의학박사 학위논문

Effect of PNPLA3 I148M Polymorphism on Histologically Proven Nonalcoholic Fatty Liver Disease in Liver Transplant Recipients

PNPLA3 I148M Polymorphism이 간이식 환자에서 조직학적으로 증명된 비알코올성 지방간질환에 미치는 영향

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김 혜 영

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The Department of Surgery

Seoul National University

College of Medicine

Hyeyoung Kim

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위 원 장 장진영 (인)

부 위 원 장 서경석 (인)

위 원 윤정환 (인)

위 원 이재영 (인)

위 원 김동식 (인)

ABSTRACT

Effect of PNPLA3 I148M Polymorphism on Histologically Proven Nonalcoholic Fatty Liver Disease in Liver Transplant Recipients

Hyeyoung Kim

The Department of Surgery

Seoul National University

College of Medicine

Aim: PNPLA3 I148M polymorphism (rs738409 C>G) is the most important and the best known polymorphism for nonalcoholic fatty liver disease (NAFLD). However, little is known about the effect of this polymorphism on NAFLD after liver transplantation (LT). We aimed to evaluate the association between this polymorphism and post-LT NAFLD.

Methods: We designed a prospective case-control study. Among adult recipients who underwent LT between April 2014 and October 2015, those whose whole blood were preoperatively collected for genotyping in both recipients and coupled donors and those who have undergone protocol biopsy at post-LT 1 year were enrolled.

Results: A total of 32 recipients were finally enrolled. Histologically proven steatosis (≥5%) were present in 28.1% of patients at a mean time of 12.7±2.0 months after LT. Moderate and more steatosis (≥33%) was present in 9.4%. One year after LT, steatosis was present in 50.0% of homozygous recipients with rs738409-G allele. It was present in 27.3% of heterozygous recipients with rs738409-G allele, and in 9.1% (p=0.041) of recipients with rs738409-CC. Genotype of donor was not significantly (p=0.647) associated with post-LT NAFLD. When both recipient and coupled donor showed heterogeneous or homozygous genotype of rs738409-G allele, there was significantly more post-LT NAFLD compared to that in others (47.1% vs. 6.7%; p=0.018). In uni- and multi-variate analysis, only the presence of rs738409-G risk allele in both donor and recipient was a significant risk factor for post-LT NALFD (relative risk, 26.95; p=0.048).

Conclusions: PNPLA3 I148M polymorphism can significantly affects histologically proven NAFLD at post-LT 1 year.

Key words: biopsy, fatty liver, genotype, hepatic steatosis, transplant

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Introduction

Nonalcoholic fatty liver disease (NAFLD) affects a substantial proportion of the general population in the world. It is the leading cause of chronic liver disease in developed countries. In the context of liver transplantation (LT), recipients are at increased risk of developing a number of metabolic syndromes such as diabetes, weight gain, hypertension, and hyperlipidemia. They are more predisposed to NAFLD when compared to the general population.[1-4] However, NAFLD in liver transplant recipients is equivocal. Very little has been published regarding this condition with increasing numbers of liver transplant recipients.[3, 5]

The etiology of NALFD and its progression are caused by complex interplay between genetic factors and environmental factors.[6] Recently, an independent genome-wide association study has identified a non-synonymous sequence variation (rs738409 C>G) encoding an isoleucine-to-methionine substitution at position 148 in adiponutrin/patatin-like phospholipase-3 (PNPLA3) gene. It appears to be the strongest determinant of human steatosis.[7-10]

Liver transplant recipients are unique in being truly chimeric individuals. Both recipient and donor genotypes can potentially impact the phenotype.[11] Therefore, PNPLA3 I148M polymorphism (rs738409 C>G) of donor and/or recipient might be significant risk factors for the development of histologically proven NAFLD after LT. The objective of this study were: 1) to evaluate the prevalence and distribution of PNPLA3 I148M polymorphism

(rs738409-G) in donors and recipients, 2) to evaluate the effect of rs738409-G of donor and recipient on NAFLD in recipients after the first year of LT, and 3) to determine peri-transplant risk factors including PNPLA3 I148M polymorphism related with post-LT NALFD in relatively early phase after LT.

Patients and Methods

This was a prospective case-control study. Adult recipients who underwent living donor LT between April 2014 and October 2015 in our institute were enrolled. Eligible patients were recipients who have preoperatively collected whole blood for sequencing of PNPLA3 (rs738409) genotype with coupled live donors under informed written consent. Among them, recipients who have undergone protocol biopsy at post-LT 1 year were finally enrolled in the present study.

