의학박사 학위논문

Impact of PNPLA3 (rs738409-G) polymorphism on post-transplant outcomes after liver transplantation for alcohol-related liver disease

알콜성간질환으로 간이식수술을 받은 환자에서 PNPLA3 단일유전자다형성이 술후 임상결과에 미치는 의미

2020년 **7**월

서울대학교 대학원
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유 태 석

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지도교수 이 광 웅

이 논문을 의학박사 학위논문으로 제출함
2020년 **7**월

서울대학교 대학원
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유태석의 박사 학위논문을 인준함
2020년 **7**월

위 원 장 김윤준 (인)
부 위 원 장 이광웅 (인)
위 원 이남준 (인)
위 원 이경분 (인)
위 원 박성길 (인)

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2020년 7 월

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5cm 유 태 석

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2020 년 7 월

서울대학교 대학원

의학과 외과학전공

유 태 석

유태석의 박사 학위논문을 인준함

2020 년 7 월

위 원 장	김윤준	(인)
부위원장	이광웅	(인)
위 원	이남준	(인)
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위 원	박성길	(인)

<Table of contents>

Abstract

Impact of PNPLA3 (rs738409-G) polymorphism on post-transplant outcomes after liver transplantation foralcohol-related liver disease

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Background

PNPLA3 (rs738409-G) polymorphism is one of the strongest genetic factors for alcohol-related liver disease (ALD). However, little is known about the effect of this polymorphism on post-transplant outcomes in cases with alcohol relapse (AR) after liver transplantation (LT). We aimed to evaluate the association between this polymorphism and post-LT clinical outcomes related to AR.

Methods

We retrospectively analyzed data from patients receiving LT for ALD from 04/2014 to 12/2017. The severity of AR was assessed by a separate questionnaire using the high-risk alcoholism relapse scale (HRAR). Liver-related clinical outcomes were assessed by the gamma-glutamyltransferase (GGT) level and alcohol-related liver failure (ARLF). Genotyping was performed using prospectively collected DNA samples in both donors and

recipients.

Results

A total of 83 recipients were enrolled. Post-LT AR occurred in 31 patients (37.3%). Twenty-three patients (14 AR, 9 abstainers) showed elevated GGT levels, and 3 AR patients experienced ARLF. In the multivariate analysis, rs738409 G allele carrier and heavy drinking (HRAR score≥4) were independent risk factors for elevated GGT levels (odds ratio [OR]=8.69, $p<0.01$; OR=13.07, p=0.01) and ARLF (OR=4.52, p=0.04; OR=19.62, p=0.03). Among 15 heavy AR patients, being an rs738409 G allele carrier was related to GGT elevation (p=0.03) and ARLF (p=0.04), but it was not to GGT elevation in mild drinkers (n=16) or abstainers (n=52).

Conclusions

PNPLA3 polymorphism of the recipient genotype can independently affect the post-LT prognosis of LT patients for ALD, especially in heavy AR patients. Therefore, strong abstinence education is strongly recommended in patients with this single nucleotide polymorphism.

Keywords: Liver transplantation; Alcoholic liver disease; Alcohol relapse

Student Number: 2017-36053

I. Introduction

According to the World Health Organization, alcohol-related liver disease (ALD) is one of the most serious diseases that accounts for 20% to 50% of liver cirrhosis prevalence, with a reported 2.25 million deaths annually in the case of end-stage liver disease [1-2]. The only fundamental treatment for end-stage liver disease is liver transplantation (LT), and the results are excellent enough to show graft and patient survival rates of ≥90% per year [3]. However, despite these results, post-LT alcohol relapse (AR) has been a major obstacle for LT patients with ALD. The incidence of AR reaches up to 10%-50%, and AR has been associated with increased damage to transplanted liver allografts and therefore, has a poor effect on patients' clinical outcomes and survival [4].

The development and progression of ALD are caused by complex interactions between genetic and environmental factors. Recently, an independent genome-wide association study has identified single nucleotide polymorphism (SNP) in encoding patatin-like phospholipase domain - containing 3 (PNPLA3) and it appears to be a strong risk factor for alcoholic hepatitis, advanced fibrosis, and a higher incidence of end stage liver disease [5]. The PNPLA3 gene is located on the long arm of chromosome 22 at band 13.31 (22q13.31). It lies on the Watson (plus) strand and is 40,750 bases in length. PNPLA3 also known as adiponutrin, acylglycerol O-acyltransferase or calcium-independent phospholipase A2-epsilon is an enzyme that in humans is encoded by the PNPLA3 gene. This adiponutrin is a single-pass type II membrane protein and is a multifunctional enzyme with both triacylglycerol lipase and acylglycerol O-acyltransferase activities.

Figure. 1 Ideogram human chromosome 22

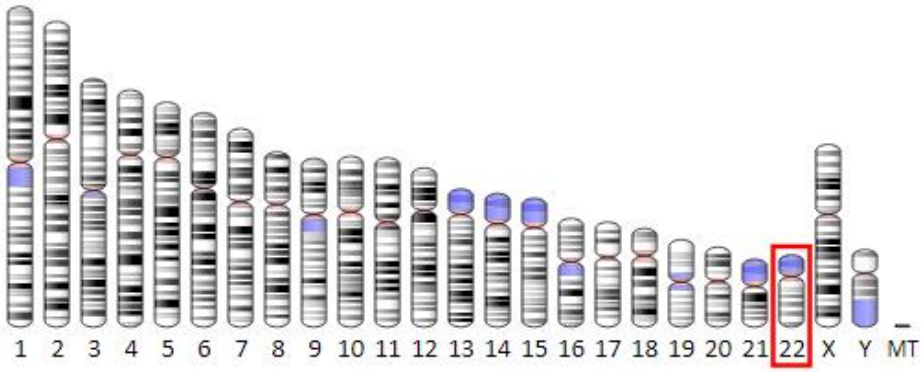
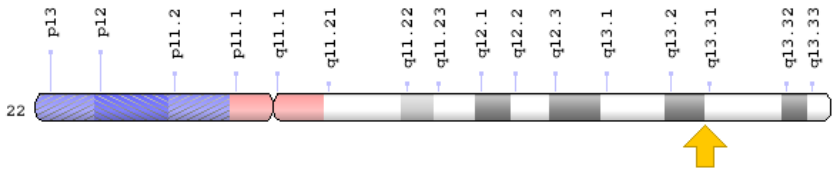


