Interaction Between the Patatin-Like Phospholipase Domain-Containing Protein 3 Genotype and Coffee Drinking and the Risk for Acute Alcoholic Hepatitis

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Only a subset of subjects with excessive alcohol consumption develops alcoholic liver disease (ALD). One of the major risk factors for ALD is the genetic variant of the patatin-like phospholipase domain-containing protein 3 (*PNPLA3*) gene. Coffee is one of the most commonly consumed beverages, and coffee consumption has been associated with lower levels of serum alanine aminotransferase. The aim of this study was to investigate the role of coffee drinking and *PNPLA3* rs738409 and their association with alcoholic hepatitis (AH) in a well-characterized cohort of subjects from the Translational Research and Evolving Alcoholic Hepatitis Treatment consortium. AH subjects and heavy drinking controls without a history of liver disease who were enrolled between May 2013 and May 2016 were included (n = 339), and the details of alcohol and coffee consumption were assessed. The *PNPLA3* variant was determined among participants of European ancestry (n = 183). Relationships between baseline data and AH status were determined, and multivariable logistic regression modeling was performed. During the study period, 189 cases with AH and 150 heavy drinking controls were prospectively enrolled. The prevalence of regular coffee consumption was significantly lower in patients with AH compared to controls (20% versus 43%; *P* < 0.0001). The overall minor allele frequency of the *PNPLA3* variant was higher in AH cases. Multivariable logistic regression revealed that coffee consumption and PNPLA3 were significantly associated with AH status at baseline after adjusting for relevant patient characteristics. *Conclusion:* We found a higher prevalence of AH among heavy drinkers with *PNPLA3* G/G and G/C genotypes regardless of coffee consumption status and a higher prevalence of AH among heavy drinkers who were not regular coffee drinkers. These findings remained after considering relevant baseline patient characteristics. Further studies are needed to confirm our observation. (*Hepatology Communications* 2018;2:29-34)

Introduction

Alcoholic liver disease (ALD) comprises a spectrum of disorders in individuals with acute and chronic alcohol consumption, ranging from alcoholic steatosis to alcoholic hepatitis (AH) and cirrhosis.[1] AH is the most florid manifestation of ALD and is associated with a high mortality.[1] ALD only develops in a subset of subjects with excessive alcohol use, suggesting that other factors in addition to alcohol are involved in disease pathogenesis.[2] A recent meta-analysis showed the patatin-like phospholipase domain-containing protein 3 (PNPLA3) variant (rs738409, C>G) as the main genetic determinant of alcoholic cirrhosis.[3] In our Translational Research and Evolving Alcoholic Hepatitis Treatment (TREAT) consortium study (TREAT001; NCT#02172898),[4] we found a higher overall allelic

frequency of PNPLA3 rs738409 among AH cases when compared to heavy drinkers without liver disease (0.34 versus 0.22; P = 0.007),[4] with PNPLA3 G/C and G/G genotypes conferring an increased risk for AH among heavy drinkers.[5]

This genetic variant, however, does not explain all the risk for AH, suggesting that other genetic or environmental factors play a role. Coffee is one of the most commonly consumed beverages, and coffee drinking was inversely associated with nonalcoholic and alcoholic liver cirrhosis.[5-7] Coffee improves serum transaminases and is inversely associated with the severity of steatohepatitis in patients with nonalcoholic fatty liver disease.[8] The aim of the present study was to investigate the role of coffee drinking and its association with PNPLA3 rs738409 as risk factors for AH.

Methods

STUDY COHORT

The analyses were performed on subjects who were recruited as part of the TREAT001 study. Detailed data were collected on demographics, alcohol use, regular coffee consumption (defined as subjects who drank ≥4 times per week for 5 years), and laboratory values, as described.[4] We used the following criteria for AH diagnosis: (1) average daily ethanol consumption of >40 and >60 grams/day for women and men, respectively; (2) consuming alcohol for at least 6 months and within the 6 weeks before enrollment; and (3) total bilirubin >2 mg/dL and aspartate aminotransferase (AST) >50 U/L at admission.[4] Controls were recruited from the Fairbanks Drug and Alcohol Treatment Center (Indianapolis, IN). They were at least 21 years old without underlying medical illnesses, such as chronic obstructive pulmonary disease, congestive heart failure, diabetes, cancer, or chronic renal failure. These subjects had normal results for AST, alanine aminotransferase, total bilirubin, albumin, platelet count, and international normalized ratio and had no past history of jaundice or signs of portal hypertension.[4]

GENOTYPING FOR PNPLA3

Determination of PNPLA3 rs738409 (C>G) polymorphism was performed using the Illumina MEGA BeadChip (Illumina Inc., San Diego, CA) at the Mammalian Genotyping Core, University of North Carolina at Chapel Hill.