In the present study, we excluded patients who underwent event-driven biopsy or those who were less than 18 years of age at the time of LT. Patients were also excluded if their clinical, histological, and/or biological features were suggestive of recurrent liver diseases or the conditions related to hepatic fat accumulation after LT, such as 1) relapse of significant alcohol consumption, 2) HBs-Ag positivity, HCV-RNA positivity, and recurrent primary biliary cirrhosis, 3) biliary complication needed to intervention, and 4) using mTOR inhibitor or therapeutic dose of steroid.

A flow chart for the patient enrollment process is shown in Fig. 1. Among the 130 recipients who underwent living donor LT between April 2014 and

October 2015, a total of 91 recipients with coupled live donors underwent gene sequencing for PNPLA3 (rs738409) genotyping. Among them, a total of 61 recipients were excluded because of no protocol biopsy at 1 year after LT (n=37), biliary complication (n=11), recurrent primary liver disease (5 cases of hepatitis C virus-RNA positivity, 3 hepatocellular carcinoma), use of mTOR inhibitor (n=1), follow-up loss (n=1), and expire during follow-up (n=1).

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Institutional Review Board of Seoul Nation University Hospital.

Each recipient received induction with 20 mg of intravenous basiliximab within two hours before LT and on postoperative day 4. Basal immunosuppressive regimen was based on calcineurin inhibitor (tacrolimus), steroids, and Mycophenolate mofetil. Calcineurin inhibitor was started within 5 days after LT. Tacrolimus doses were adjusted according to individual clinical need with respective target whole blood trough levels around 8-12 ng/mL for the first month after LT, followed by 5-8 ng/mL thereafter. Mycophenolate mofetil dose was 1.0g daily (500mg twice a day) for most patients. Intravenous methylprednisolone 500 mg was given intra-operatively before portal perfusion. It was tapered from 200mg to 20mg within 6 days. Thereafter, oral prednisolone was continued at 20mg daily. It was tapered to 0-5mg/day within about 6 months after LT.

Outpatient follow-up were usually conducted once a week for the first month after discharge. It was gradually lengthened to every 3 or 4 months.

Additional visits were scheduled when clinically necessary. Complete laboratory examinations, including liver function tests and blood tacrolimus trough level were conducted at each follow-up.

As part of routine management, protocol liver biopsies were performed from postoperative days 7 to 14. They were performed afterwards when clinically necessary. Since August 2010, this practice was augmented with additional biopsies at 1, 3, 5, and 10 years post-LT. In all cases, an informed consent was obtained from each patient before biopsy.

Genomic DNA was isolated from whole-blood samples prospectively collected before LT in recipients and their coupled live donors. Donor and recipient DNAs were analyzed for rs738409-G (NM_025225.2:c.444C>G NP_079501.2:p.Ile148Met) single nucleotide polymorphism in the PNPLA3 gene based on Sanger Sequencing (BioFact Co. Daejeon, Korea). To genotype rs738409 single nucleotide polymorphism, the following primers were used: forward 5'-GCCAGCTGTG GCTACTCTGT-3' and reverse '3-TGTGGTGACC CAGTGTGACT C-5'. Polymerase chain reaction was carried out using the following conditions: denaturation at 95℃ for 3 minutes followed by 35 cycles of 20 seconds at 95℃, 40 seconds at 60℃, 30 seconds at 72℃, and a final extension at 72℃ for 5 minutes. The amplification size was 500bp. For quality control, genotyping was performed in duplicates. Both concordance rate and the overall success rate were 100%.

Hepatic steatosis was defined as micro- and macro-vesicular lipid accumulations of 5% or more on biopsy specimens. The severity of steatosis

were evaluated using the steatosis grading and scoring system devised by the Pathology Committee of the Nonalcoholic Steatohepatitis Clinical Research Network: normal to minimal steatosis (<5%), mild steatosis (5-33%), moderate steatosis (>33-66%), marked or severe steatosis (>66%) [12-14]. Sono-guided fine needle aspiration liver biopsies was performed by specialized radiologists. The biopsy specimens were stained with hematoxylin-eosin and Masson trichrome stains. Pathologic reviews were performed by two hepatopathologists at the institution.

From medical records, past history such as alcohol or smoking, underlying diseases such as diabetes or hypertension, laboratory data including liver function tests, and post-LT clinical course were obtained. Obesity was defined by BMI equal to or over 25.0kg/m^2. Pre-existing donor graft steatosis was evaluated at the time of initial organ procurement via liver biopsy. Hypertension was defined as blood pressure ≥130/85mmHg or by antihypertensive prescription, and diabetes was defined as fasting blood glucose ≥126mg/dl, serum glucose ≥200mg/dl, or if patient was taking diabetes medication.

Statistical analyses were performed using SPSS version 21.0. Continuous variables were compared with Student's *t* test while categorical variables were compared using Pearson's Chi-square test or Fisher's exact test. Linear by linear association was used if a variable had more than two categories. For multivariate analysis, we used linear logistic regression analyses. All *p*-values were two-sided. Statistical significance was considered when *p* value was less than 0.05.