Figure. 2 PNPLA3 gene location: 22q13.31, which is the long (q) arm of chromosome 22 at position 13.31; Molecular location: base pairs 43,923,805 to 43,947,582 on chromosome 22



It is involved in the triacylglycerol hydrolysis in adipocytes and may play a role in energy metabolism. The mature protein is 481 amino acids in length and the predicted molecular weight is 52.865 kiloDaltons. Two the isoforms have been described but the functional significance is not known [5]. The role of this genetic polymorphisms in determining liver disease risk and outcome has received considerable attention in recent years. A common SNP exhibits a rs738409; C>G in the gene PNPLA3 resulting in a substitution of an isoleucine residue for methionine at position 148 of the protein (Ile148Met; I148M). There is considerable evidence that carriage of the risk allele, rs738409:G, plays an important role in determining the risk of developing ALD from individual studies [1-5], a meta-analysis [9] and, most recently, a genome-wide association study [5]. In addition, there is growing evidence that rs738409:G influences several other important aspects of ALD; thus, carriage of the G allele is associated with earlier development of cirrhosis, independently of the age of onset of at-risk alcohol consumption; more rapid progression towards decompensated disease; a reduction in transplantation-free survival; and, even poorer outcomes following development of hepatocellular carcinoma [9]. However, there has been no study of the role of PNPLA3 in patients with AR after LT.

Therefore, we investigated two major clinical factors of AR patients and association between these factors and PNPLA3. First factor is Gamma-glutamyltransferase (GGT) which one of the best studied markers to detect AR on the basis of objective laboratory finding. Second is post-LT alcohol related liver failure (ARLF) which can be one of the major causes of death due to continuous alcohol drinking. Therefore, in this study, we aimed to

evaluate the effect of PNPLA3 I148M single nucleotide polymorphism (SNP, rs738409-G) on clinical outcomes in patients with AR after LT.

II. Materials and Methods

1. Patients

Informed consent was obtained from the participants, and this study conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the Institutional Review Board of Seoul National University Hospital (1805-177-949). Between April 2014 and December 2017, 115 patients underwent LT due to ALD at Seoul National University Hospital, South Korea. Thirty-two patients were excluded because of the absence of consent from anyone or both coupled donor and recipient (n=10), biliary complication (n=8), acute or chronic rejection (n=6), early post-LT mortality (n=2), recurrent original liver disease (hepatitis C virus RNA positive, n=1; hepatocellular carcinoma, n=1) and follow-up loss (n=2). A total of 83 patients were enrolled in this study.

2. Diagnosis of ALD and pre- and post-LT management

ALD was diagnosed based on a history of habitual and excessive alcohol consumption combined with corresponding clinical and laboratory data before LT. Moreover, a transplant psychologist evaluated all patients by regularly conducting

face-to-face psychological interviews when patients with ALD were considered for transplantation. A specified period of alcohol abstinence prior to LT was not regularly applied. However, when there was a suspicion of drinking any time prior to transplantation, patients were sent to alcohol rehabilitation centers for further treatment. Patients, who denied consultation, were taken from the list because of noncompliance and were asked to strictly abstain from alcohol consumption, and sobriety was assessed at every interview with their families. Outpatient follow-up was usually performed once a week for the first month after discharge. It was gradually lengthened to every 3 or 4 months. Additional visits were scheduled when required. Complete laboratory examinations, including liver function tests and blood immunosuppression trough level, were performed at each follow-up. From the medical records, information on underlying diseases such as diabetes (DM) or hypertension, laboratory and radiologic data, and post-LT clinical course was retrospectively collected. Preexisting donor graft steatosis was evaluated at the time of organ procurement by liver biopsy. Hypertension was defined as blood pressure ≥130/85 mmHg or by antihypertensive prescription, and DM was defined as fasting blood glucose ≥126 mg/dL, serum glucose ≥200 mg/dL, or if the patient was taking diabetes medication.

3. AR after LT

During follow-up at the out-patient clinic, diagnosis and severity of AR was assessed by a separate questionnaire using the high-risk alcoholism relapse scale (HRAR, Table. 1), which was reported from a study of relapse following treatment for

Table. 1 High-risk alcoholism relapse scale

Items	Score
Duration of heavy drinking (years)	
<11	0
11-25	1
>25	2
Daily drink number (1 drink = 12g of ethanol)	
<9	0
9-17	1
>17	2
Previous alcohol in-patient treatments number	
0	0
1	1
>1	2

alcoholism of a cohort of male US veterans [2]. This scale includes the following three factors: duration of drinking, usual number of drinks per day, and number of treatments for alcoholism. Each item is scored 0 - 2, with a total possible score ranging from 0 to 6. In this study, the heavy drinking group consisted of patients with HRAR scores ≥4, whereas the mild drinking group

comprised patients with HRAR scores <4.