STATISTICAL ANALYSIS

Basic descriptive statistics, including mean, SD, and frequencies (percentages) were used to characterize the

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TABLE 1. BASELINE DEMOGRAPHIC AND CLINICAL CHARACTERISTICS OF THE STUDY COHORT

Variables	Controls (n = 150)			AH Cases (n = 189)			P Value
Age (years)	44.2 ± 12.3			46.7 ± 11.0			NS
Men, n (%)	95 (63.3%)			113 (59.8%)			NS
Race, Caucasian, n (%)	126 (84.0%)			165 (87.3%)			NS
BMI (kg/m^2)	28.6 ± 7.1			29.3 ± 8.3			NS
Regular coffee consumption, n (%)	65 (43.3%)			38 (20.4%)			<0.0001
PNPLA3	CC	GC	GG	CC	GC	GG	0.03
	57	32	4	39	41	10	
	(61.3%)	(34.4%)	(4.3%)	(43.3%)	(45.6%)	(11.1%)	
WBC (×10^3 cells/mm^3)	7.2 ± 2.6			11.4 ± 7.9			<0.0001
Hemoglobin (g/dL)	13.0 ± 2.1			10.1 ± 2.0			<0.0001
Platelet counts (×10^3 cells/mm^3)	244.2 ± 71.8			144.2 ± 85.7			<0.0001
Total bilirubin (mg/dL)	0.5 ± 0.3			13.9 ± 11.5			<0.0001
INR	1.0 ± 0.2			1.8 ± 0.5			<0.0001
AST (U/L)	27.7 ± 9.2			140.1 ± 88.2			<0.0001
ALT (U/L)	25.8 ± 10.4			62.9 ± 65.8			<0.0001
Alkaline phosphatase (U/L)	76.1 ± 31.3			193.6 ± 138.1			<0.0001
Albumin (g/dL)	3.9 ± 0.6			2.8 ± 0.7			<0.0001
Total protein (g/dL)	6.5 ± 0.8			6.1 ± 0.9			<0.0001
Creatinine (mg/dL)	0.9 ± 0.3			1.0 ± 0.9			NS
MELD Scores	7.1 ± 2.3			22.1 ± 7.0			<0.0001
Severity of liver diseases (MELD >20), n (%)	0			110 (58%)			NA

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; INR, international normalized ratio; MELD, Model for End-Stage Liver Disease; WBC, white blood count.

data set. The chi-square and Student t tests were used for comparison between groups for categorical and continuous variables, respectively. We examined the relation of AH with variables of interest by first comparing the prevalence of AH among the levels of these variables. To further study the strength and form of association between coffee consumption and PNPLA3 gene status, we employed multivariable logistic regression modeling that controlled for the potential effects of other variables related to AH. The modeling procedure was conducted as follows: First, the variables PNPLA3, coffee consumption, body mass index, age, and sex were tested in univariate models (race was not included because all patients with PNPLA3 information were Caucasian) with AH status at baseline as the outcome. Clinical and laboratory results were not included as potential adjustments because it is well known that these variables are associated with AH status and this was not our primary focus. Variables for which the univariate P satisfied $P < 0.20$ were considered for inclusion in the final multivariable model. Next, all possible models composed of combinations of main effects from the screened variables were fit, and the model with the minimum Akaike information criterion was selected as optimal.[9] After selection of the optimal model, an interaction term between PNPLA3 and coffee consumption was added to assess evidence for its inclusion in the final model. The

resulting model was then used to calculate the odds ratios (ORs) and 95% confidence intervals (CIs). A P value of <0.05 was considered statistically significant in the final multivariable model. All analyses were performed with SAS software version 9.3 (Cary, NC).

Results

CLINICAL CHARACTERISTICS

During the study period from May 2013 to May 2016, 189 cases with AH and 150 controls were prospectively enrolled. The detailed demographic and clinical characteristics of the study cohort are summarized in Table 1. Among regular coffee drinkers, the average duration of regular coffee consumption for controls and cases with AH was 26.7 ± 12.6 and 26.3 ± 13.8 years, respectively. Controls drank ∼3.4 ± 2.1 (mean ± SD) cups, while cases with AH consumed ∼2.6 ± 3.0 cups daily.

