rate after LDLT correlated with higher rates of preoperative renal failure (P = 0.01) and sarcopenia (P = 0.02; Table 2). In the multivariate analysis, only sarcopenia (hazard ratio = 2.06, P = 0.047) was an independent prognostic factor. Age, BMI, Child-Pugh score, MELD score, and GV/SLV ratio did not influence overall survival after LDLT.

Sarcopenia and Postoperative Sepsis

Twenty-five of the 204 patients experienced postoperative sepsis. The rate of postoperative sepsis was 17.7% (17/96) for patients with sarcopenia and 7.4% (8/108) for patients without sarcopenia (P = 0.03). Risk factors for postoperative sepsis were investigated. In the univariate analysis, recipient age (P < 0.001), donor age (P = 0.046), recipient status (P = 0.03), preoperative renal failure (P = 0.01), a MELD score $\geq$ 20 (P = 0.04), and sarcopenia (P = 0.03) were significant. A logistic regression analysis revealed that a lower recipient age (P < 0.001), a higher BMI (P = 0.02), and sarcopenia (P = 0.009) were significant risk factors (Table 3).

The effects of early enteral nutrition on postoperative sepsis were investigated in patients with sarcopenia and patients without sarcopenia. Early enteral nutrition within the first 48 hours after LDLT was performed for 24.2% (24/99) in 2003-2007 and for 100% (105/105) in 2008-2011. The incidence of postoperative sepsis was 18.2% (18/99) in 2003-2007 and 6.7% (7/105) in 2008-2011 (P = 0.02). In the

TABLE 1. Comparison of the Clinical Characteristics of Patients With Sarcopenia and Patients Without Sarcopenia

Variable	No Sarcopenia (n = 108)	Sarcopenia (n = 96)	Univariate Analysis: *P* Value	Multivariate Analysis: Hazard Ratio	Multivariate Analysis: 95% Confidence Interval	Multivariate Analysis: *P* Value
Recipient age (years)*	53.9 ± 10.5	54.8 ± 8.5	0.48	1.03	1.00-1.07	0.05
Donor age (years)*	34.4 ± 9.8	35.2 ± 11.2	0.59	1.01	0.98-1.04	0.50
Recipient sex: male/female [% (n)]	39.8 (43)/60.2 (65)	62.5 (60)/37.5 (36)	0.001	3.34	1.75-6.41	<0.001
Recipient status: hospitalized/home [% (n)]	20.4 (22)/79.6 (86)	38.5 (37)/61.5 (59)	0.005	1.95	0.90-4.23	0.09
Preoperative renal failure: yes/no [% (n)]	2.8 (3)/97.2 (105)	10.4 (10)/89.6 (86)	0.04	2.02	0.44-9.23	0.37
Recipient BMI (kg/m^2)*	24.2 ± 3.6	22.8 ± 3.1	0.004	0.86	0.78-0.95	0.002
Child-Pugh class: A + B/C [% (n)]	38.9 (42)/61.1 (66)	24.0 (23)/76.0 (73)	0.02	1.42	0.68-2.97	0.35
MELD score: ≥20/<20 [% (n)]	10.2 (11)/89.8 (97)	24.0 (23)/76.0 (73)	0.01	2.46	0.95-6.37	0.06
GV/SLV ratio (%)*	40.7 ± 7.7	41.3 ± 8.5	0.62	0.99	0.96-1.03	0.72

*The data are presented as means and standard deviations.

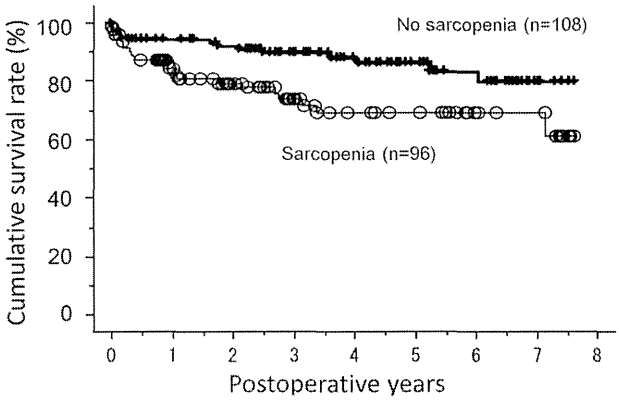

Figure 2. Overall survival and sarcopenia. Patients with sarcopenia had significantly worse overall survival than patients without sarcopenia ($P = 0.02$).

subgroup of patients without sarcopenia, the incidence of postoperative sepsis was 11.7% (7/60) in 2003-2007 and 2.1% (1/48) in 2008-2011 ($P = 0.07$). In the subgroup of patients with sarcopenia, the incidence of postoperative sepsis was 28.2% (11/39) in 2003-2007 and 10.5% (6/57) in 2008-2011 ($P = 0.03$; Table 4).

DISCUSSION

To determine sarcopenia, we measured the major and minor axes of the psoas muscle; we did not use any area-measuring software. Using such software is sometimes a little complicated; in particular, the tracing of the psoas muscle area may not always be correct. In the current study, the data were normally distributed well, and so they were considered to be reliable. There is no apparent definition of sarcopenia based on the psoas muscle area.[5] In many reports, the definition of sarcopenia has been decided subjectively on the basis of data from examinees.[5,11] In the current study, on the basis of data from our previous study, sarcopenia was defined as less than the 5th percentile value of the psoas muscle area of healthy donors of each sex.[9] The data for the psoas muscle area of the donors, both males and females, were also normally distributed,[9] so it was reasonable to define a cutoff value for patients with sarcopenia. Although an area less than the 5th percentile of the psoas muscle area of healthy donors was defined as sarcopenia, 58.3% of male recipients and 35.6% of female recipients were diagnosed with sarcopenia. Not surprisingly, more recipients than healthy donors had central muscle loss.

In this study, preoperative sarcopenia was an independent predictor of mortality after LDLT. Associations with sarcopenia and a poor prognosis have been reported not only for transplant patients[5,12] but also for cancer patients.[11,13] Sarcopenia seems to reflect a surgeon's clinical impression of disease severity. Actually, in the current study, the Kaplan-Meier curve for patients with sarcopenia was significantly lower than the curve for patients without sarcopenia in the early period after LDLT, and approximately 40% of the deaths were due to postoperative sepsis or bleeding.

It has been reported that approximately 40% of patients with cirrhosis suffer from sarcopenia.[4] Although the mechanism of sarcopenia in patients with cirrhosis has not been clarified, one of the most important causes is thought to be malnutrition. A poor nutritional status has been suggested to increase the risk of posttransplant complications or mortality.[14,15] Malnutrition has been reported in 60% to 80% of patients

TABLE 2. Univariate and Multivariate Analyses of the Impact of Sarcopenia and Other Clinical Characteristics on Overall Survival

Variable	All Patients	Univariate Analysis: *P* Value	Multivariate Analysis: Hazard Ratio	95% Confidence Interval	*P* Value
Recipient age (years)*	54.4 ± 9.6	0.81	1.00	0.96-1.04	0.99
Donor age (years)*	34.8 ± 10.4	0.16	1.02	0.99-1.05	0.21
Recipient sex: male/female (n)	103/101	0.41	1.09	0.54-2.19	0.81
Recipient status: hospitalized/home (n)	59/145	0.37	1.00	0.44-2.28	0.99
Preoperative renal failure: yes/no (n)	13/191	0.01	2.60	0.78-8.62	0.12
BMI (kg/m^2)*	23.6 ± 3.4	0.18	1.09	0.98-1.20	0.10
Child-Pugh class: C/A + B (n)	139/65	0.39	1.10	0.48-2.56	0.81
MELD score: ≥20/<20	34/170	0.15	1.15	0.45-2.95	0.77
GV/SLV ratio (%)*	41.0 ± 8.1	0.80	0.99	0.95-1.03	0.63
Sarcopenia: yes/no (n)	96/108	0.02	2.06	1.01-4.20	0.047

*The data are presented as means and standard deviations.