4. Liver-related clinical outcomes after LT

We considered elevated GGT level during follow-up and the development of alcohol-related liver failure (ARLF) as indicators of AR-related clinical outcomes. Elevated GGT was defined as a GGT level higher than the upper range of normal (65 IU/L). Non-AR related causes such as biliary complications, drugs, or rejection were excluded. Plasma GGT activities were determined by routine clinical chemistry methods according to the recommendations of the International Committee for Clinical Laboratory Standards [1]. ARLF was defined as AR patients presenting with one of the following complications (uncontrolled ascites, variceal bleeding, or hepatic encephalopathy) but without any other potential liver failure causes (viral hepatitis, graft rejection, liver vascular or biliary complications, malignancy) [6,3,7]. Uncontrolled ascites was defined as clinically prominent ascites with the necessity of intermittent tapping to relieve symptoms of abdominal distension even with continuous use of diuretics. However, combined chronic renal failure was excluded. Variceal bleeding was defined as endoscopically proven bleeding from esophageal or gastric varices with hematemesis, heart rate >100 beats/min, systolic blood pressure >100 mmHg, or need for blood transfusion. Hepatic encephalopathy was based on an altered level of recipient consciousness as a result of liver failure. After ruling out other potential non-hepatic causes of ARLF, the diagnosis of hepatic encephalopathy was confirmed by the patient's clinical presentation (changes in mood, depressed consciousness, movement problems, and so on) usually supported by the blood

ammonia level and/or a continuous reaction time test.

5. PNPLA3 analysis

Analysis of the I148M PNPLA3 polymorphism (rs738409-G) was performed as described in a previous report [8]. Genomic DNA was isolated from whole-blood samples collected in recipients and their coupled donors. Donor and recipient DNAs were analyzed for rs738409-G (NM_025225.2:c.444C>GNP_079501.2:p.Ile148Met) single nucleotide polymorphism in the PNPLA3 gene based on Sanger Sequencing (BioFact, Daejeon, Korea). To genotype the rs738409 single nucleotide polymorphism, the following primers were used: forward, 50-GCCAGCTGTGGCTACTCTGT-30; and reverse, 30-TGTGGTGACCCAGTGTGACT C-50. Polymerase chain reaction was carried out using the following conditions: denaturation at 95°C for 3 min followed by 35 cycles of 20 s at 95°C, 40 s at 60°C, 30 s at 72°C, and a final extension at 72°C for 5 min. The amplification size was 500 bp. For quality control, genotyping was carried out in duplicate. Both the concordance rate and overall success rate were 100%.

6. Statistical analysis

Statistical analysis was performed using SPSS version 18 (IBM, Armonk, NY, USA). Continuous variables were compared with Student's t-test, and categorical variables were compared using Pearson's χ2-test; however, if the expected values in any of the cells of a contingency table were <5, the Fisher's exact test was used. Cox logistic regression models were used (using Bonferroni correction) in the multivariate analysis. All P-values were

two-sided. Statistical significance was considered when the p-value was <0.05.

III. Results

1. Baseline characteristics and incidence and severity of AR

The baseline characteristics of 83 recipients with coupled donors are summarized in Table. 2. The mean age was 52.05 ± 8.01 years for recipients and 38.66 ± 15.07 years for donors. Sixteen recipients (19.35%) had hepatitis B infection, two (2.41%) were positive for hepatitis C virus RNA, and 16 (19.28%) had hepatocellular carcinoma before LT. Fifty-four recipients underwent living donor LT, whereas 29 underwent deceased donor LT. Post-LT AR occurred in 31 (37.35%) patients; of these, 14 patients(45.16%) showed elevated GGT levels when an AR occurred. Among the 31 patients who relapsed, 3 (9.68%) had ARLF and all were heavy drinkers (HRAR score ≥4). The median follow-up period was 26.32 months (range, 8.21-52.34 months).

2. Prevalence and distribution of PNPLA3 polymorphism

The prevalence and distribution of rs738409-G are also shown in Table. 2. The genotype and allele frequencies of rs738409 C/G polymorphism in this study fit with the Hardy - Weinberg equilibrium. Genotypes of PNPLA3 were 19.28% (GG), 31.32% (GC), and 49.40 (CC) for the recipients and 27.71% (GG), 37.35%

Table. 2 Baseline characteristics of liver transplant recipients & donors

	Recipients (n=83) N, (%)	Donors (n=83) N, (%)
Gender, male : female	69 : 14	53 : 30
Age, years	52.05±8.01	38.66±15.07
Obesity, BMI ≥ 25 kg/m^2	26 (31.33)	24 (28.92)
Underlying diseases		
Hypertension	13 (15.66)	2 (2.41)
Diabetes Mellitus	19 (22.89)	2 (2.41)
Hepatitis B virus	16 (19.28)	0
Hepatitis C virus	2 (2.41)	0
Combined HCC	16 (19.28)	0
MELD	19.41±8.67	-
ABO incompatible LT	6 (7.23)	-
LT type (Living : Deceased)	54 : 29	-
Alcohol relapse (post-LT)	31 (37.35)	-
Alcohol related liver failure (Post-LT)	3 (3.61)	
PNPLA3 genotype		
GG	16 (19.28)	23 (27.71)
GC	26 (31.32)	31 (37.35)
CC	41 (49.40)	29 (34.94)
G allele frequency, %	50.60	65.06

(GC), and 34.94% (CC) for the donors. The frequency of rs738409-G was 50.60% in the recipients and 65.06% in the donors. There was no significant difference in the frequency of the rs738409-G allele between the recipients and donors (p=0.13).

