LIVER

PNPLA3 Gene Polymorphism Is Associated With Predisposition to and Severity of Alcoholic Liver Disease

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OBJECTIVES: **The genetic polymorphism with an isoleucine-to-methionine substitution at position 148 (rs738409 C>G) in the *patatin-like phospholipase domain protein 3 (PNPLA3)* gene confers risk of steatosis. PNPLA3 polymorphism is shown to be associated with alcoholic liver disease (ALD). We performed a systematic review and meta-analysis to examine association of this genetic polymorphism with ALD spectrum and its severity.**

METHODS: **Medline, Embase, and Cochrane Library were searched for studies on association of PNPLA3 polymorphism and ALD spectrum: alcoholic fatty liver (AFL), alcoholic liver injury (ALI), alcoholic cirrhosis (AC), and hepatocellular carcinoma (HCC). Pooled data are reported as odds ratio (OR) with 95% confidence interval. Heterogeneity was assessed using the I^2 statistics and publication bias using Egger's test and Begg and Mazumdar's test. Individual participant data obtained from five studies were used for subgroup analyses.**

RESULTS: **Among 10 studies included in this pooled analysis, compared with controls, OR for rs738409 CG and GG among ALI patients was 1.45 (1.24–1.69) and 2.22 (1.50–3.28), respectively, compared with CC. Respective OR among AC patients was 2.09 (1.79–2.44) and 3.37 (2.49–4.58) and among AC patients with HCC was 2.87 (1.61–5.10) and 12.41 (6.99–22.03). Data for AFL were inconsistent. Among ALD patients, OR of CG and GG genotypes was 2.62 (1.73–3.97) and 8.45 (2.52–28.37), respectively, for AC compared with fatty liver (FL) patients. Similar OR for AC compared with ALI was 1.98 (1.24–3.17) and 3.86 (1.18–12.60). The OR for CG and GG genotypes among AC patients for HCC occurrence was 1.43 (0.76–2.72) and 2.81 (1.57–5.01), respectively. Individual participant data analysis showed age to predispose to AC among ALI patients.**

CONCLUSIONS: **PNPLA3 genetic polymorphism (rs738409 C>G) is associated with increased risk for the entire spectrum of ALD among drinkers including ALI, AC, and HCC. Studies are needed to clarify association of PNPLA3 polymorphism and steatosis in alcoholics. *PNPLA3* gene may potentially be a therapeutic target in ALD.**

SUPPLEMENTARY MATERIAL is linked to the online version of the paper at http://www.nature.com/ajg

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INTRODUCTION

Alcohol abuse is a common cause of cirrhosis and indication for liver transplantation. Clinical spectrum of alcohol-related liver disease includes simple steatosis or alcoholic fatty liver (AFL), alcoholic liver injury with elevated transaminases but without cirrhosis (ALI), alcoholic cirrhosis (AC), and hepatocellular carcinoma (HCC). Amount of alcohol consumption is the most important factor in the development of ALI and AC (1). However, only ~10–20% of heavy drinkers develop AC, suggesting a role of other host factors and comorbidities predisposing to ALI and its progression to AC and HCC (2). Data on genetic predisposition to alcoholism and alcoholic liver disease (ALD) among monozygotic twins (3), and on ethnic variations on ALD-related mortality independent of amount of alcohol use, support the role of genetic factors in mediating ALD (4–6). Single-nucleotide polymorphisms within genes of cytokines, alcohol-metabolizing enzymes, and antioxidant enzymes have been shown to be associated with progression of alcohol-induced liver fibrosis (7). However, data on association of these genetic factors in the development of ALD have remained conflicting until a few years ago (8).