TABLE 3. Univariate and Multivariate Analyses of Risk Factors for Postoperative Sepsis

Variable	All Patients	Univariate Analysis: *P* Value	Multivariate Analysis: Hazard Ratio	95% Confidence Interval	*P* Value
Recipient age (years)*	54.4 ± 9.6	<0.001	0.88	0.83-0.94	<0.001
Donor age (years)*	34.8 ± 10.4	0.046	1.01	0.97-1.05	0.66
Recipient sex: male/female (n)	103/101	0.39	0.83	0.30-2.32	0.72
Recipient status: hospitalized/home (n)	59/145	0.03	2.20	0.70-6.91	0.18
Preoperative renal failure: yes/no (n)	13/191	0.01	2.45	0.49-12.2	0.28
BMI (kg/m^2)*	23.6 ± 3.4	0.66	1.19	1.03-1.38	0.02
Child-Pugh class: C/A + B (n)	139/65	0.82	0.43	0.12-1.61	0.21
MELD score: ≥20/<20	34/170	0.04	1.71	0.49-5.95	0.40
GV/SLV ratio (%)*	41.0 ± 8.1	0.23	1.05	0.99-1.12	0.13
Sarcopenia: yes/no (n)	96/108	0.03	5.31	1.53-18.4	0.009

*The data are presented as means and standard deviations.

TABLE 4. Incidence of Postoperative Sepsis

	Postoperative Sepsis [% (n/N)]		
	2003-2007 (n = 99)	2008-2011 (n = 105)	*P* Value
All patients (n = 204)	18.2 (18/99)	6.7 (7/105)	0.02
Patients without sarcopenia (n = 108)	11.7 (7/60)	2.1 (1/48)	0.07
Patients with sarcopenia (n = 96)	28.2 (11/39)	10.5 (6/57)	0.03

with cirrhosis. However, assessing the nutritional status of patients with liver dysfunction is difficult because of fluid collections caused by impaired protein synthesis in the liver.[16-18] The albumin and prealbumin levels do not necessarily reflect the nutritional status because hepatocellular protein synthesis is usually impaired in these patients. The assessment and interpretation of body weight are also difficult because of the presence of ascites, pleural effusion, and peripheral edema. Besides, sarcopenic, obese patients with respiratory and gastrointestinal tumors have recently been reported to have worse survival.[19] These facts may be the reasons that younger, high-BMI, and sarcopenic patients are at high risk for postoperative sepsis.

The incidence of postoperative sepsis was reduced even in patients with sarcopenia after the routine application of early enteral nutrition. However, the incidence was still high (10.5%). One of the reasons may be the lack of glutamine, especially in patients with sarcopenia. Glutamine is mainly synthesized in

skeletal muscle, and that is reduced in sarcopenic patients.[20] Additionally, we used an enteral nutrition formula that does not include glutamine, and it is thought that patients with sarcopenia suffer from glutamine depletion. Glutamine is an important nutrient in constructing the intestinal wall: a decrease in glutamine can weaken the intestinal wall, and postoperative sepsis due to bacterial translocation may occur.[21] Besides, it has recently been reported that portal glucose delivery stimulated not liver but instead muscle protein synthesis in an in vivo study.[22] Protein synthesis in patients with sarcopenia must be lower than that in patients without sarcopenia. Now, a prospective study using early enteral nutrition with or without glutamine is being planned and promoted.

As for the benefits of this study, the most important difference between DDLT and LDLT may be the timing of liver transplantation. It can be easier to control the timing of the operation with LDLT. If a patient with sarcopenia is diagnosed in a candidate for LDLT, liver transplantation can be deferred, and previous treatments for sarcopenia (ie, nutritional and physical therapy) can be applied. The diagnosis of sarcopenia before transplantation can be more useful in LDLT versus DDLT. Branched-chain amino acids (BCAAs) are a source of energy, modulate signal transduction as messengers in skeletal muscle, and prevent muscle atrophy.[16,23,24] On the other hand, previous studies have shown the impact of changes in BCAA levels on the immune system. In vitro studies have shown that the omission of a single BCAA from a medium of cultured lymphocytes completely abolishes protein synthesis and cellular proliferation.[25-27] Kakazu et al.[28,29] demonstrated that an increased concentration of BCAAs could restore the functions of dendritic cells harvested from patients with cirrhosis both in vitro and ex vivo. Preoperative BCAA supplementation may have effects not only in preventing central muscle loss but also in restoring immune function in patients with advanced liver cirrhosis.

In conclusion, sarcopenia is an independent predictor of mortality and a risk factor for sepsis after LDLT. The incidence of postoperative sepsis was reduced even in patients with sarcopenia after the routine application of early enteral nutrition. Sarcopenia may be an objective evaluation of malnutrition in transplant candidates, and the treatment of malnutrition may improve mortality rates after liver transplantation. Further studies with larger numbers are required.

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Portal Vein Thrombosis After Hepatectomy

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Abstract
Background Although various complications after hepatectomy have been reported, there have been no large studies on postoperative portal vein thrombosis (PVT) as a complication. This study evaluated the incidence, risk factors, and clinical outcomes of PVT after hepatectomy.
Methods The preoperative and postoperative clinical characteristics of patients who underwent hepatectomy were retrospectively analyzed.
Results A total of 208 patients were reviewed. The incidence of PVT after hepatectomy was 9.1 % ($n = 19$), including main portal vein (MPV) thrombosis ($n = 7$) and peripheral portal vein (PPV) thrombosis ($n = 12$). Patients with MPV thrombosis had a significantly higher incidence of right hepatectomy ($p < 0.001$), larger resection volume ($p = 0.003$), and longer operation time ($p = 0.021$) than patients without PVT ($n = 189$). Multivariate analysis identified right hepatectomy as a significant independent risk factor for MPV thrombosis (odds ratio 108.9; $p < 0.001$). Patients with PPV thrombosis had a significantly longer duration of Pringle maneuver than patients without PVT ($p = 0.002$). Among patients who underwent right hepatectomy, those with PVT ($n = 6$) had a significantly lower early liver regeneration rate than those without PVT ($n = 13$; $p = 0.040$), and those with PVT had deterioration of liver function on postoperative day 7. In all patients with MPV thrombosis who received anticoagulation therapy, PVT subsequently resolved.
Conclusions Postoperative PVT after hepatectomy is not rare. It is closely related to delayed recovery of liver function and delayed liver regeneration.

Introduction

Hepatectomy is a widely accepted treatment for patients with primary liver tumors and well-preserved liver function [1]. It is the only curative treatment for patients with resectable metastatic liver tumors [2]. Despite the developments in surgical techniques and postoperative management, hepatectomy remains an invasive procedure with a relatively high postoperative complication rate, which has a negative impact on postoperative mortality [3].