3. Peri-transplant risk factors for clinical outcomes

The results of the risk factor analysis for elevated GGT level and ARLF are summarized in Table. 3. In the univariate analysis,

Table. 3 Risk factor analysis according to serum GGT levels & alcohol related liver dysfunction among liver transplant recipients (n=83), (a): univariate analysis, (b): multivariate analysis

(a) Univariate analysis

Variables	Serum GGT status			Alcohol related liver failure		
	Elevated GGT (n=23) N, (%)	Normal GGT (n=60) N, (%)	p-value	Positive (n=3) N, (%)	Negative (n=80) N, (%)	p-value
Pre-LT recipient factor						
Gender, male	19 (82.61)	50 (83.33)	1.00	3 (100)	66 (82.50)	1.00
Mean age, years	49.00± 8.07	53.22± 7.74	0.13	48.67± 6.35	52.18± 8.07	0.46
Obesity,	6	20	0.52	0 (0)	26	0.55

BMI ≥ 25 kg/m^2	(26.09)	(33.33)			(32.50)	
Underlying diseases						
Hyper-tension	4 (17.39)	9 (15.00)	0.75	0 (0)	13 (16.25)	1.00
DM	6 (26.09)	13 (21.67)	0.67	0 (0)	19 (23.75)	1.00
Hepatitis B	2 (8.69)	14 (23.33)	0.21	0 (0)	16 (19.28)	1.00
Hepatitis C	1 (4.35)	1 (1.67)	0.48	0 (0)	2 (2.50)	1.00
Combined HCC	6 (26.09)	10 (16.67)	0.66	0 (0)	26 (32.50)	0.55
MELD	18.46± 8.10	19.78± 8.91	0.54	25.90± 9.06	19.17± 8.62	0.19
GRWR	1.74± 0.45	1.75± 0.44	0.49	2.00± 0.00	1.74± 0.44	0.31
ABO incom-patible LT	1 (4.35)	5 (8.33)	1.00	0 (0)	6 (7.50)	1.00
Pre-LT donor factors						
Gender, male	11 (47.83)	42 (70.00)	**0.06**	3 (100)	50 (62.50)	0.55
Age, years	40.96± 13.72	37.76± 15.58	0.39	60.00± 15.62	37.85± 14.54	0.13
Obesity, BMI ≥ 25 kg/m^2	8 (34.78)	16 (26.67)	0.49	1 (33.33)	23 (28.75)	1.00
Pre-existing graft steatosis ≥ 5%	2 (8.69)	5 (8.33)	1.00	0 (0)	7 (8.75)	1.00

Post-LT recipient factors						
LT type, LDLT	13 (56.52)	41 (68.33)	0.27	0 (0)	54 (67.50)	**0.04**
Post-LT alcohol relapse						
No drinking	9 (39.13)	43 (71.67)		0 (0)	52 (65.00)	
Mild drinking (HRAR<4)	4 (17.39)	12 (20.00)		1 (33.33)	14 (17.50)	
Heavy drinking (HRAR≥4)	10 (43.48)	5 (8.33)	**<0.01**	2 (66.67)	14 (17.50)	**0.05**
Use of steroid at alcohol drinking	4 (17.39)	5 (8.33)	0.59	0 (0)	9 (11.25)	1.00
Smoking	10 (43.48)	20 (33.33)	1.00	1 (33.33)	29 (36.25)	1.00
Genetic factors						
Recipient PNPLA3 genotype GGorGC : CC	18:5	24:36	**<0.01**	2:1	41:39	0.01
Donor PNPLA3 genotype GGorGC : CC	14:9	40:20	0.62	1:2	53:27	0.28

(b) Multivariate analysis

Variables	Serum GGT status			Alcoholic liver failure		
	Relative risk	95% Confidence interval	p-value	Relative risk	95% Confidence interval	p-value
GGorGC : CC	8.69	2.13-35.46	**<0.01**	4.52	1.52-8.21	**0.04**
Post-LT alcohol drinking						
No drinking	Reference		-	Reference		
Mild drinking (HRAR < 4)	1.68	0.39-7.24	0.49	1.48	0.01-5.01	0.99
Heavy drinking (HRAR ≥ 4)	13.07	2.65 - 64	**<0.01**	19.62	5.01-42.1	**0.03**