MULTIVARIATE LOGISTIC REGRESSION MODELING

All patient characteristic variables, namely age, body mass index, and sex, did not pass the univariate screen for inclusion into the final multivariable model. Only

TABLE 2. UNIVARIATE MODEL RESULTS AND MULTIVARIABLE MODEL ESTIMATES (*PNPLA3* VARIANT AND COFFEE CONSUMPTION AND THEIR ASSOCIATION WITH AH)

Variable	Estimate	Standard Error	P Value	Odds Ratio	95% CI Lower	95% CI Upper
			Univariate Models			
Age	0.02	0.01	0.21	1.01	0.99	1.04
Body mass index	0.007	0.02	0.70	1.00	0.97	1.05
Sex (female vs male)	−0.08	0.30	0.78	0.92	0.51	1.67
PNPLA3 genotype (GG/GC vs CC)	0.73	0.30	0.01	2.07	1.15	3.74
Coffee consumption	−0.93	0.32	0.004	0.39	0.21	0.74
			Multivariable Model			
PNPLA3 variant (GC/GG vs CC)	0.69	0.31	0.02	2.00	1.09	3.65
Coffee consumption (Yes vs No)	−0.90	0.33	0.005	0.41	0.21	0.77

PNPLA3 and coffee consumption exhibited univariate P values less than 0.20 ($P = 0.0156$ and $P = 0.0036$, respectively; Table 2). The multivariable logistic regression model that included only the main effects for coffee consumption and PNPLA3 status was selected as optimal and provided a better fit than the multivariable logistic regression model that included the main effects and an interaction (Akaike information criterion, 245.78 versus 247.75, respectively). The results implied by the final multivariable model are summarized in the following subsections.

PNPLA3 POLYMORPHISM IN AH AND CONTROLS

The distribution of the *PNPLA3* CC, CG, and GG genotypes[10] and its allelic frequencies were determined in a subset of 90 cases with AH and 93 controls of European ancestry.[4] The overall minor allele frequency of *PNPLA3* rs 738409 was 0.34 and 0.22 for AH cases and controls, respectively. We found evidence that the distribution of PNPLA3 varied by AH case status. Particularly, it seems that a higher percentage of AH cases have at least one G variant ($P = 0.0301$; Table 1). In the multivariable analyses, the presence of the GG/GC allele was associated with increased prevalence of AH (OR, 2.00; 95% CI, 1.09-3.65; Table 2) after adjusting for coffee consumption.[4]

COFFEE CONSUMPTION AND AH

We found that the prevalence of regular coffee consumption was significantly lower in patients with AH when compared to controls (20% versus 43%; $P < 0.0001$; Table 1). The association persisted in the multivariable logistic regression analysis after adjusting for

PNPLA3 polymorphism (OR, 0.41; 95% CI, 0.21-0.77; Table 2).

INTERACTION BETWEEN *PNPLA3* POLYMORPHISM AND COFFEE CONSUMPTION AND THE RISK FOR AH

We investigated the relationship between *PNPLA3* polymorphism and coffee consumption and the prevalence of AH among the pooled cohort of 183 heavy drinkers with and without AH. Among heavy drinkers who carried the *PNPLA3* CC genotype and were regular coffee drinkers, the prevalence of AH was 27%. The prevalence estimate increased to 86% for nonregular coffee drinkers with the *PNPLA3* GG genotype; however, multivariable logistic regression modeling showed that there was insufficient evidence for inclusion of an interaction effect between PNPLA3 and coffee consumption, after adjusting for relevant baseline characteristics.

Discussion

ALD, a major complication of excessive alcohol use, is one of the leading causes of chronic liver disease with substantial morbidity and mortality.[11] Continued drinking can lead to alcoholic cirrhosis in up to 15%-20% of excessive drinkers in their lifetime,[12] suggesting that other factors in addition to excessive alcohol drinking are involved in progression. We found an important gene (*PNPLA3*) and environmental (coffee drinking) effect and an association with AH among those who consume alcohol heavily. Although previous studies have shown independent relationships between *PNPLA3* variants[3] and coffee drinking and various

liver diseases,[8,13] to our knowledge this is the first report to investigate them together as risk factors for AH.