Results

Baseline characteristics

The baseline characteristics of the 32 patients with coupled donors finally enrolled in this study are summarized in Table 1. The mean age of recipients was 55.25±8.89 months (range, 25-70 months). The mean age of donors was 35.38±11.68 months (range, 19-58 months). Pre-existing steatosis was present in 40.7% of recipients and 21.9% of donors.

Prevalence and distribution of rs738409-G

The prevalence and distribution of rs738409-G are shown in Table 1. The genotype and allele frequencies of rs738409 polymorphism in this study fit with the Hardy-Weinberg equilibrium. The frequency of rs738409-G was 48.5% in recipients and 56.3% in donors (Table 1). There was no significantly difference in the frequency of rs738409-G allele in recipients and donors (P=0.479).

Prevalence and degree of steatosis after LT

Histologically proven steatosis (≥5%) was present in 28.1% (n=9) of patients at a mean period of 12.7±2.0 months between LT and biopsy (Table 2). Mild, moderate, and severe steatosis were present in 18.8% (n=6), 9.4% (n=3), and 0.0% (n=0) of patients, respectively (Table 2). Additionally, other histological features were present in table 2.

Proportion of post-LT steatosis according to the PNPLA3 (rs738409) genotype

The proportions of post-LT steatosis in recipients according to rs738409 genotype in recipients and donors are shown in Fig. 2 (A) and Fig. 2 (B), respectively. At post-LT 1 year, there was a significant (P=0.041) difference in the prevalence of histologically proven steatosis according to the rs738409 genotype in recipients. Steatosis was present in 9.1% of recipients with rs738409-CC homozygous genotype. It was present in 27.3% of recipients with rs738409-CG heterogeneous genotype and in 50.0% of recipients with rs738409-GG homozygous genotype (Fig. 2-A). According to rs738409 genotype in donors, steatosis was present in 0.0% of recipients when their donors had rs738409-CC genotype. It was present in 50.0% of recipients when their donors had rs738409-CG and in 18.2% of recipients when their donors had rs738409-GG. Genotype of donor was not significantly (P=0.647) associated with post-LT NAFLD (Fig. 2-B). However, when both recipients and coupled donors showed heterogeneous or homozygous genotype of the rs738409-G risk allele, histologically proven steatosis at post-LT 1 year had significantly higher frequency compared to that in other groups (47.1% vs. 6.7%; P=0.018). The presence of G allele in both donor and recipient was significantly (P=0.026) associated to post-LT steatosis (Fig. 2C).

Risk factors for steatosis at post-LT 1 year

Results of risk factor analysis for NAFLD are summarized in Table 3. In

univariate analysis, among the various clinical parameters, only genetic factors showed significant relation with steatosis at post-LT 1 year (Table 3 (A)). Recipient PNPLA3 genotype (rs738409) was significantly different between the group of steatosis and normal group (CC:CG:GG = 43.5:34.8:21.7% vs. 11.1:33.3:55.6%, *P*=0.041). As previously described, genotype of donor was not significantly (*P*=0.647) associated with post-LT NAFLD. The presence of rs738409-G risk allele in both recipient and coupled donor was a significant factor for histologically proven post-LT NAFLD (88.9%; *P*=0.018). Factor of three and more rs738409-G alleles was also significant for histologically proven post-LT NAFLD (66.7%; *P*=0.049). Except genetic factors, other peri-transplant factors including age of recipient, pre-LT steatosis or pre-existing graft steatosis, pre-LT alcoholic liver cirrhosis, and obesity at the time of biopsy failed to show significant differences between the group of steatosis and normal group.

In multivariate analysis with known potential risk factors for steatosis (recipient's age ≥50 years, pre-LT steatosis, pre-existing graft steatosis, pre-LT alcoholic liver cirrhosis, obesity at the time of biopsy, weight gain after LT, and genotype of PNPLA3), only the presence of rs738409-G risk allele in both recipient and coupled donor (heterogeneous or homozygous) was significant independent risk factor for post-LT NALFD (relative risk, 26.95; 95% confidence interval 1.04 to 701.40; *P*=0.048) (Table 3 (B)).

Discussion

Single nucleotide polymorphism rs738409 in the PNPLA3 gene was first identified as a risk factor for steatosis in 2008 by the Dallas Heart Study.[7] Rs738409-G is a well-established genetic risk factor for NAFLD in the general population.[7-10, 15-18] However, NAFLD in liver transplant recipients is equivocal. Very little has been published regarding this condition.[3-5, 19-21] To date, few studies have reported the association between PNPLA3 I148M polymorphism and post-LT NAFLD.