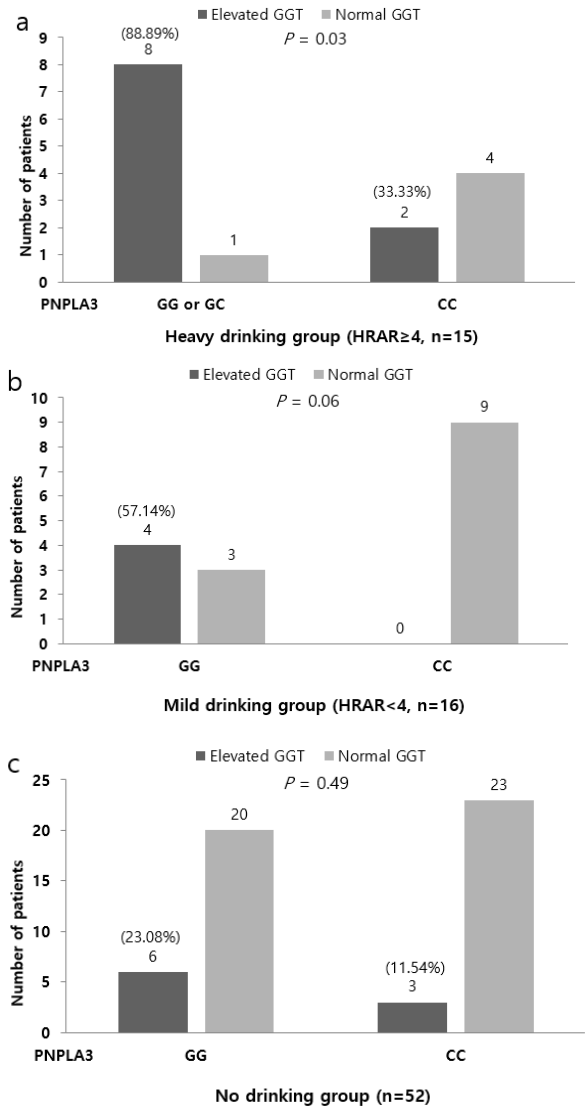
recipient PNPLA3 genotype (rs738409) was significantly different between the group with GGT elevation and the normal group (GG or GC:CC=18:5 vs. 24:36, $p<0.01$) as well as between the group with ARLF and the group without ARLF (GG or GC:CC=2:1 vs. 41:39, $p=0.01$). However, donor genotype and the presence of rs738409-G risk allele in both recipient and coupled donor were not significantly different. Except for genetic factors, only post-LT alcohol habitus showed significant differences between the elevated GGT and the normal GGT group ($p<0.01$). Whereas, the alcohol habitus after LT ($p=0.05$) and types of LT

(p=0.04) showed a significant difference between the ARLF and non-ARLF groups. In the multivariate analysis, the presence of the rs738409-G risk allele in recipient PNPLA3 genotype [odds ratio (OR)=8.69, 95% confidence interval (CI)=2.13 - 35.46, p<0.01] and heavy alcohol drinking habit (HRAR score ≥4) after LT (OR=13.07, 95%CI=2.65 - 64.00, p<0.01) were significant independent risk factors for GGT elevation. The presence of the rs738409-G allele in recipient PNPLA3 (OR=4.52, 95%CI=1.52 - 8.21, p=0.04) and heavy alcohol drinking habit (HRAR score≥4) (OR=19.62, 95%CI=5.01 - 42.10, p=0.03) were also significant risk factors for the development of ARLF.

4. Effect of rs738409-G/C on clinical outcomes according to alcohol drinking habits after LT

The effect of the PNPLA3 genotype (GG or GC, CC) on serum GGT levels according to the degree of alcohol drinking (heavy, mild, and no drinking) is shown in Figure. 3. The patients with GG or GC SNP showed a significantly higher rate of GGT elevation in the heavy drinking group (p=0.03) and borderline significance in the mild drinking group (p=0.06). However, PNPLA3 genotype did not show any effect on the elevation of GGT in the no drinking group.

Figure. 3 The effect of PNPLA3 genotype (GG or GC, CC) on serum GGT levels according to the degree of alcohol drinking (Heavy, mild and no drinking). The patients with GG or GC SNP showed a significant higher rate of GGT elevation in heavy drinking group and borderline significance in mild drinking group.



IV. Discussion

PNPLA3 (rs738409-G) polymorphism is one of the strongest genetic factors for ALD. However, little is known about the effect of this polymorphism on post-transplant outcomes in cases with AR after LT. In this study, we found that PNPLA3 polymorphism of recipient genotype can independently affect the post-LT prognosis of LT patients for ALD, especially in heavy AR patients. Also, heavy alcohol relapse after LT is significantly correlated with elevated GGT level during follow-up and even ARLF.

It is well known that AR after LT negatively impacts the graft and its disease progression, eventually resulting in poor prognosis. In a recent meta-analysis for AR after LT [9], relapsers compared to abstainers had higher odds for graft steatosis (OR=4.1), alcoholic hepatitis (OR=9.3), and advanced fibrosis or cirrhosis (OR=8.4). Moreover, one study by Erard-Poinsot et al. involving heavy alcoholic relapsers after LT strongly supported that severe AR is responsible for the rapid progression of cirrhosis, fibrosis, and even liver failure after LT [10]. In addition, the hepatic decompensation after AR increases the risk of liver failure and patients' mortality [11]. One of the recently reported prospective studies on 712 (128 severe alcohol relapsers) patients transplanted for ALD demonstrated the survival rate of severe relapsers compared to that of abstainers at 10 years of follow-up (49% vs. 70%, $p<0.01$), and the most common cause of death (60/128, 46.88%) was liver graft failure related to continuous alcohol use [7]. In our study, post-LT AR occurred in 31 patients (37.35%), and most of the recipients with AR (45.16%) showed

elevated GGT levels. Especially, among the 31 relapsers, 3 recipients (9.68%) eventually suffered from ARLF after LT.

The reason why we chose GGT as one of the main clinical outcomes is that GGT is an important factor for the follow-up of ALD patients. Several studies have reported a notable positive correlation between alcohol intake and serum GGT activity in the clinical course, and its sensitivities have exceeded those of other commonly used markers. In a recent study on 210 non-alcohol heavy drinkers, 272 heavy drinkers, and 76 patients with alcoholic cirrhosis investigating the kinetics of alcohol markers, the diagnostic accuracy of GGT to detect drinking was moderate (area under the curve=0.7) and was significantly associated with the amount of alcohol drinking, which is in contrast to the AST/ALT ratio [1]. Hietala et al. also analyzed that the sensitivity of GGT (76%) in classifying 165 heavy drinkers for ALD exceeded that of the mean corpuscular volume (45%), AST (47%), ALT (50%), and even CDT (carbohydrate deficient transferrin, 67%) [12]. Given that this test is cost-effective and easy to manage in hospital laboratories, it becomes a suitable and widely used marker for routine screening of alcohol consumption. Moreover, GGT is one of the risk factors for AR patients whose condition progressed to alcohol-related end stage liver disease. Yates et al. reported in their study that significant GGT elevations were found in alcoholic liver cirrhotic patients, who were continuously consuming alcohol for 5 years [13]. Surely, these reports were not in a setting of LT. However, in our study, we observed an association between elevated GGT level and post-LT alcohol intake. The heavy drinking group (HRAR score ≥ 4) showed a higher proportion of elevated GGT level than the no