The *PNPLA3* gene is located on the long arm of chromosome 22.[14] *PNPLA3* rs738409 (C>G) has been shown to be associated with alcoholic cirrhosis,[15-17] and we have found that those patients with the GG/GC allele are associated with AH. Our observation that coffee drinking may exert a protective effect against AH is interesting and deserves further discussion. Several studies have found an inverse relationship between coffee consumption and levels of gamma glutaryltransferase.[18,19] In patients with chronic hepatitis C infection, higher coffee consumption was associated with less steatosis on liver biopsy, lower serum AST/ alanine aminotransferase ratio, and lower alpha-fetoprotein and insulin.[20] Increasing coffee intake was associated with a decrease in adverse outcomes, such as ascites, liver disease-related death, and complications of portal hypertensions, or a ≥2-point increase in the Ishak fibrosis score during follow-up biopsies in patients with hepatitis C infection.[20] Mechanistically, it was shown that caffeine, the main component found in coffee, has anti-inflammatory properties.[13,21] Treatment with caffeine significantly attenuated the increase in serum aminotransferases and ameliorated hepatocyte damage and hepatic steatosis through inhibition of lipogenic genes in ethanol-fed mice.[21] Further, caffeine reduces intrahepatic lipid content and stimulates β-oxidation in hepatocytes by an autophagy–lysosomal pathway.[22] In mice fed a high-fat diet, caffeine markedly reduced steatosis by inducing autophagy and lipid uptake in lysosomes.[22] Caffeine can also inhibit the production of tumor necrosis factor α and reactive oxygen species by Kupffer cells.[21] Lastly, caffeine's protective mechanism may be mediated through its antagonistic effect on adenosine receptors and thus inhibition of collagen synthesis by hepatic stellate cells.[23] While caffeine might be the mechanistically important component of coffee, some studies have suggested that the protective effect of coffee against hepatic injury may be secondary to noncaffeine-mediated mechanisms,[13,24] as demonstrated by the protective effects of decaffeinated coffee. However, the evidence is weaker overall for decaffeinated than for regular coffee.[13]

Our results suggest that coffee drinking favorably modifies the adverse effects of *PNPLA3* minor alleles among heavy drinkers, but the mechanistic basis for this phenomenon is unclear. PNPLA3 plays a role in the hydrolysis of glycerolipids, and its variant leads to a loss of this function.[14] The *PNPLA3* variant also is associated with the presence of large hepatic lipid droplets, and the accumulation of inactive PNPLA3 on lipid droplets may cause hepatic triglyceride accumulation.[25] It is plausible that heavy drinkers with the *PNPLA3* variant who are not regular coffee drinkers may have increased lipogenesis; however, they may not be able to effectively export the increase in the amount of lipid leading to subsequent liver injury. This hypothesis warrants further mechanistic investigation.

Our study has several strengths and limitations. The strengths are the prospective study design. We also included a cohort of heavy drinker controls without a history or laboratory evidence of liver disease. We acknowledge several limitations of our study. First, the self-reported questionnaire was determined at baseline at the time of enrollment, and we did not capture long-term or lifetime coffee consumption. Additionally, we do not have information on decaffeinated coffee consumption in our study cohort. Second, the diagnosis of AH was based on clinical criteria as reported by the TREAT consortium[4] instead of using liver biopsy. Given the lack of histopathologic finding, we cannot rule out the possibilities that the *PNPLA3* GG variant may be associated with underlying alcoholic cirrhosis rather than AH. Third, our sample size is relatively small. Despite this limitation, we found a higher prevalence of those with the GG variant in AH subjects.

In conclusion, we found a higher prevalence of AH among heavy drinkers with the *PNPLA3* GG and GC genotypes, regardless of coffee consumption status, and a higher prevalence of AH among heavy drinkers who are not regular coffee drinkers, regardless of PNPLA3 status. We recommend that our observations be confirmed by other investigators and that *in vitro* and *in vivo* experiments be undertaken to further explore the interaction between *PNPLA3* variants and coffee drinking and the risk for ALD.

REFERENCES

1) Liangpunsakul S, Haber P, McCaughan GW. Alcoholic liver disease in Asia, Europe, and North America. Gastroenterology 2016;150:1786-1797.

2) Gao B, Bataller R. Alcoholic liver disease: pathogenesis and new therapeutic targets. Gastroenterology 2011;141:1572-1585.

3) 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: 571-581.

4) Liangpunsakul S, Puri P, Shah V, Kamath P, Sanyal A, Urban T, et al.; Translational Research and Evolving Alcoholic Hepatitis Treatment Consortium. Effects of age, sex, body weight, and quantity of alcohol consumption on occurrence and severity of alcoholic hepatitis. Clin Gastroenterol Hepatol 2016;14:1831-1838.e3.

5) Ruhl CE, Everhart JE. Coffee and caffeine consumption reduce the risk of elevated serum alanine aminotransferase activity in the United States. Gastroenterology 2005;128:24-32.