In a previous study, we have reported that histologically proven NAFLD is present in 27.1% of recipients at a mean period of 35.4 months between LT and biopsy.[5] Even though the timing of biopsy was different, histologically proven NALFD was present with very similar prevalence (28.1%) in this study at a relatively early phase after LT (mean of 12.7 months). There was no NASH in our study population. That may be due to relatively early phase after LT. To the best of our knowledge, this is the first study about the association between PNPLA3 I148M polymorphism and histologically proven post-LT NAFLD. Liver biopsy remains the best method for diagnosing NALFD.[22] In the present study, rs738409-G genotype of recipient was significantly (P=0.041) associated with NAFLD at one years after LT. However, genotype of donor was not significantly (P=0.647) associated with NAFLD at one year after LT. As previously described, steatosis was present in 50.0% of homozygous recipients with rs738409-G allele. It was present in 27.3% of heterozygous recipients and in 9.1% of recipients with rs738409-CC. When the rs738409-G alleles in both recipients and coupled donors were heterogeneous or homozygous, their combination was significantly associated

with NAFLD at one years after LT (relative risk, 26.95; 95% confidence interval 1.04 to 701.40; *P*=0.048). Regarding fibrosis, PNPLA3 polymorphism has been known to be associated with liver fibrosis in various liver disease.[10, 17, 23, 24] However, in the present study, there was no significant association between PNPLA3 polymorphism and fibrosis at 1 year after LT.

In the present study, all recipients and donors are Asians (Korean). There are more homozygous recipients and donors with rs738409-G allele in this study population compared to the Western people. [9, 23, 25] The frequency of rs738409-G allele of PNPLA3 genotype has been reported from 22.6% to 42.5% in the previous Western studies,[9, 23, 25] which is less than that in this study population (48.5% in recipients and 56.3% in donors). There was no significant difference between recipients and donors in the present study (*P*=0.479).

Finkensteadt et al. have reported that liver transplant recipients who carry rs738409-GG in PNPLA3 are at increased risk for hepatic triglyceride accumulation, independent of the graft PNPLA3 genotype.[20] In their study of 95 recipients and coupled donors, at 5 years after LT, steatosis is present in 63.2% of patients homozygous for the rs738409-G allele. It is present in 31.4% of heterozygous recipients and in 12.0% of rs738409-CC recipients (*P*=0.002).[20] They have suggested that reduced PNPLA3 activity in extrahepatic tissues may be associated with hepatic fat accumulation in LT setting.[20] They have reported that there is no association between PNPLA3 genotype and steatosis at 1 year or 3 years after LT, which is different from our results. In the present study, for the first time, an association between

PNPLA3 and post-LT NALFD in recipients was found. This might be due to the fact that NALFD was diagnosed by unenhanced computed tomography in their retrospective study.[20] Computed tomography is inaccurate and less sensitive diagnostic tool for hepatic steatosis, especially for mild degree of hepatic steatosis. Biopsy still remains the gold standard of diagnosis for NAFLD even though it is slightly invasive. The severity of hepatic steatosis can be accurately determined radiologically (unenhanced computed tomography or ultrasonography) only when there is moderate or severe fatty infiltration of the liver documented by a liver biopsy.[26-28] We used histologic diagnosis to access post-LT steatosis. Histological diagnosis is a more accurate and more sensitive diagnostic tool for NAFLD compared to unenhanced computed tomography. Therefore, contrary to their suggestion, graft PNPLA3 genotype might be associated with the post-LT steatosis, especially when in combination with that of the recipient (Table 3). We suggested that reduced PNPLA3 activities in extrahepatic and hepatic tissues in combination might be associated with hepatic fat accumulation in early phase after LT. More studies are needed to determine the effect of PNPLA3 genotype in recipients or donor on post-LT NAFLD.

Recently, Liu et al. have reported that PNPLA3 I148M can affects *de novo* NAFLD occurrence with a prominent interaction with obesity after studying 65 long-term recipients with a survival exceeding 10 years.[29] However, hepatic steatosis was diagnosed by inaccurate and subjective tool of ultrasound in their study.[29] Even though fewer patients were used on the relationship between PNPLA3 I148M and NAFLD after LT in this study, our

results suggested that PNPLA3 I148M had somewhat significant effect on post-LT NAFLD.