drinking (66.67% vs. 17.32%, p<0.01) or mild drinking groups (66.67% vs. 25.00%, p=0.03). Moreover, of the 3 recipients with AR whose graft eventually progressed to ARLF, they all presented high GGT levels. Therefore, recipients with AR after LT themselves should be cautious about GGT elevation, as AR with GGT elevation may result in irreversible liver dysfunction, such as ARLF. In fact, the risk factors for GGT elevation in AR patients can be preventable, and the liver damage due to AR is also modifiable by breaking harmful alcohol abuse and lifestyle modification. Regular healthy diet and physical activity are important management strategies for ALD after LT. However, assessment of alcohol consumption is difficult and even harder to evaluate for LT recipients who are strongly advised to remain abstinent and may therefore feel guilty to declare alcohol intake. Indirect and accessible biomarker such as serum GGT could be helpful to estimate possible alcohol intake. Moreover, physicians can gradually introduce psychiatric counseling and promote familial support to prevent the resumption of drinking after LT [2]. Therefore, follow-up of serial GGT level after LT may be essential for every recipient with a potential risk of AR and especially, we should focus on the recipients with AR with GGT elevation during the follow-up period.

Several studies on PNPLA3 concluded that PNPLA3 rs738409 G allele carriers should be considered as a genetically defined subpopulation prone to developing a wide spectrum of ALD. Buch et al., who performed one of the main genome-wide association studies for ALD, identified PNPLA3 as a risk locus for alcohol-related liver injury and its progression [5]. This same variant has also been found to be strongly associated with the

risk of developing ALD (OR=1.95) and cirrhosis (OR=2.25) in the meta-analysis [14]. Moreover, ALD patients with heavy drinking habits or high GGT level during follow-up can be significantly associated with PNPLA3 genetic polymorphism. Kolla et al. reported that only PNPLA3 genetic polymorphism is associated with increased risk for ALD in a cohort of heavy drinkers with a long-term history of alcohol consumption [15], and Zhang et al. [16] similarly reported that PNPLA3 polymorphism is an important genetic marker for ALD and there is also significant association between the rs738409 PNPLA3 G-allele and increased levels of GGT for ALD. In our study, we observed that a rs738409 G allele carrier in the recipient was an independent risk factor for elevated GGT level together with heavy drinking habits (HRAR score ≥ 4) (OR=8.69, 95%CI=2.13 - 35.46, $p<0.01$; OR=13.07, 95%CI=2.65 - 64.00, $p<0.01$). Along with this variant, there are several genes influencing ALD progression in a similar way. TM6SF2, MBOAT7, GCKR and HSD17B13 genes were recently shown to be associated with hepatic fat accumulation and an increased risk of progressive fatty liver disease [17]. Interestingly, in case of the HSD17B13 gene, this gene interacted with PNPLA3 I148M, such that additional HSD17B13 TA alleles reduced the risk of liver damage conferred by PNPLA3 mRNA suppression [18]. However, and there have been very few studies to prove the effect of these gene variants in the ALD recipients. Therefore, further studies are needed to assess whether other genetic variants will affect the post-transplant outcomes in LT settings.

Recent genetic association studies have highlighted that the genetic roles of the individual variability in the predisposition to

non-alcoholic fatty liver disease (NAFLD) and ALD are largely shared [8]. This concept was first reported for the PNPLA3 variant and this is the major genetic determinant of hepatic fat content that increases susceptibility to liver damage followed by inflammation, fibrosis, and finally end stage liver disease [19] [20]. Moreover, a practical implication of these observations is that therapies aimed at fatty liver disease resolution targeting a specific pathway leading to liver damage may have a preventive effect against liver disease progression. Linden et al. demonstrated that the pharmacological downregulation of the PNPLA3 mutant protein reduces the risk of liver disease progression in PNPLA3 knock-in mice carrying the mutant protein fed with a NAFLD-inducing diet [21]. Therefore, this treatment can be potentially effective in humans with NAFLD or ALD. Further study is needed to clarify this hypothesis.

In our study, we observed that a rs738409 G allele carrier in the recipient rather than in the donor was an independent risk factor for an elevated GGT level. This result may contradict the findings of several reports with a non-LT setting that reported a common mechanism of PNPLA3 SNP which is related to the accumulation of enlarged lipid droplets located on the surface of the hepatocytes and hepatic stellate cells [22,23]. One of the potential mechanism of our finding is the extrahepatic malfunction of enzymes which are encoded by PNPLA3 SNP. In other words, recipient's PNPLA3 SNP may a play role in extrahepatic function

of these enzymes in an LT setting. Karlas et al. reported that a reduced PNPLA3 activity in extrahepatic adipocyte tissues is associated with hepatic fat accumulation and post-LT fatty liver disease and that, prior to transplantation patients carrying the risk allele have an increased susceptibility for liver damage due to alcohol even without the presence of the metabolic syndrome [24].