Recently, a genome-wide screen in a population of Hispanic, African, and European Americans from the Dallas Heart Study identified a strong association between a single-nucleotide polymorphism, rs738409 C>G (causing an isoleucine-to-methionine substitution at position 148, I148M), in the *patatin-like phospholipase domain containing protein 3* (*PNPLA3*) gene and hepatic fat content (9). In addition, a strong association between PNPLA3 genotype and elevated alanine aminotransferase levels as well as hepatic fat content was confirmed in other populations (10–16). Although this gene was discovered to be associated with steatosis in patients with nonalcoholic fatty liver disease (12), data are emerging on its association with other liver diseases, including hepatitis C virus cirrhosis (17) and alcoholic cirrhosis (18). We performed a systematic review and meta-analysis of studies to examine the association between the presence of the PNPLA3 rs738409 polymorphism and the spectrum of ALD.

METHODS

Identification and retrieval of primary studies

We performed search of PubMed/Medline, Embase, and Cochrane for full-length articles in English examining PNPLA3 polymorphism association among ALD patients. We followed the MOOSE (Meta-analysis of Observational Studies in Epidemiology) guidelines (19). The initial medical subject heading search terms were "liver cirrhosis, alcoholic," and "adiponutrin, human" and expanded search using "rs738409" and "patatin-like phospholipase domain-containing 3 protein, human." All databases were searched from their inception through September 2014. Articles were selected for full text review based on title and abstract. Manual search of the bibliographies of retrieved publications was done by two independent investigators (H.S. and A.K.S.) to increase yield of potentially relevant articles.

Inclusion and exclusion criteria

Inclusion and exclusion criteria were defined at the time of study conception and before data collection. For inclusion into the meta-analysis, a study had to (i) include patients with alcohol dependence; (ii) assess the presence of the PNPLA3 variant (rs738409 C>G); (iii) define the ALD spectrum to AFL: steatosis on liver ultrasound in the absence of elevated liver enzymes, ALI: liver steatosis accompanied by elevated amino transferases in the absence of cirrhosis, AC: confirmed by biopsy or clinical evaluation supported by hematological, biochemical, and/or radiologic imaging findings, and HCC: diagnostic findings on triple phase magnetic resonance imaging or computed tomography or on histological examination of the liver tissue; and (iv) be published as a full-length article. We excluded studies (i) without gene frequency and/or odds ratio (OR) data and (ii) including subjects with other liver disease etiologies without separate data among ALD patients.

Study selection and data extraction

Two reviewers (H.S. and A.K.S.) independently examined the studies for the inclusion and exclusion criteria and also extracted the data from the studies included in the analysis. The reviewers resolved discrepancies by jointly reviewing the study in question. Studies included in the analysis were reviewed for (i) study characteristics: author and year of publication, and study design (population based or not, using controls or not); (ii) study population: ALD spectrum and the sample sizes; (iii) frequencies of PNPLA3 polymorphism genotypes (rs738409 CC, CG, and GG): among healthy controls and ALD patients; and (iv) ORs: for association of PNPLA3 polymorphism and spectrum of ALD and for severity of ALD among diseased drinkers. Corresponding authors of studies included in the analysis were contacted to obtain the individual participant data.

End points and outcomes

Our study end points were (i) alcohol-related fatty liver (FL), ALI, AC, or HCC among drinkers compared with healthy controls and (ii) severity of ALD among drinkers. Healthy controls were defined as subjects without evidence of AFL, ALI, AC, or HCC irrespective of their drinking status as our primary aim was to examine the association of PNPLA3 polymorphisms with the predisposition to ALD.

We also performed the subgroup analyses for patient demographics (age, gender, and ethnicity) using the individual participant data.

Assessment of study quality

The quality of included studies was assessed independently by two authors (H.S. and A.K.S.) using the Newcastle–Ottawa Quality Assessment Scale for case–control studies (20). This scale has two different instruments for assessing case–control and cohort studies. Each instrument includes measures of quality in three domains: selection, comparability, and exposure. A study can receive up to one point for each of four areas meas-

ured within the selection domain and for each of three areas measured within the exposure domain. A maximum of two points can be assigned within the comparability domain. The highest possible score is nine. High-quality studies were considered to have a score of seven or greater. Any discrepancies between the two authors were addressed by a joint reevaluation of the original article.