Nongenetic factors previously identified as predictors of post-LT steatosis include pre-LT hypertension, diabetes mellitus, alcoholic cirrhosis, donor age, pre-LT graft steatosis, weight gain or obesity after LT, tacrolimus-based regimen, and increased post-LT triglyceride concentrations.[3-5, 21, 30] Among them, pre-LT alcoholic cirrhosis (odds ratio 8.03, *P*=0.003), obesity at biopsy (odds ratio 3.87, *P*=0.001), and preexisting donor graft steatosis (odds ratio 3.15, *P*=0.022) have been found to be significant risk factors for histologically proven NAFLD at a mean 35.4 months after LT.[5] However, in the present study, genetic factor of PNPLA3 genotype was the only risk factor for NAFLD at 1 year after LT. The association between PNPLA3 genotype and post-LT NAFLD was independent of pre-LT alcoholic cirrhosis, age, preexisting donor graft steatosis, obesity after LT, or pre-LT steatosis.

It is well known that NAFLD can progress to non-alcoholic steatohepatitis, cirrhosis, or hepatocellular carcinoma,[25, 31-34] and obesity and metabolic syndrome, even though somewhat controversial.[31, 35, 36] NAFLD is an independent risk factor for cardiovascular disease and emerging data show that NAFLD plays important roles in the pathogenesis of chronic kidney disease.[37-41] Recognizing post-LT NAFLD is important because it may significantly affect graft and patient survival by promoting fibrosis and cirrhosis and by related cardiovascular disease and chronic kidney disease.[4, 37] NAFLD is modifiable through lifestyles such as body weight reduction. Therefore, healthy diet and physical activity are the cornerstones in the

treatment of NAFLD.[42, 43] Shen et al. have suggested that, although PNPLA3 rs738409-GG genotype confers a higher risk of NALFD, these patients are more sensitive to beneficial effects of lifestyle modification and should be encouraged to do so.[44, 45] Since NAFLD was present in a considerable portion (28.1%) of patients in a relatively early phase (1 year) after LT, genetic factor might play a significant role. If we know the PNPLA3 genotype of both recipient and donor, we can predict the risk for post-LT NAFLD and actively recognize post-LT NAFLD through protocol biopsy so that we can prevent or treat it through lifestyle modification from an early phase after LT. These efforts can reduce post-LT NAFLD. They might improve long-term post-transplant survival of recipients.

The present study had several limitations, especially in respect to relatively small number of patients in study cohort. The post-transplant course can be complex and heterogeneous in cases. This might not have been adequately adjusted for in the analysis. Even though we excluded other possible risks for secondary hepatic steatosis, there might be a selection bias in the process of identification of post-LT NAFLD. Nevertheless, the current study had several strengths. To the best of our knowledge, the present study was the first one that reported the association between PNPLA3 genotype and histologically proven NAFLD after LT. Even though a relatively small number of study population (n=32) was used in this study, the prevalence and distribution of PNPLA3 genotype in finally enrolled patients was similar to that in initially enrolled patients (n=91). A total of 37 patients who were excluded from this study because of no biopsy made their decision on whether to performed

protocol biopsy at 1 year after LT or not entirely by themselves (i.e., there was no intervention of researchers in the process of protocol biopsy). Therefore, even if the number of finally enrolled patients is increased in our study population, the results might be similar to that of the present study. In addition, this is the prospectively designed case control study. Therefore, we believe that this study could serve as a useful background for future studies in this field. We plan to continue this study and follow the study cohort with serial protocol biopsies at specific time points such as post-LT 3 years, 5 years, and 10 years.

In conclusion, PNPLA3 I148M polymorphism of recipient alone or combined with coupled donor can significantly affect histologically proven NAFLD at post-LT 1 year. This finding supports the importance of PNPLA3 genotype as a genetic risk factor for post-LT NAFLD, especially in relatively early phase after LT.

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TABLE 1. Baseline Characteristics

	Recipients (n=32)	**Donors (n=32)**
Characteristics at LT		
Gender (male: female)	22:10 (68.8%:31.3%)	17:15 (53.1%:46.9%)
Age (year)	55.25±8.89 (range, 25-70)	35.38±11.68 (range, 19-58)
Obesity (BMI ≥25kg/m^2)	7 (21.9%)	11 (34.4%)
Hypertension	8 (25.0%)	3 (9.40%)
Diabetes	7 (21.9%)	1 (3.1%)
Underlying liver diseases		
Hepatitis B related	23 (71.9%)	0 (0.0%)
Hepatitis C related	1 (3.1%)	0 (0.0%)
Alcoholic liver cirrhosis	11 (34.4%)	0 (0.0%)
Steatosis ≥5%	13 (40.7%)	7 (21.9%)
Combined HCC	17 (53.1%)	0 (0.0%)
ABO incompatible LT	8 (25.0%)	8 (25.0%)
Characteristics at biopsy		
Hypertension	11 (34.4%)	
Diabetes	12 (37.5%)	
Obesity (BMI ≥25kg/m^2)	6 (18.8%)	
Dyslipidemia	0 (0.0%)	
Use of steroid	10 (31.2%)	
PNPLA3 genotype		