Moreover, theoretically, the frequency of the rs738409-G allele which is known as a high-risk factor of ALD, should be higher in the ALD recipients than in the donors or general population. However, the frequencies between the recipients and donors were not different in this study. The frequencies of G allele are similar to those of the general LT recipients in Korea[8]. The exact reason of this finding is unknown, however, multiple factors may have affected the aggravation of ALD before LT.

Finally, in heavy drinker group analysis, we found that most of the rs738409-G allele carrier patients showed high GGT level (8/9, 88.89%), whereas, major of CC genotype had no GGT elevation (4/6, 66.67%), indicating that if the patients presented as rs738409 G allele carriers in PNPLA3, the patients who drink after LT showed risk of GGT elevation. However, in the CC genotype group, heavy drinking may not be harmful for GGT elevation. Therefore, post-LT patients who are rs738409 G allele carriers should especially make an effort to cease alcohol drinking, even though no drinking is an essential step for preventing ARLF.

The present study undoubtedly had several limitations. First, it was a retrospective study involving a relatively small number of patients. Some patients were lost to follow-up (<5%) and several donors and recipients did not provide consent to participate in this study. Nevertheless, to minimize selection bias, assessment of alcohol consumption was performed using prospective questionnaires, which are based on the HRAR, and we also checked the reports from both patients and their relatives separately for the accuracy of responses. Second, serum GGT has some limited clinical value for detecting alcohol consumption for ALD. Even though serum GGT may help to distinguish alcoholics with or without liver disease [20], owing to the lack of specificity, GGT might be a poor marker when alcohol consumption needs to be screened in patients with nonalcoholic liver diseases. To overcome this weakness, recent studies have suggested a formulated equation from GGT-CDT combination [12]. However, in this study, we could not perform the CDT measurement in every potential LT patient with alcoholism, because the CDT assay kit is expensive and not covered by the national health insurance. Third, several confounding factors can lead to false results of GGT measurements. Even if we analyzed and found no correlation between the GGT level and well-known confounding factors, such as patients' age, BMI, and smoking in this study (Table. 3), other issues might influence the GGT values and changes. Finally, the research population of this study focused on Korean LT patients for ALD. Our population was 100% Korean; therefore, the applicability of these genetic tests to other ethnic populations cannot be assumed and would require specific study.

In conclusion, PNPLA3 polymorphism of recipient genotype can

independently affect the post-LT prognosis of LT patients for ALD, especially in heavy AR patients. Moreover, heavy alcohol relapse after LT (HRAR score ≥4) is significantly correlated with elevated GGT level during follow-up and even ARLF progression. Therefore, strong abstinence education is strongly recommended not only in patients with this single nucleotide polymorphism but also in AR patients with GGT elevation. Thus far, the gold standard for avoiding alcohol resumption after LT is to secure the sufficient period of prohibition before LT. However, the effectiveness of the 'abstinence rule policy', e.g. 6 month abstinence rule before LT in ALD is still controversial in terms of reducing the risk of AR. However, from our findings, the abstinence rule can be selectively applied in ALD patients with the rs738409-G allele patients, because the long-term outcome will be satisfactory even with the presence of mild to moderate AR in patients with rs738409-C allele.

V. References

1. Staufer K, Yegles M. Biomarkers for detection of alcohol consumption in liver transplantation. *World J Gastroenterol.* 2016;22(14):3725-3734.
2. Onishi Y, Kimura H, Hori T, et al. Risk of alcohol use relapse after liver transplantation for alcoholic liver disease. *World J Gastroenterol.* 2017;23(5):869-875.
3. Singal AK, Chaha KS, Rasheed K, Anand BS. Liver transplantation in alcoholic liver disease current status and controversies. *World J Gastroenterol.* 2013;19(36):5953-5963.
4. Karim Z, Intaraprasong P, Scudamore CH, et al. Predictors of relapse to significant alcohol drinking after liver transplantation. *Can J Gastroenterol.* 2010;24(4):245-250.
5. Buch S, Stickel F, Trepo E, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. *Nat Genet.* 2015;47(12):1443-1448.
6. Wells JT, Said A, Agni R, et al. The impact of acute alcoholic hepatitis in the explanted recipient liver on outcome after liver transplantation. *Liver Transpl.* 2007;13(12):1728-1735.
7. Dumortier J, Dharancy S, Cannesson A, et al. Recurrent alcoholic cirrhosis in severe alcoholic relapse after liver transplantation: a frequent and serious complication. *Am J Gastroenterol.* 2015;110(8):1160-1166; quiz 1167.
8. Kim H, Lee KW, Lee K, et al. Effect of PNPLA3 I148M

polymorphism on histologically proven non-alcoholic fatty liver disease in liver transplant recipients. *Hepatol Res.* 2017.

9. Kodali S, Kaif M, Tariq R, Singal AK. Alcohol Relapse After Liver Transplantation for Alcoholic Cirrhosis-Impact on Liver Graft and Patient Survival: A Meta-analysis. *Alcohol Alcohol.* 2017:1-7.
10. Erard-Poinsot D, Guillaud O, Hervieu V, et al. Severe alcoholic relapse after liver transplantation: What consequences on the graft? A study based on liver biopsies analysis. *Liver Transpl.* 2016;22(6):773-784.
11. Jepsen P, Ott P, Andersen PK, Sorensen HT, Vilstrup H. Clinical course of alcoholic liver cirrhosis: a Danish population-based cohort study. *Hepatology.* 2010;51(5):1675-1682.
12. Hietala J, Koivisto H, Anttila P, Niemela O. Comparison of the combined marker GGT-CDT and the conventional laboratory markers of alcohol abuse in heavy drinkers, moderate drinkers and abstainers. *Alcohol Alcohol.* 2006;41(5):528-533.
13. Yates WR, Petty F, Brown K. Risk factors for alcohol hepatotoxicity among male alcoholics. *Drug Alcohol Depend.* 1987;20(2):155-162.
14. Chamorro AJ, Torres JL, Miron-Canelo JA, Gonzalez-Sarmiento R, Laso FJ, Marcos M. Systematic review with meta-analysis: the I148M variant of patatin-like phospholipase domain-containing 3 gene (PNPLA3) is significantly associated with alcoholic liver cirrhosis. *Aliment Pharmacol Ther.* 2014;40(6):571-581.