Portal vein thrombosis (PVT) is a common complication of liver cirrhosis and is associated with decreased liver function and aggravated portal hypertension. The reported prevalence of PVT in individuals with liver cirrhosis ranges from 10 to 25 % [4]. It is generally accepted that decreased portal venous flow is the primary factor leading to PVT in patients with cirrhosis [5]. Although PVT is often overlooked, it is potentially life-threatening and may lead to mesenteric ischemia and sepsis [6]. Various postoperative complications after hepatectomy have been reported including liver failure [7], bile leakage [8], pulmonary complications [9], ascites [10], and venous thromboembolism [11], but no large studies have reported postoperative PVT as a complication.

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The aim of this study was to clarify the incidence, risk factors, and clinical outcomes of postoperative PVT after hepatectomy.

Methods

Patients with primary or metastatic liver tumors who underwent hepatectomy without simultaneous splenectomy between January 2009 and June 2012 at Kyushu University Hospital (Fukuoka, Japan), and underwent contrast-enhanced computed tomography (CT) on postoperative day (POD) 7, were eligible for review.

Surgical procedures

The surgical procedure was selected according to the following criteria. The extent of resection was determined based on the expectation of an R0 resection. Patients did not undergo trisectionectomy if the indocyanine green retention rate at 15 min ($ICGR_{15}$) was >15 %, bisectionectomy if the $ICGR_{15}$ was >25 %, monosectionectomy if the $ICGR_{15}$ was >35 %, or subsectionectomy if the $ICGR_{15}$ was >45 %. Laparoscopic hepatectomy was performed if the tumor measured <3 cm.

Hepatic parenchymal transection was performed using an ultrasonic dissector (CUSA; Integra Lifesciences, Plainsboro, NJ, USA) and a TissueLink Monopolar Sealer (TissueLink Medical, Dover, NH, USA). For laparoscopic hepatectomy, although we had performed parenchymal division with laparoscopic CUSA and TissueLink Monopolar Sealer, we recently started using an EnSeal (Ethicon Endo-Surgery, Cincinnati, OH, USA) and water-dripping bipolar forceps [12, 13]. Small vessels were sealed with EnSeal, and large vessels, including Glissonian pedicles, were sealed with Hem-o-lok clips. The Pringle maneuver and/or the hanging maneuver were occasionally performed to increase the safety of the operation. The Pringle maneuver was performed as follows: The entire hepaticoduodenal ligament was encircled and tightened with a rubber tourniquet. It was then subjected to 15 min of hepatic inflow occlusion followed by 5 min of reperfusion, repeated as needed. Sectionectomy and subsectionectomy were performed with the Glissonian approach. All operations were performed under low central venous pressure conditions.

Diagnosis of PVT

Contrast-enhanced CT was routinely performed on POD 7 as one of the examinations to detect complications such as small bilomas and parenchymal congestion. Radiologists reviewed the CT images, diagnosed cases of PVT, and identified the location of the PVT. After discharge, asymptomatic patients with PVT were followed up with monthly contrast-enhanced CT scans. Main portal vein (MPV) thrombosis was defined as thrombus only in the MPV or in the MPV and superior mesenteric vein. Peripheral portal vein (PPV) thrombosis was defined as thrombus in the portal vein stump or branches of the portal vein.

Anticoagulation therapy for PVT

As hepatectomy can result in coagulopathy and increased postoperative bleeding, patients were not given routine postoperative anticoagulation therapy. In patients with MPV thrombosis, anticoagulation therapy was initiated when the thrombus extended to the superior mesenteric vein or reduced portal venous flow. In patients with PPV thrombosis, anticoagulation therapy was initiated when the thrombus was localized in the umbilical portion and reduced portal venous flow, or if the portal vein stump thrombus extended into a major branch of the portal vein. All patients with MPV or PPV thrombus, stump thrombus, or prolonged coagulopathy were carefully observed, and anticoagulation therapy was initiated if extension of the thrombus was detected.

Anticoagulation therapy consisted of low-molecular-weight heparin followed by oral warfarin, targeting a prothrombin time-international normalized ratio (PT-INR) between 2 and 3. Patients were followed up with monthly contrast-enhanced CT scans until resolution of the PVT.

Three-dimensional volumetry and estimation of liver regeneration rate

Three-dimensional volumetry has been described elsewhere [14, 15]. Briefly, multidetector helical CT (MDCT) images were obtained using 2 mm thick slices. Enhancement was achieved using an intravenous bolus injection of nonionic contrast medium. Three-dimensional reconstruction of the liver and tumor were obtained from the MDCT data using Zio M900 software (Zio Software, Tokyo, Japan), which allowed manual adjustment of the cutoff line. The liver regeneration rate was calculated as postoperative liver volume on POD 7 divided by the preoperative functional liver volume. Preoperative functional liver volume was calculated by subtracting the tumor volume from the preoperative total liver volume.

Statistical analysis

All statistical analyses were performed using SAS software (JMP 9.0.1; SAS Institute, Cary, NC, USA). All variables are expressed as the mean ± standard error. Categoric

variables were compared using the χ^2 test, and continuous variables were compared using the nonparametric Wilcoxon test or the parametric t test. Logistic regression analyses were performed to identify independent risk factors. A value of $p < 0.050$ was considered statistically significant.

Results

Patients

A total of 222 patients without a preoperative diagnosis of PVT underwent hepatectomy between January 2009 and June 2012. Six of the patients were excluded because they underwent simultaneous splenectomy, and eight were excluded because they did not undergo contrast-enhanced CT on POD 7. The remaining 208 patients were enrolled in this study.

The patients included 153 men and 55 women, with a mean age of 66.7 ± 0.8 years. The indications for hepatectomy were primary liver tumor in 160 patients and metastatic liver tumor in 48 patients. The operative procedure was trisectionectomy in 2 patients (1.0 %), bisectionectomy in 38 patients (18.3 %), monosectionectomy in 35 patients (16.8 %), subsectionectomy in 30 patients (14.4 %), and partial hepatectomy in 103 patients (49.5 %). A total of 43 patients (20.7 %) underwent laparoscopic hepatectomy.

PVT after hepatectomy

Postoperative PVT occurred in 19 patients (9.1 %) who had undergone hepatectomy, including MPV thrombosis in 7 patients and PPV thrombosis in 12 patients (Fig. 1). In patients with MPV thrombosis, thrombus was limited to the MPV in five cases and extended from the MPV to the superior mesenteric vein in two cases. In patients with PPV thrombosis, thrombus was in the umbilical portion of the portal vein in four cases and in the portal vein stump in eight cases. Details of the 19 patients with PVT are shown in Table 1.

Comparison of patients with and without MPV thrombosis

Univariate analyses showed that patients with MPV thrombosis ($n = 7$) had a significantly higher proportion of right hepatectomy (85.7 vs. 6.9 %; $p < 0.001$), larger resection volume (665 ± 138 vs. 243 ± 27 g; $p = 0.003$), and longer operation time (385 ± 37 vs. 335 ± 9 min; $p = 0.021$) than patients without PVT ($n = 189$; Table 2). There were no significant differences in preoperative clinical characteristics between patients with MPV thrombosis and patients without PVT. Multivariate analysis identified right hepatectomy as a significant independent risk factor for MPV thrombosis [odds ratio (OR) 108.886 (95 % confidence interval 10.54–2,906.57); $p < 0.001$]. On the other hand, resection volume [OR 1.001 (0.999–1.004); $p = 0.413$] and operation time [OR 1.001 (0.993–1.005); $p = 0.728$] were not significantly associated.