CC	11 (34.4%)	7 (21.9%)
CG	11 (34.4%)	14 (43.8%)
GG	10 (31.2%)	11 (34.4%)
G allele frequency	48.5%	56.3%

Data are presented as the mean ± SD

Abbreviation: LT, Liver transplantation; BMI, Body mass index; HCC, hepatocellular carcinoma

TABLE 2. Histological Features of Nonalcoholic Fatty Liver Disease (NAFLD) in Recipients After 1 Year

NAFLD	Cases (n = 32)	Prevalence
Steatosis		
Normal to minimal (<5%)	23	71.9%
Mild (5%-33%)	6	18.8%
Moderate (33%-66%)	3	9.4%
Severe (>66%)	0	0.0%
Lobular inflammation		
No	20	62.5%
Mild	11	34.4%
Moderate	1	3.1%
Severe	0	0.0%
Hepatocyte ballooning	4	12.5%
Fibrosis stage		
Stage 0 – 1B	24	75.0%
Stage 1C	8	25.0%
Stage 2	0	0.0%
Stage 3	0	0.0%
Stage 4 (cirrhosis)	0	0.0%

TABLE 3. Risk Factor Analysis for Nonalcoholic Fatty Liver Disease

(A) Univariate Analysis.

Variables	**No Steatosis (n=23)**	**Steatosis (n=9)**	***P* values**
PRE-LT RECIPIENT FACTORS			
Gender (male)	16 (69.6%)	6 (66.7%)	1.000
Recipient age at LT (year)	55.43 ± 9.51	54.78 ± 7.60	0.855
Obesity (BMI ≥25 kg/m^2)	4 (17.4%)	3 (33.3%)	0.370
Underlying liver disease			
Hepatitis B related	16 (69.6%)	7 (77.8%)	1.000
Hepatitis C related	0 (0.0%)	1 (11.1%)	0.281
Alcoholic liver cirrhosis	8 (34.8%)	3 (33.3%)	1.000
Fatty liver (steatosis ≥5%)	8 (34.8%)	5 (55.6%)	0.427
Combined HCC	12 (52.2%)	5 (55.6%)	1.000
ABO incompatible LT	5 (21.7%)	3 (33.3%)	0.654
Hypertension	5 (21.7%)	3 (33.3%)	0.654
Diabetes	5 (21.7%)	2 (22.2%)	1.000
Dyslipidemia	0 (0.0%)	0 (0.0%)	1.000
GENETIC FACTORS			
Recipient PNPLA3 genotype			
CC : CG : GG	10:8:5 (43.5:34.8:21.7%)	1:3:5 (11.1:33.3:55.6%)	**0.041**
CC : CG or GG	10:13 (43.5:56.5%)	1:8 (11.1:88.9)	0.115

CC or CG : GG	18:5 (78.3:21.7%)	4:5 (44.4:55.6%)	0.096
Donor PNPLA3 genotypes			
CC : CG : GG	7:7:9 (30.4:30.4:39.1%)	0:7:2 (0.0:77.8:22.2%)	0.647
CC : CG or GG	7:16 (30.4:69.6%)	0:9 (0.0:100.0%)	0.149
CC or CG : GG	14:9 (60.9:39.1%)	7:2 (77.8:22.2%)	0.441
Recipient & donor genotype			
G allele in both	9 (39.1%)	8 (88.9%)	**0.018**
Three and more G alleles	6 (26.1%)	6 (66.7)	**0.049**
PRE-LT DONOR FACTORS			
Donor gender (male)	12 (52.2%)	5 (55.6%)	1.000
Donor age (year)	35.96±11.76	33.89±12.06	0.660
Donor obesity (BMI ≥25kg/m^2)	10 (43.5%)	1 (11.1%)	0.115
Graft to recipient weight ratio	1.37±0.36	1.16±0.31	0.131
Pre-existing graft steatosis	5 (21.7%)	2 (22.2%)	1.000
RECIPIENT FACTORS at Bx.*			
Hypertension	8 (34.8%)	3 (33.3%)	1.000
Diabetes	8 (34.8%)	4 (44.4%)	0.946
Obesity (BMI ≥25kg/m^2)	4 (17.4%)	2 (22.2%)	1.000
Dyslipidema	3 (13.0%)	1 (11.1%)	1.000
Weight gain after LT	6 (26.1%)	4 (44.4%)	0.407
Use of steroid[#]	6 (26.1%)	4 (44.4%)	0.407

Data are presented as the mean ± standard deviations or numbers with percentages in parentheses unless otherwise indicated. * Timing at a mean

period of 12.7±2.0 months between LT and biopsy. [#]Physiologic dose in all cases.

Abbreviation: LT, liver transplantation; BMI, body mass index; HCC, hepatocellular carcinoma.