6) Corrao G, Lepore AR, Torchio P, Valenti M, Galatola G, D'Amicis A, et al. The effect of drinking coffee and smoking cigarettes on the risk of cirrhosis associated with alcohol consumption. A case-control study. Provincial Group for the Study of Chronic Liver Disease. Eur J Epidemiol 1994;10:657-664.

7) Corrao G, Zambon A, Bagnardi V, D'Amicis A, Klatsky A. Coffee, caffeine, and the risk of liver cirrhosis. Ann Epidemiol 2001;11:458-465.

8) Saab S, Mallam D, Cox GA, Tong MJ. Impact of coffee on liver diseases: a systematic review. Liver Int 2014;34:495-504.

9) Akaike H. Information theory and an extension of the maximum likelihood principle. In: Petrov BN, Csaki F, eds. Second International Symposium on Information Theory. Budapest, Hungary: Akademiai Kiado; 1973:267-281

10) Romeo S, Kozlitina J, Xing C, Pertsemlidis A, Cox D, Pennacchio LA, et al. Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 2008;40: 1461-1465.

11) Rehm J, Samokhvalov AV, Shield KD. Global burden of alcoholic liver diseases. J Hepatol 2013;59:160-168.

12) Mills SJ, Harrison SA. Comparison of the natural history of alcoholic and nonalcoholic fatty liver disease. Curr Gastroenterol Rep 2005;7:32-36.

13) Kennedy OJ, Roderick P, Buchanan R, Fallowfield JA, Hayes PC, Parkes J. Systematic review with meta-analysis: coffee consumption and the risk of cirrhosis. Aliment Pharmacol Ther 2016;43:562-574.

14) Huang Y, Cohen JC, Hobbs HH. Expression and characterization of a PNPLA3 protein isoform (I148M) associated with nonalcoholic fatty liver disease. J Biol Chem 2011;286:37085-37093.

15) Tian C, Stokowski RP, Kershenobich D, Ballinger DG, Hinds DA. Variant in PNPLA3 is associated with alcoholic liver disease. Nat Genet 2010;42:21-23.

16) Stickel F, Buch S, Lau K, Meyer zu Schwabedissen H, Berg T, Ridinger M, et al. Genetic variation in the PNPLA3 gene is associated with alcoholic liver injury in caucasians. Hepatology 2011;53:86-95.

17) Buch S, Stickel F, Trepo E, Way M, Herrmann A, Nischalke HD, et al. A genome-wide association study confirms PNPLA3 and identifies TM6SF2 and MBOAT7 as risk loci for alcohol-related cirrhosis. Nat Genet 2015;47:1443-1448.

18) Nilssen O, Forde OH, Brenn T. The Tromso Study. Distribution and population determinants of gamma-glutamyltransferase. Am J Epidemiol 1990;132:318-326.

19) Tanaka K, Tokunaga S, Kono S, Tokudome S, Akamatsu T, Moriyama T, e al. Coffee consumption and decreased serum gamma-glutamyltransferase and aminotransferase activities among male alcohol drinkers. Int J Epidemiol 1998;27:438-443.

20) Freedman ND, Everhart JE, Lindsay KL, Ghany MG, Curto TM, Shiffman ML, et al.; HALT-C Trial Group. Coffee intake is associated with lower rates of liver disease progression in chronic hepatitis C. Hepatology 2009;50:1360-1369.

21) Lv X, Chen Z, Li J, Zhang L, Liu H, Huang C, et al. Caffeine protects against alcoholic liver injury by attenuating inflammatory response and oxidative stress. Inflamm Res 2010;59:635-645.

22) Sinha RA, Farah BL, Singh BK, Siddique MM, Li Y, Wu Y, et al. Caffeine stimulates hepatic lipid metabolism by the autophagy-lysosomal pathway in mice. Hepatology 2014;59: 1366-1380.

23) Yamaguchi M, Saito SY, Nishiyama R, Nakamura M, Todoroki K, Toyo'oka T, et al. Caffeine suppresses the activation of hepatic stellate cells cAMP-independently by antagonizing adenosine receptors. Biol Pharm Bull 2017;40:658-664.

24) Xiao Q, Sinha R, Graubard BI, Freedman ND. Inverse associations of total and decaffeinated coffee with liver enzyme levels in National Health and Nutrition Examination Survey 1999-2010. Hepatology 2014;60:2091-2098.

25) Smagris E, BasuRay S, Li J, Huang Y, Lai KM, Gromada J, et al. Pnpla3I148M knockin mice accumulate PNPLA3 on lipid droplets and develop hepatic steatosis. Hepatology 2015;61:108-118.