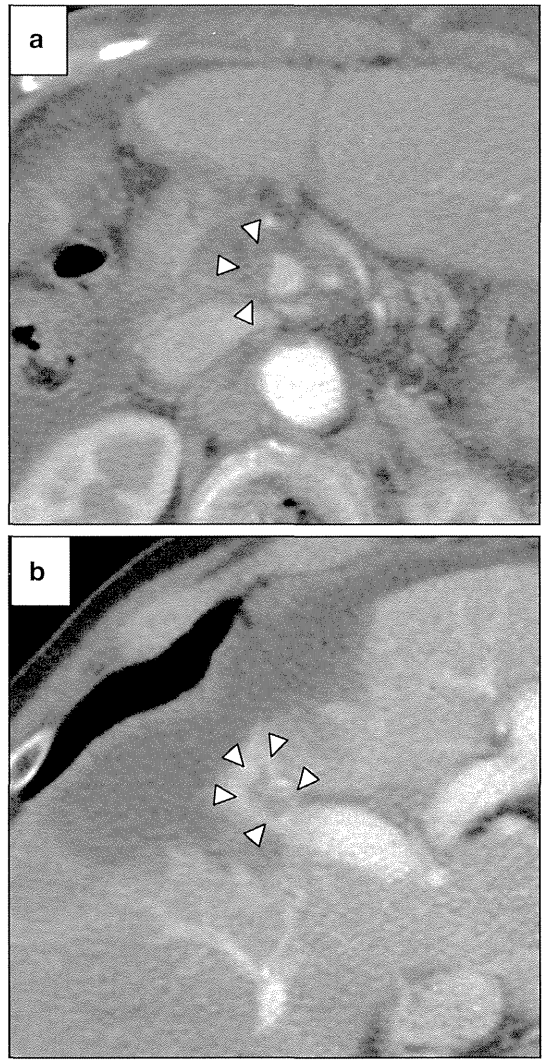

Fig. 1 Postoperative portal vein thrombosis (PVT) after hepatectomy was classified as main portal vein (MPV) thrombosis when there was thrombus in the MPV and superior mesenteric vein (**a**). It was classified as peripheral portal vein (PPV) thrombosis when there was thrombus in the portal vein stump or branches of the portal vein (**b**). *Arrowheads* indicate the PVT

Comparison of patients with and without PPV thrombosis

Univariate analyses showed that patients with PPV thrombosis ($n = 12$) had a significantly longer duration of the Pringle maneuver than patients without PVT (76 ± 11 vs. 43 ± 3 min; $p = 0.002$; Table 2).

Regeneration

To evaluate the impact of PVT on early clinical outcomes, laboratory data and liver regeneration rate on POD 7 were investigated in patients who underwent right hepatectomy ($n = 19$). There were no significant differences in preoperative clinical characteristics, including the estimated tumor volume and resection with or without the middle hepatic vein, between patients with PVT ($n = 6$) and without PVT ($n = 13$). Interestingly, patients with PVT had a significantly lower rate of liver regeneration than patients without PVT (46.9 ± 3.4 vs. 56.4 ± 2.4 %;

Table 1 Characteristics of patients with PVT

Cases	Age (years)	Sex	Extent of resection	PVT location	Anticoagulation	Outcome
1	63	Male	Right lobe	MPV + SMV	Yes	Resolved
2	77	Male	Left lateral section + S8	MPV + SMV	Yes	Resolved
3	70	Male	Right lobe	MPV	Yes	Resolved
4	71	Male	Right lobe	MPV	No	Resolved
5	37	Male	Right lobe	MPV	No	Resolved
6	77	Female	Right lobe	MPV	No	Resolved
7	75	Male	Right lobe	MPV	No	Resolved
8	73	Male	Anterior section	PPV, UP	Yes	Resolved
9	65	Male	S4	PPV, UP	Yes	Resolved
10	76	Female	Partial S3/4	PPV, UP	Yes	Resolved
11	65	Female	S4	PPV, UP	Yes	Resolved
12	40	Male	Posterior section	PPV, Stump-Rt	Yes	Resolved
13	76	Male	S8	PPV, Stump-Rt	Yes	Resolved
14	64	Male	S8	PPV, Stump	No	Resolved
15	81	Male	Left lateral section	PPV, Stump	No	Resolved
16	77	Male	Left lobe + partial S5	PPV, Stump	No	Stable
17	71	Male	Left lateral section	PPV, Stump	No	Stable
18	65	Male	S8	PPV, Stump	No	Stable
19	64	Female	Partial S2 + S7	PPV, Stump	No	Stable

MPV main portal vein, *PPV* peripheral portal vein, *PVT* portal vein thrombosis, *Rt* right branch of the portal vein, *S* segment, *Stump* stump of the portal vein, *SMV* superior mesenteric vein, *UP* umbilical portion of the portal vein

Table 2 Univariate analysis of relations between clinical factors and PVT

Factors	Without PVT ($n = 189$)	MPV thrombosis ($n = 7$)	PPV thrombosis ($n = 12$)	p^a	p^b
Age (years)	66.6 ± 0.8	67.1 ± 4.3	68.1 ± 3.3	0.895	0.652
Male sex	138 (73.0 %)	6 (85.7 %)	9 (75.0 %)	0.427	0.880
Primary tumor, yes	144 (76.2 %)	6 (85.7 %)	10 (83.3 %)	0.538	0.557
Albumin (g/dl)	4.0 ± 0	4.1 ± 0.2	3.9 ± 0.1	0.544	0.640
AST (IU/l)	39 ± 2	48 ± 10	38 ± 7	0.388	0.861
ALT (IU/l)	37 ± 2	39 ± 11	37 ± 9	0.824	0.997
Total bilirubin (mg/dl)	0.8 ± 0	0.7 ± 0.2	0.8 ± 0.2	0.677	0.990
Platelet count ($\times 10^4/\mu l$)	17.4 ± 0.7	18.7 ± 3.9	17.4 ± 2.9	0.742	0.999
PT-INR	1.05 ± 0.01	1.07 ± 0.03	1.02 ± 0.03	0.541	0.370
$ICGR_{15}$ (%)	13.2 ± 0.6	9.1 ± 2.8	16.3 ± 2.2	0.159	0.176
Liver cirrhosis, yes	35 (18.5 %)	0	3 (25.0 %)	0.094	0.591
Rt. hepatectomy, yes	13 (6.9 %)	6 (85.7 %)	0	**<0.0001**	0.181
Resection volume (g)	243 ± 27	665 ± 138	232 ± 103	**0.003**	0.916
Operation time (min)	335 ± 9	451 ± 49	385 ± 37	**0.021**	0.194
Blood loss (g)	541 ± 42	562 ± 226	406 ± 170	0.927	0.442
Duration of Pringle maneuver (min)	43 ± 3	66 ± 14	76 ± 11	0.078	**0.002**
Number of intraoperative Pringle maneuvers	133 (70.4 %)	6 (85.7 %)	10 (83.3 %)	0.348	0.312

Boldface numbers indicate significance in Tables 2 and 3

ALT alanine aminotransferase, *AST* aspartate aminotransferase, $ICGR_{15}$ indocyanine green retention rate at 15 min, *PT-INR* prothrombin time-international normalized ratio, *Rt.* right

[a] Comparisons between patients with MPV thrombosis and without PVT

[b] Comparison between patients with PPV thrombosis and without PVT

Table 3 Impact of PVT on recovery of liver function and early liver regeneration after right hepatectomy