(B) Multivariate Analysis.

Variables	*P* values	Relative risk	95% Confidence interval
Age ≥ 50 years	0.282	0.146	0.004–4.870
Pre-LT steatosis of recipient	0.330	3.013	0.328–27.681
Pre-LT alcoholic liver cirrhosis	0.497	0.442	0.042– 4.658
Obesity (BMI ≥25kg/m^2) at Biopsy	0.139	14.823	0.417–527.012
Pre-LT graft steatosis of donor	0.181	0.152	0.010–2.411
Weight gain after LT	0.393	2.753	0.269-28.162
G allele in both donor and recipient	**0.048**	**26.953**	**1.036–701.395**

Abbreviation: LT, liver transplantation; BMI, body mass index.

Figure 1.

Flow chart showing the enrollment of patients for the study.

LT, liver transplantation; HCC, hepatocellular carcinoma; HIV, human immunodeficiency virus.

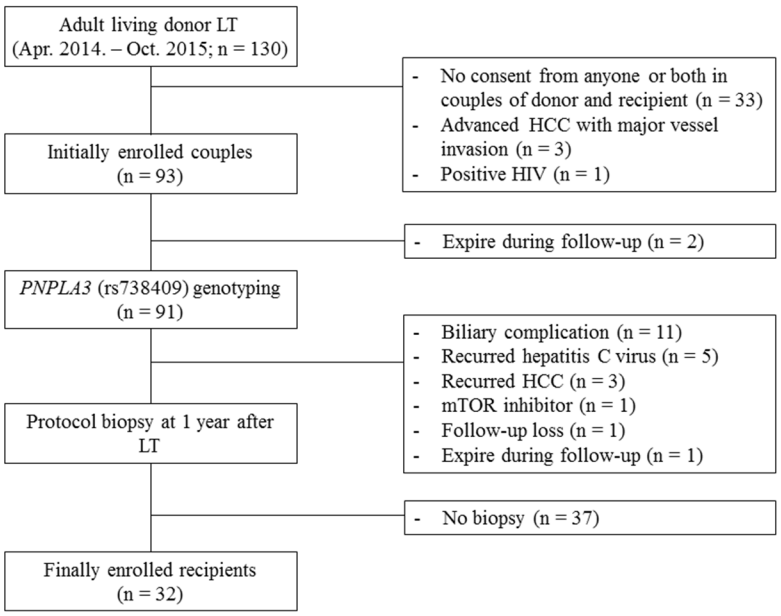


Figure 2.

Steatosis and PNPLA3 of recipients and donors.

Proportion of histologically proven steatosis in recipients at post-transplant 1 year, according to the PNPLA3 genotype of recipients (A), donors (B), and recipient & donors (C).

(A)

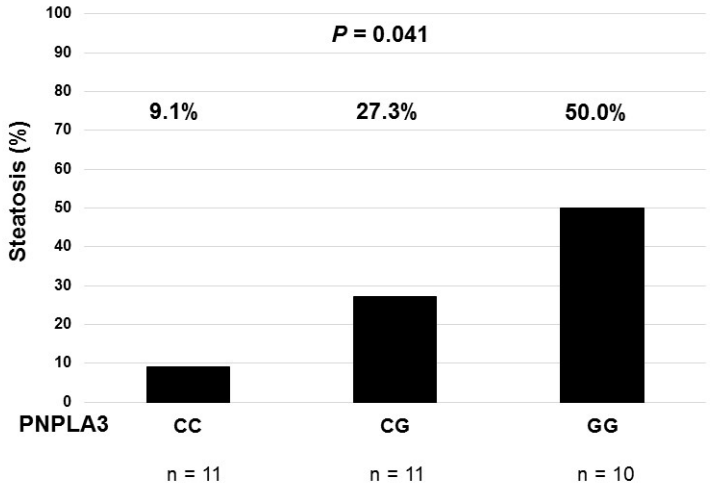


Figure 2.

(B)