15. Kolla BP, Schneekloth TD, Biernacka J, et al. PNPLA3 Association with Alcoholic Liver Disease in a Cohort of Heavy Drinkers. *Alcohol Alcohol.* 2018;53(4):357-360.
16. Zhang Y, Guo T, Yang F, et al. Single-nucleotide rs738409 polymorphisms in the PNPLA3 gene are strongly associated with alcoholic liver disease in Han Chinese males. *Hepatol Int.* 2018;12(5):429-437.
17. Romeo S, Sanyal A, Valenti L. Leveraging Human Genetics to Identify Potential New Treatments for Fatty Liver Disease. *Cell Metab.* 2020;31(1):35-45.
18. Abul-Husn NS, Cheng X, Li AH, et al. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. *N Engl J Med.* 2018;378(12):1096-1106.
19. Romeo S, Kozlitina J, Xing C, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. *Nat Genet.* 2008;40(12):1461-1465.
20. Atkinson SR, Way MJ, McQuillin A, Morgan MY, Thursz MR. Homozygosity for rs738409:G in PNPLA3 is associated with increased mortality following an episode of severe alcoholic hepatitis. *J Hepatol.* 2017;67(1):120-127.
21. Linden D, Ahnmark A, Pingitore P, et al. Pnpla3 silencing with antisense oligonucleotides ameliorates nonalcoholic steatohepatitis and fibrosis in Pnpla3 I148M knock-in mice. *Mol Metab.* 2019;22:49-61.
22. Pirazzi C, Valenti L, Motta BM, et al. PNPLA3 has

retinyl-palmitate lipase activity in human hepatic stellate cells. *Hum Mol Genet.* 2014;23(15):4077-4085.

23. Pingitore P, Dongiovanni P, Motta BM, et al. PNPLA3 overexpression results in reduction of proteins predisposing to fibrosis. *Hum Mol Genet.* 2016;25(23):5212-5222.

24. Karlas T, Kollmeier J, Bohm S, et al. Noninvasive characterization of graft steatosis after liver transplantation. *Scand J Gastroenterol.* 2015;50(2):224-232.

25. Niemela O. Biomarkers in alcoholism. *Clin Chim Acta.* 2007;377(1-2):39-49.

요약(국문초록)

알콜성간질환으로 간이식수술을 받은 환자에서 PNPLA3 단일유전자다형성이 술후 임상결과에 미치는 의미

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배경

PNPLA3 (rs738409-G) 단일유전자변이는 알콜성간질환에 있어 가장 강력한 유전인자 중 하나이다. 하지만, 간이식수술후 알코올 재중독된 환자에 있어 유전자변이가 임상결과에 어떤 영향을 주는지에 대한 연구는 드문 실정이다. 따라서 본 연구에서는 알콜성간질환으로 간이식수술을 받은 환자에서 수술후 결과가 PNPLA3 단일유전자변이에 어떤 영향을 미치는지 조사하고자 한다.

방법

본 연구에서는 2004년 4월부터 2017년 12월까지 알콜성 간질환으로 간이식수술을 받은 환자의 데이터를 후향적으로 분석하였다. 재중독의 정도는 고위험 재중독 척도(HRAR)를 이용하여 설문방식을 통하여 각 환자에게 측정하였다. 간이식후 임상결과들 중 혈중 GGT 수치와 수술후 재중독으로 인한 간부전을 조사하였다. 유전체 검사는 간기증자와 수혜자 모두에서 전향적으로 혈액을 수지하여 분석하였다.

결과

총 83명의 환자가 연구에 속하였다. 간이식후 재중독은 31명(37.3%)의 환자에서 나타탔다. 전체 환자 중 23명의 환자에서 GGT 수치 상승을 보였고 3명의 재중독 환자에서 알콜성 간부전을 보였다. 다변량 분석에서 rs738409 G 유전인자를 가지고 있거나 과한 재중독 환자 (HRAR ≥4) 는 수술후 경과중 혈중 GGT상승(odds ratio [OR]=8.69, p<0.01; OR=13.07, p=0.01)과 알콜성 간부전(OR=4.52, p=0.04; OR=19.62, p=0.03)의 독립적인 위험인자로 나타났다. 또한 15명의 과한 재중독 환자에서 rs738409 G 유전인자는 혈중 GGT 상승과 알콜성 간부전과 연관을 보였지만 과하지 않은 재중독 환자나 재중독 되지 않은 환자에서는 GGT 상승을 보이지 않았다.

결론

간수혜자의 PNPLA3 단일유전자변이는 알콜성간질환으로 간이식받은 환자의 예후에 독립적인 영향을 미치며 특별히 과한 재중독에서 그 영향이 크다. 따라서 이와 같은 단일유전자변이를 가지고 있는 환자에 있어서는 특별히 강력한 금주교육이 요구되어진다.

주요어 : 간이식; 알콜성간질환; 알콜성재중독

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