Factors	Without PVT ($n = 13$)	With PVT ($n = 6$)	p
Liver regeneration (%)	56.4 ± 2.4	46.9 ± 3.4	**0.040**
Albumin (g/dl)	3.4 ± 0.1	2.9 ± 0.1	**0.019**
Total bilirubin (mg/dl)	1.2 ± 0.2	1.8 ± 0.2	**0.034**
AST (IU/l)	42 ± 6	36 ± 6	0.461
ALT (IU/l)	109 ± 20	89 ± 29	0.581
PT-INR	1.13 ± 0.03	1.36 ± 0.05	**0.002**
PHLF, yes	2 (15.4 %)	5 (83.3 %)	**0.004**
PHLF grades (A/B/C)	2/0/0	1/4/0	**0.033**

PHLF posthepatectomy liver failure

$p = 0.040$). Laboratory data on POD 7 also indicated delayed liver regeneration in patients with PVT compared with patients without PVT (Table 3). Patients with PVT had a significantly lower serum albumin level (2.9 ± 0.1 vs. 3.4 ± 0.1 g/dl; $p = 0.019$), higher serum total bilirubin level (1.8 ± 0.2 vs. 1.2 ± 0.2 mg/dl; $p = 0.034$), and higher PT-INR (1.36 ± 0.05 vs. 1.13 ± 0.03; $p = 0.002$) than patients without PVT. There were no significant differences between patients with and without PVT regarding the aspartate aminotransferase level (36 ± 6 vs. 42 ± 6 IU/l; $p = 0.461$) or alanine aminotransferase level (89 ± 29 vs. 109 ± 20 IU/l; $p = 0.581$). Posthepatectomy liver failure, PHLF [7] occurred significantly more frequently in patients with PVT than without PVT (83.3 vs. 15.4 %; $p = 0.004$). Among patients with PVT, four had grade B PHLF and one had grade A PHLF. Among the patients without PVT, two had grade A PHLF ($p = 0.033$). There were no postoperative deaths of patients with or without PVT.

Clinical course of PVT

Nine patients received anticoagulation therapy for PVT (Table 1). These patients had a mean follow-up of 4.6 ± 1.9 months, and the PVT resolved in all patients after a mean treatment period of 1.6 ± 0.5 months. Interestingly, the PVT also resolved after a mean period of 3.0 ± 0.6 months in the six patients who did not receive anticoagulation therapy. There were no cases of PVT progression.

Discussion

Although PVT is widely recognized as a common complication of liver cirrhosis, it is unclear whether postoperative PVT is a complication of hepatectomy, and the incidence of PVT after hepatectomy is unknown. In the current study, the rate of postoperative PVT occurring after hepatectomy was 9.1 %. Previous studies have reported a postoperative pneumonia rate of 13 % (17/555) [9] and a venous thromboembolism rate of 2.9 % (167/5,706) [11] after hepatectomy. Compared with other posthepatectomy complications it is clear that postoperative PVT after hepatectomy is not rare.

For diagnosis of PVT, abdominal CT is preferable to color Doppler ultrasonography because of its high sensitivity (90 %) and specificity (99 %) [6, 16]. Although the point at which the postoperative PVT starts to develop is not known, the results of the current study showed that it is reasonable to screen patients on POD 7 because those with PVT did not have symptoms indicating mesenteric ischemia (e.g., acute or colicky abdominal pain or bloody stools [17]) at that time. Contrast-enhanced CT on POD 7 is therefore recommended for screening patients for PVT.

The etiology of PVT can be categorized based on Virchow's triad of venous stasis, the hypercoagulable state, and endothelial injury. These three factors may be interdependent and often coexist [6, 18]. In the current study, PPV was detected in the portal vein stump in 8 of 12 patients, suggesting that venous stasis and endothelial injury at the stump induced PPV thrombosis. The Pringle maneuver can result in portal vein endothelial injury and stasis, and the duration of the Pringle maneuver was a significant risk factor for PPV thrombosis. It is also hypothesized that blood clots may be formed during clamping of the hepatoduodenal ligament, which embolize to the stumps of PPVs to form PPV thrombosis. Patients who underwent right hepatectomy tended to have a larger resection volume, smaller remnant liver volume, and more frequent Pringle maneuver than patients who underwent other hepatectomy procedures. Recently, a correlation was reported between small remnant liver volume and an increased von Willebrand factor/disintegrin ratio and metalloproteinase with thrombospondin type 1 motif (ADAMTS13), which may induce thrombogenesis [18]. Patients who undergo right hepatectomy therefore have increased risks of thrombogenesis, portal venous stasis, and endothelial injury. Also, right hepatectomy may be an independent risk factor for MPV thrombosis.

Among patients who underwent right hepatectomy, the liver regeneration rate was 46.9 % in patients with PVT. In contrast, the liver regeneration rate was 56.4 % in patients without PVT, which is consistent with previously reported rates [19, 20]. As many studies have indicated the importance of portal venous flow for liver regeneration [21–23], it is possible that reduced portal venous flow due to PVT results in delayed liver regeneration. Smaller liver volume also results in decreased portal venous flow and increased

intrahepatic vascular resistance [24], which may result in progression of the PVT and deterioration of liver function. In the current study, 83 % (5/6) of patients with PVT who underwent right hepatectomy had PHLF according to the consensus definition of the International Study Group of Liver Surgery [7].

Luca et al. [25] studied the natural course of PVTs in patients who had cirrhosis but no malignancy and who did not receive anticoagulation therapy. They reported that partial PVT worsened in 48 % of patients (20/42), improved in 45 % of patients (19/42), and was stable in 7 % of patients (3/42). Spontaneous resolution of PVT was thought to be due to thrombus shrinkage rather than lysis because of changes in vessel size. In the current study, PVT resolved spontaneously in the six patients (60 %) who did not receive anticoagulation therapy, which is consistent with the previous literature [25, 26]. In all patients who received anticoagulation therapy, the PVT resolved during follow-up. However, a recent prospective study reported that recanalization of the portal vein occurred in only 38 % of patients with symptomatic PVT who received anticoagulation therapy [27]. Portal vein occlusion in patients with PVT with extension into the superior mesenteric vein may result in mesenteric ischemia, sepsis, and death [6]. Turnes et al. [28] reported that among patients with acute PVT who had cirrhosis but no malignancy those who received early anticoagulation therapy had a higher frequency of recanalization than those who did not receive early anticoagulation therapy. As many reports have recommended immediate initiation of anticoagulation therapy after a definitive diagnosis of PVT [6, 28–30], the high rate of recanalization in this study may be a result of the early initiation of anticoagulation therapy in patients with PVT.

The main limitation of this study is its retrospective nature, which limited the data available for analysis. Portal hemodynamics may have had an impact on PVT, and it was unclear how the local hemodynamics affected changes in PVT. This study also included a relatively small number of inhomogeneous cases. Although various surgical procedures were included in this study, the analyses did not account for potential differences in background characteristics among patients who underwent different procedures. Further analysis of a larger number of patients from multiple centers, and of differences between procedures, is necessary to confirm these findings.

Conclusions

The findings of this study suggest that most cases of PVT can be stabilized or improved, with recovery of liver function. However, the small number of patients with severe PVT indicates that these patients should be carefully observed because of the possibility of worsening liver function.

Conflict of interest None.