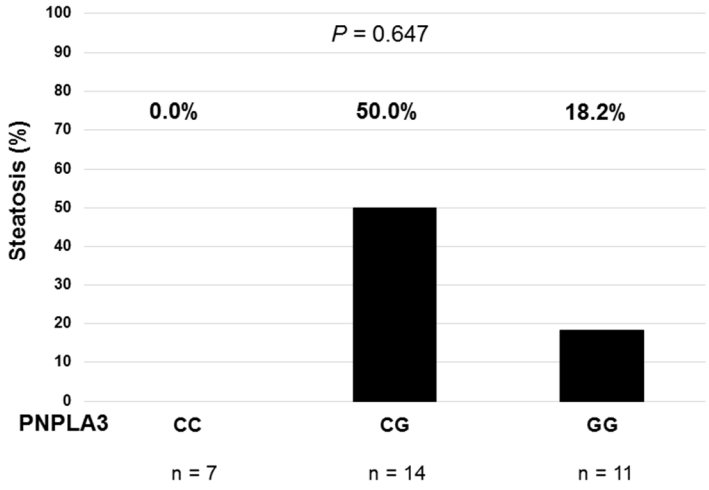
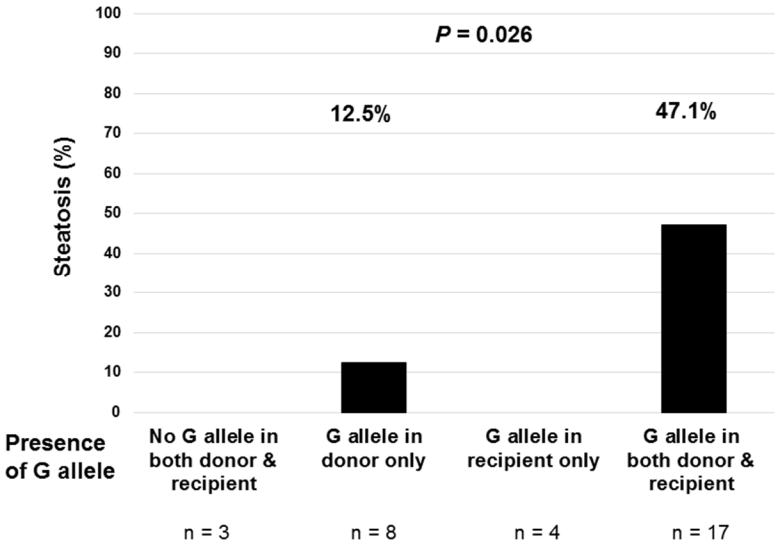

100
90
80
70
60
50
40
30
20
10
0
Steatosis (%)
P = 0.647
0.0%
50.0%
18.2%
PNPLA3
CC
CG
GG
n = 7
n = 14
n = 11

Figure 2.

(C)


100
90
80
70
60
50
40
30
20
10
0
Steatosis (%)
P = 0.026
12.5%
47.1%
Presence of G allele
No G allele in both donor & recipient
G allele in donor only
G allele in recipient only
G allele in both donor & recipient
n = 3
n = 8
n = 4
n = 17

국문 초록

서론: PNPLA3 I148M polymorphism (rs738409 C>G)은 비알코올성 지방간질환의 가장 중요하고 잘 알려진 polymorphism이다. 그러나, 이 polymorphism이 간이식 후의 비알코올성 지방간질환에 미치는 영향에 관해서는 알려진 바가 거의 없다. 본 연구는 PNPLA3 I148M polymorphism과 간이식 후 비알코올성 지방간질환과의 연관성을 알아보고자 하였다.

방법: 본 연구는 전향적 환자-대조군 연구이다. 2014 년 4 월부터 2015 년 10 월까지 서울대학교병원에서 간이식을 받은 성인 환자들 중에서, 환자 및 그의 기증자 모두에서 genotyping 을 위해 전향적으로 모은 전혈이 있고 간이식 후 1 년에 시행된 프로토콜 조직검사를 받은 간이식 환자를 대상으로 하였다.

결과: 총 32명의 간이식 환자가 최종 연구대상이 되었다. 이식 후 평균 12.7±2.0 개월에, 조직학적으로 증명된 steatosis (≥5%)는 28.1%의 환자에서 있었다. 중등도 이상(≥33%)의 steatosis는 9.4%였다. 이식 후 1년에, rs738409-G allele의 homozygous 이식환자의 50%에서 steatosis가 있었다. rs738409-G allele의 heterozygous 이식환자의 27.3%와 rs738409-CC를 가진 이식환자의 9.1%에서 steatosis가 있었다($p = 0.041$). 기증자의 genotype은 이식 후 비알코올성 지방간과 유의하게 관련되지 않았다($p = 0.647$). 환자 및 그의 기증자 모두에서 rs738409-G allele의 heterogeneous 혹은 homozygous genoypte인 경우가

간이식 후 비알코올성 지방간질환이 유의하게 많았다(47.1% vs. 6.7%; p = 0.018). 단변수 및 다변수분석 결과, 이식환자 및 그의 기증자 모두에서 rs738409-G risk allele을 보이는 것이 간이식 후 비알코올성 지방간질환의 유일하게 유의한 위험인자였다(상대위험비, 26.95; p = 0.048).

결론: PNPLA3 I148M polymorphism은 간이식 후 1년에 조직학적으로 증명된 비알코올성 지방간질환에 유의하게 영향을 미칠 수 있다.

주요어: 조직검사, 지방간, 유전자형, 간지방증, 이식

학 번: 2014-30613