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Interferon-lambda4 genetic polymorphism is associated with the therapy response for hepatitis C virus recurrence after a living donor liver transplant

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SUMMARY. The standard therapy against hepatitis C virus (HCV) recurrence postliver transplantation includes interferon (IFN)α and ribavirin. *IFNL4 ss469415590* polymorphism has been reported as a novel predictor of the response to IFN therapy for chronic HCV infection. We examined the impact of IFNL4 polymorphism on the responsiveness to IFN therapy after liver transplantation. Tissue specimens were collected from 80 HCV-infected recipients and 78 liver donors, and their *IFNL4 ss469415590* genotype, hepatic *IFNL4* and interferon-stimulated genes' mRNA expression levels were examined. The association of the polymorphism and expression levels in terms of the IFN therapy response to HCV recurrence was analysed. Most individuals who had *rs8099917* risk alleles also had *ss469415590* risk alleles ($R^2 = 0.9$). Sustained virological response (SVR) rates were higher in both liver graft recipients and transplants with *ss469415590* TT/TT alleles than in those with the risk ΔG allele ($P = 0.003$ and $P = 0.005$, respectively). In recipients with *ss469415590* TT/TT, *IFNL4* TT mRNA levels showed no significant differences between livers of patients who responded to therapy and those who did not ($P = 0.4$). In recipients with the risk ΔG allele, *IFNL4* ΔG mRNA expression levels were significantly lower in SVR patients than in non-SVR patients ($P = 0.02$). Hepatic interferon stimulable genes and IFNL4 mRNA expression were correlated. Our findings suggest that analysing the *ss469415590* genotype and *IFNL4* ΔG expression provides a novel prediction strategy for the possible response to IFN therapy after liver transplantation.

Keywords: genetic polymorphism, HCV, IFNL4, interferon therapy, living donor liver transplantation.

INTRODUCTION

Hepatitis C virus (HCV) infection affects 170 million people worldwide. Most HCV infections become chronic, with some progressing to liver cirrhosis or hepatocellular carcinoma. These diseases are the leading reasons for liver transplantation (LT) worldwide [1,2]. Hepatitis C virus re-infection occurs in almost all LT recipients and has significant negative effects on LT and liver graft recipients' survival [3,4].

The current standard therapy against HCV recurrence after LT includes pegylated interferon (PEG-IFN)α and ribavirin (RBV) [5]; however, many patients cannot tolerate curative doses, and the sustained virological response (SVR) rate of this therapy is very poor [4,6]. Because of the substantial cost, the poor efficacy and the adverse side effects of the therapy, being able to predict which patients will respond well to IFN therapy for recurrent HCV, are desirable.

A genome-wide association study showed that single nucleotide polymorphisms (SNPs) located near *IL28B* are strongly associated with a virological response (VR) to IFN therapy and spontaneous clearance of HCV [7–11]. In Japanese patients, especially *rs8099917* is closely associated with the efficacy of the IFN therapy; patients with the TT genotype have a stronger response to IFN therapy than those with TG or GG genotypes [9].

Abbreviations: cDNA, complementary DNA; DAA, direct-acting antiviral; GV/SLV, graft volume/standard liver volume; HCV, hepatitis C virus; ISG, interferon-stimulated gene; LDLT, living donor liver transplantation; LT, liver transplantation; MELD, model for end-stage liver disease; PEG-IFN-α, pegylated interferon-α; RBV, ribavirin; RT-PCR, reverse transcription polymerase chain reaction; SNP, single nucleotide polymorphism; SVR, sustained virological response; VR, virological response.

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We have previously reported that, even after living donor liver transplantation (LDLT), the efficacy of therapy is associated with both the recipient's and the donor's *IL28B* SNPs [12]. Patients with *IL28B* TG or GG genotypes express interferon-stimulated genes (ISGs) highly [13–15]. Some ISGs, such as *ISG15* and *USP18*, have been reported to inhibit IFN activity and help HCV replication [16–18].

Most recently, Prokunina-Olsson *et al.* reported that *IFNL4 ss469415590* genetic polymorphism, TT or ΔG, is significantly associated with the IFN therapeutic outcome [19]. *ss469415590* is located in the exon region of the *IFNL4* gene; therefore, the ΔG allele leads to a translational frameshift. Patients with this ΔG allele in *ss469415590* express a novel IFN-like antiviral *IFNL4* gene, while patients with a TT homozygous haplotype express an antiviral-less unknown function gene. Prokunina-Olsson *et al.* also reported that IFNL4 ΔG would inhibit IFN-involving therapy. Therefore, patients with the ΔG risk allele cannot respond to IFN therapy [19].

In this study, we analysed *IFNL4 ss469415590* polymorphism and *IFNL4* and ISGs mRNA expression levels in the resected liver. We examined the impact of the polymorphism and expression on the responsiveness to IFN and RBV combination therapy for HCV recurrence after LDLT.

PATIENTS AND METHODS

Patients

Eighty recipients were enrolled in the study and underwent LDLT for HCV-related liver disease at our institute between January 2004 and December 2010. Simultaneous splenectomies for 62 recipients (78%) were performed to prevent pancytopenia due to antiviral therapy. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Sample collection and all the experiments were performed after obtaining informed consent from recipients and donors, according to an established protocol approved by the Ethics Committee of Kyushu University. Data do not contain any information that could lead to the identification of patients.

Antiviral treatment and assessment of the therapeutic effects

PEG-IFN-α2b (Pegintron®; Merck & Co., Inc., Whitehouse Station, NJ, USA) was started at a dose of 0.5–1.0 μg/kg/week with 200–400 mg/day of ribavirin (Rebetol®; Merck & Co., Inc.). Doses were escalated in a stepwise manner up to 1.5 μg/kg/week and 800 mg/day, respectively, according to individual tolerability. A SVR was defined as a lack of HCV-RNA at 6 months after completion of the treatment. Non-SVR patients include patients who did not respond the therapy or occurred relapse after treatment completion.

DNA extraction and genotyping

DNA was extracted from the recipients' and donors' liver tissues. Genotyping for identifying *IL28B* (*rs8099917*) [13] and *IFNL4* (*ss469415590*) [19] genetic polymorphisms was performed using Taqman GTXpress Master Mix and Custom TaqMan SNP Genotyping Assay (Life Technologies Inc., Tokyo, Japan) according to the manufacturer's protocol.

Measurement of IFNL4 using real-time RT-PCR

Total RNA was extracted from resected liver tissue. The synthesis of first-strand complementary DNA (cDNA) was performed using a PrimeScript RT reagent kit with gDNA Eraser (Takara Bio Inc., Tokyo, Japan). Hepatic *IFNL4* [19], *ISG15* and *USP18* gene expressions were measured by real-time reverse transcription polymerase chain reaction (RT-PCR) using the Taqman Gene Expression assay (Life Technologies). *β-actin* expression was used as the endogenous reference for each sample. The shown value was normalized using qPCR Human Reference Total RNA (Clontech Laboratories, Inc., Palo Alto, CA, USA), for an internal reference.

Statistical analysis

Fisher's exact tests and Pearson's χ^2 tests were used for qualitative variables. Nonparametric Wilcoxon tests and Student's *t*-tests were used for quantitative variables.

RESULTS

Patient's characteristics and genotyping for IL28B (*rs8099917*) and IFNL4 (*ss469415590*) polymorphisms.

Table 1 shows individual patient's characteristics according to whether or not they achieved SVR. Achievement of SVR was significantly associated with both recipient and donor *rs8099917* polymorphisms. No significant differences between SVR and non-SVR patients, except for the *IL28B* genotype, were observed.

In addition to the 80 cases shown in Table 1, we also included cases in which patients were positive for HCV antibodies but HCV-RNA negative for *IFNL4 ss469415590* genotyping. *ss469415590* genotyping showed that 63 recipients and 61 donors were identified as having TT/TT homozygous alleles. Twenty-six recipients and 25 donors were identified as having the risk alleles (ΔG/TT or ΔG/ΔG). Most Japanese individuals who had *rs8099917* risk alleles also had *ss469415590* risk ΔG alleles (Table 2).

Comparisons between polymorphisms and SVR rates after LDLT

We retrospectively evaluated the association between *IFNL4* polymorphisms in recipients and the SVR rates to

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Table 1 Patient's characteristics according to whether or not they achieved SVR

	SVR (n = 40)	non-SVR (n = 40)	P-value
Pretransplantation factor			
Recipient's age, mean	57 (19–73)	57 (34–73)	n.s.
Recipient's sex (M/F), n	23/17	20/20	n.s.
Recipient's rs8099917 (TT/non-TT), n	35/5	23/17	<0.01
Donor's age, mean	32 (20–53)	35 (20–53)	n.s.
Donor's sex (M/F), n	28/12	27/13	n.s.
Donor's rs8099917 (TT/non-TT), n	34/5	21/18	<0.01
HCV genotype (1/2/unknown), n	31/9/0	33/4/3	n.s.
MELD score, mean ± SD	12.3 ± 4.6	13.2 ± 7.8	n.s.
HCV viral load (logIU/mL), mean ± SD	5.4 ± 0.8	5.7 ± 0.5	n.s.
History of IFN therapy (y/n), n	13/26	20/17	n.s.
Intraoperative factor, mean ± SD			
Intraoperative bleeding (mL)	4951 ± 3968	4151 ± 3383	n.s.
Operation time (min.)	816 ± 173	784 ± 130	n.s.
GV/SLV (%)	41.2 ± 6.7	40.9 ± 7.7	n.s.
Posttransplantation factor			
Acute cellular rejection (y/n), n	4/36	5/35	n.s.
Bile duct complication (y/n), n	7/33	7/33	n.s.
CMV infection (y/n), n	10/30	7/32	n.s.
Immunosuppressant (CsA/Tac), n	22/17	26/13	n.s.
Steroid pulse therapy (y/n), n	4/36	4/36	n.s.
Time to IFN therapy from transplantation (mo), mean ± SD	6.2 ± 5.7	6.9 ± 6.9	n.s.
Pretreatment viral load (logIU/mL), mean ± SD	6.6 ± 0.6	6.6 ± 0.5	n.s.
Pathological activity score, mean ± SD	1.2 ± 0.7	1.3 ± 0.6	n.s.
Pathological fibrosis score, mean ± SD	0.6 ± 0.8	0.4 ± 0.7	n.s.

MELD, model for end-stage liver disease; GV/SLV, graft volume/standard liver volume.
The degree of chronic hepatitis was classified according to the General Rules for the Clinical and Pathological Study Group of Japan [36].

Table 2 Correlation between *IFNL4 ss469415590* and *IL28B rs8099917* genotypes

	IFNL4 ss469415590		
	TT/TT	ΔG/TT or ΔG/G	R^2
Recipient rs8099917			
TT	63	1	0.9
TG or GG	0	26	
Donor rs8099917			
TT	60	0	0.9
TG or GG	1	25	

IFN therapy after LDLT. Sustained virological response rate was significantly higher in recipients who had *ss469415590* TT/TT alleles compared with those who had the risk ΔG allele (60% *vs* 23%; P = 0.003) (Fig. 1a). Sustained virological response rates were also significantly higher in recipients who received liver grafts from a donor carrying the TT/TT homozygous allele (59% *vs* 23%; P = 0.005) (Fig. 1b). Combined analyses revealed that SVR rates were significantly decreased when either the transplant or liver graft recipient had the risk ΔG allele (P = 0.004) (Fig. 1c).

In this study's population, most recipients and donors who had *rs8099917* risk alleles also had *ss469415590* risk ΔG alleles (Table 2). Therefore, *IL28B rs8099917* polymorphisms in both the recipient and donor significantly affected SVR rates (recipient, P = 0.003; donor, P = 0.001; combined, P < 0.001) (Fig. 1d–f). Supplementary Fig. 1 shows that *IL28B* and *IFNL4* risk alleles in both transplant and liver graft recipients were associated with SVR rates for IFN therapy.

SVR rates and IFNL4 mRNA expression in recipients with IFNL4 risk alleles

IFNL4 ss469415590 polymorphism is located in the exon region of the *IFNL4* gene. We measured both *IFNL4* TT and ΔG mRNA in the recipients' resected liver tissue and compared IFNL4 expression between SVR and non-SVR recipients of IFN therapy after LDLT. Only *IFNL4* TT mRNA, which is encoding a functionless protein [19], was

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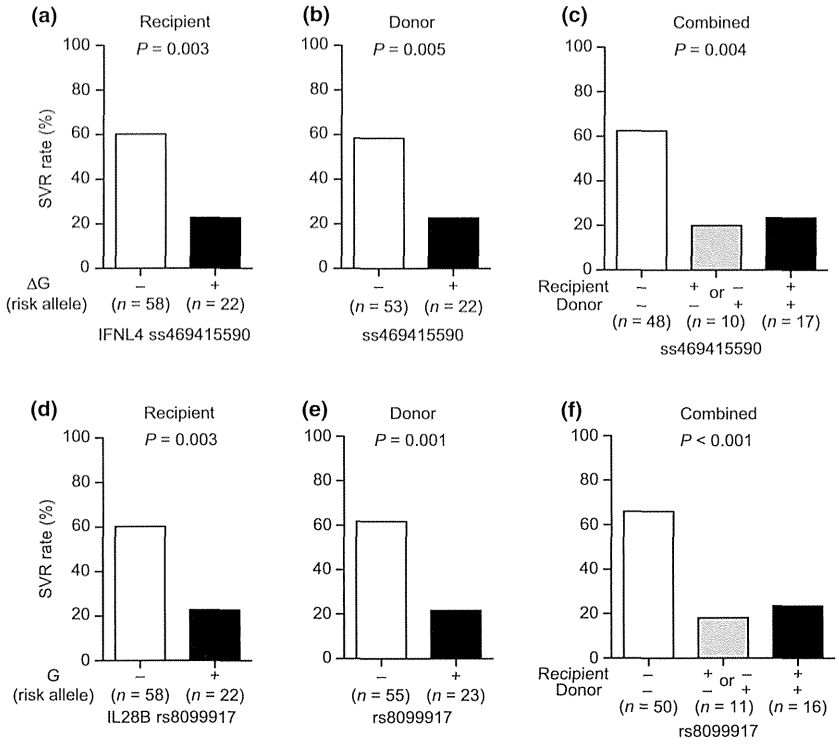

Fig. 1 Genetic polymorphisms with SVR rate to interferon therapy after LDLT. SVR rates in recipients **(a)** and donors **(b)** with *IFNL4 ss469415590* TT/TT (white bar) or risk ΔG (black) alleles. Combined analyses of the recipient and donor polymorphisms **(c)**. SVR rates in recipients **(d)** and donors **(e)** with *IL28B rs8099917* T/T (white bar) or risk G (black) alleles. Combined analyses of recipient and donor polymorphisms **(f)**.

expressed in recipients with *ss469415590* TT/TT. *IFNL4* TT mRNA expression levels showed no significant difference when compared with livers from SVR patients and non-SVR patients ($P = 0.4$) (Fig. 2a).

IFNL4 mRNA expression was also measured in recipients with the ΔG risk allele. *IFNL4* TT mRNA tended to be lower ($P = 0.3$) (Fig. 2b), and ΔG mRNA expression (reported as antiviral protein [19]) was also significantly lower in SVR patients than in non-SVR patients ($P = 0.02$) (Fig. 2c). However, the population was limited in this study. We also looked for correlations between *IFNL4* TT and ΔG mRNA in recipients with the ΔG/TT heterozygous genotype. Correlations are displayed in Fig 2d.

Association between IFNL4 and ISG mRNA expression in recipients with the IFNL4 risk allele

We measured hepatic *ISG15* and *USP18* mRNA in the liver tissue from recipients and found that *ISG15* and *USP18* mRNA were significantly higher in those who had *ss469415590* TT/TT alleles than in those with the risk ΔG allele (*ISG15*, $P < 0.001$; *USP18*, $P = 0.004$) (Fig. 3a). Interestingly, ISG expression levels appeared to be associated with whether or not a recipient was able to achieve SVR in response to IFN therapy after LDLT (*ISG15*, $P = 0.004$; *USP18*, $P = 0.003$) (Fig 3b).

We also looked for correlations between *IFNL4* ΔG mRNA and ISGs in recipients with the *ss469415590* risk ΔG allele. Correlations are displayed in Fig. 3c.

DISCUSSION

We examined the impact of *IFNL4 ss469415590* and *IL28B rs8099917* polymorphisms on responsiveness to IFN therapy after LDLT. *ss469415590* in both liver graft donors and recipients appeared significantly associated with the SVR rate after LDLT (Fig 1a–c).

Prokunina-Olsson *et al.* reported a correlation between the *IL28B rs8099917* risk G allele and the *IFNL4 ss469415590* risk ΔG allele in the Asian population [19]. The results from our study are consistent with this, showing a good correlation between risk alleles (Table 2).

We have previously reported an association between *IL28B* polymorphism and IFN sensitivity related to a HCV-RNA mutation after LDLT [12]; the polymorphism being associated with the mutation rate in the HCV-RNA core region [20], not in the IFN sensitivity-determining region [21] or the IFN/RBV resistance-determining region [22]. A combined analysis of *IL28B* polymorphisms and HCV-RNA

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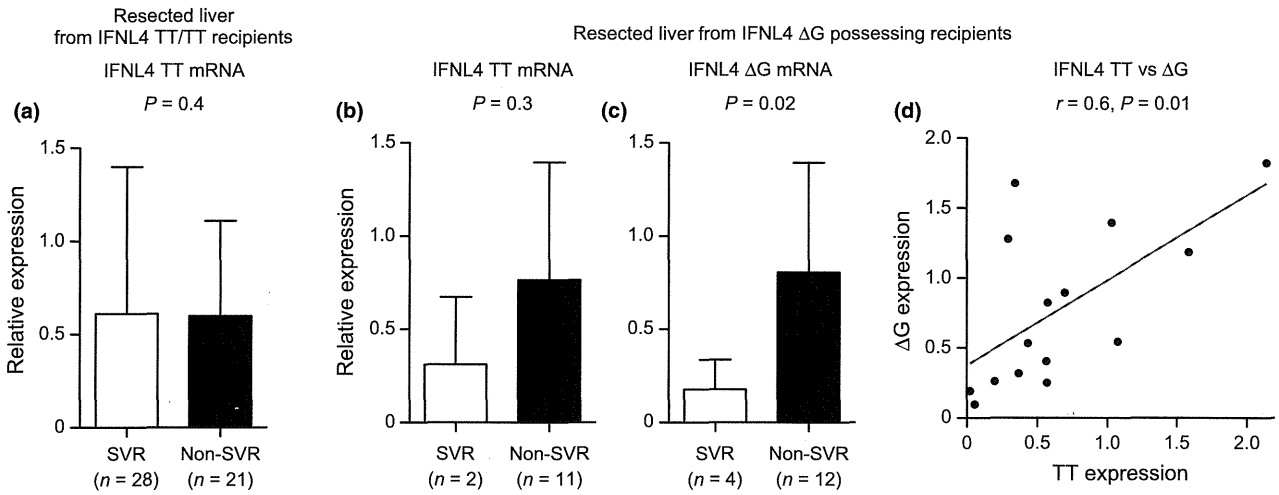

Fig. 2 Expression of *IFNL4* mRNA in recipients' livers resected at LDLT. Comparison of *IFNL4* TT mRNA expression between sustained virological response (SVR) and non-SVR recipients with IFNL4 TT/TT genotypes **(a)**. Comparison of *IFNL4* TT **(b)** and ΔG **(c)** mRNA expression between SVR and non-SVR recipients with *IFNL4* risk ΔG alleles. Correlation of TT and ΔG *IFNL4* mRNA in recipients with *IFNL4* TT/ΔG genotypes **(d)**.

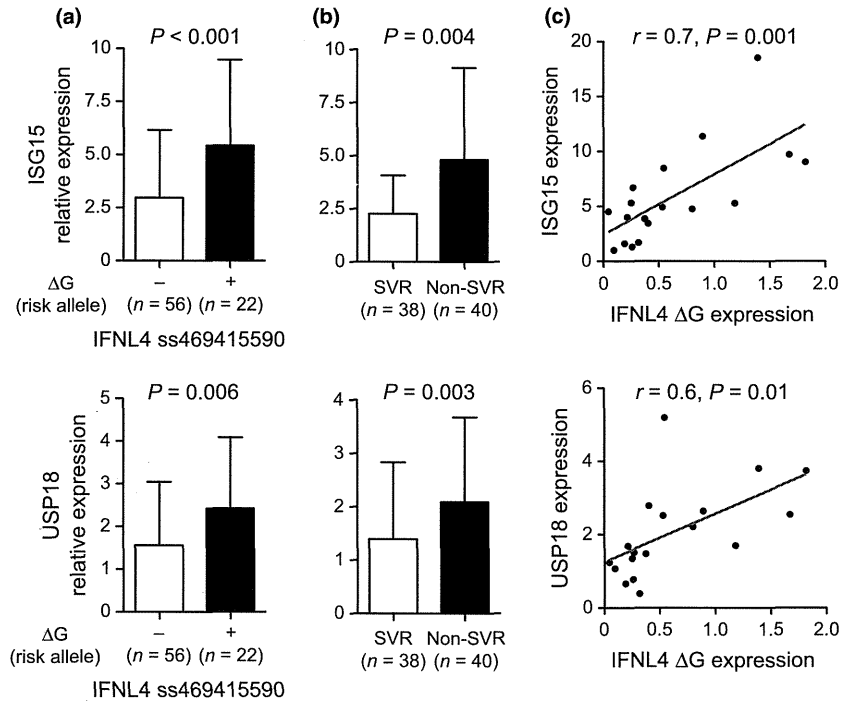

Fig. 3 Association between ISG expression and IFNL4 expression. Comparison of *ISG15* (upper graphs) and *USP18* (lower graphs) mRNA expression in patients with IFNL4 TT/TT and risk ΔG allele **(a)**. Comparison of *ISG15* and *USP18* mRNA expression between SVR and non-SVR recipients **(b)**. Correlation of *IFNL4* ΔG and ISGs (ISG15, upper and USP18, lower) mRNA expression levels **(c)**.

mutations appears more effective in predicting the response to IFN therapy than a single analysis [12]. Therefore, this study advocated that IFNL4 is associated with the HCV-RNA core mutation, and a combined analysis of IFNL4 and HCV-RNA mutations would be a more effective predictor.

IFNL4 mRNA expression was measured in liver tissue from recipients, and then, in the recipients with *IFNL4 ss469415590* TT/TT, *IFNL4* TT expression did not change between SVR and non-SVR patients (Fig. 2a). IFNL4 TT protein could not function as an antiviral IFN [19]; there-

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