We also assessed the Hardy–Weinberg equilibrium (HWE) to assess genotype frequencies in the included studies. As the deviation from HWE in controls has been associated with problems in the design and conduct of genetic association studies particularly because of population stratification, genotyping error, or selection bias (21,22), the magnitude of deviations from HWE and its statistical significance are reported. HWE analysis was performed among the healthy controls using the χ^2 test (χ^2 value <3.84 indicating allele frequency to be in HWE). If a study showed a significant deviation of HWE, we planned to perform sensitivity analyses excluding the studies which deviated from HWE (if any) as recommended (23).

Statistical analysis

The strength of the association between rs738409 and ALD prevalence was expressed by OR and their corresponding 95% confidence interval. Random effects model was used for analyzing the pooled data for all the analyses (24). Heterogeneity was measured by the between-study variance using the I^2 statistics (25) with a cutoff of ≥50% and χ^2 test with P<0.10 (25). When there was heterogeneity with only two studies in the analysis, we reviewed both the studies for any differences to explain the heterogeneity. For heterogeneous data on analysis with more than two studies, we took stepwise approach: (i) excluding study with different ethnicity on Mestizio subjects, (ii) excluding two studies with highest and lowest OR, and (iii) meta-regression if needed. To assess the extent of publication bias, both Egger's test and the Begg and Mazumdar's test were used on unadjusted analyses (26,27). Funnel plots were assessed by two independent investigators (H.S. and A.K.S.). At least two studies in the analyses were needed to examine the heterogeneity and three studies for examining any publication bias.

To examine independent association of rs738409 and ALD spectrum, generalized linear mixed models were built for patient demographics using the individual participant data. This statistical method was chosen to take into account the differences between the studies and is equivalent to the random effects model used for our main analysis. Data were not controlled for body mass index, given that only two of the five studies had information on this variable. First unadjusted odds for a particular outcome were assessed comparing the various PNPLA3 polymorphisms. Then, in a stepwise manner age and gender were entered into the model to examine independent association of PNPLA3 polymorphisms as well as individual demographics on the outcome. All statistical analyses were performed using the Comprehensive Meta-analysis software (Biostat, Englewood, NJ).

RESULTS

Baseline study characteristics

A total of 254 citations were retrieved on initial search. After reviewing article titles and abstracts, a total of 14 studies were included for full-text review (**Figure 1**). Of these, four studies (28–31) were excluded because of unavailability of data for analysis and/or were not full manuscripts, leaving 10 studies involving 4,112 patients for this meta-analysis. All 10 studies reported PNPLA3 polymorphism data on AC, 4 studies each on ALI and HCC, whereas only one study reported data on AFL (**Table 1**). Of the 10 studies, 7 recruited healthy controls (18,32–37), whereas the other 3 had no control group (38–40). Healthy controls were heavy drinkers without liver disease in two studies (18,32), or nondrinkers without liver disease in four studies (33,34,36,37). One study included both drinkers and nondrinkers without liver disease (35). The baseline characteristics of the included studies are reported in **Table 1**. All studies included subjects with Caucasian ethnicity except one study that included Mestizio subjects, people from Mexico with mixed European and Native American ancestry (32).

Assessment of study quality

Based on the Newcastle–Ottawa Scale, half of the included studies (five) were considered as high-quality studies (**Supplementary Table S1** online). In addition, all seven studies reporting genotype frequencies for control population were in HWE (**Supplementary Table S2**).

PNPLA3 polymorphism (rs738409) in ALD compared with healthy controls

A total of 7 studies involving 2,878 ALD patients and 4,091 controls were analyzed. Compared with healthy controls, prevalence of the PNPLA3 polymorphism rs738409 was assessed in patients

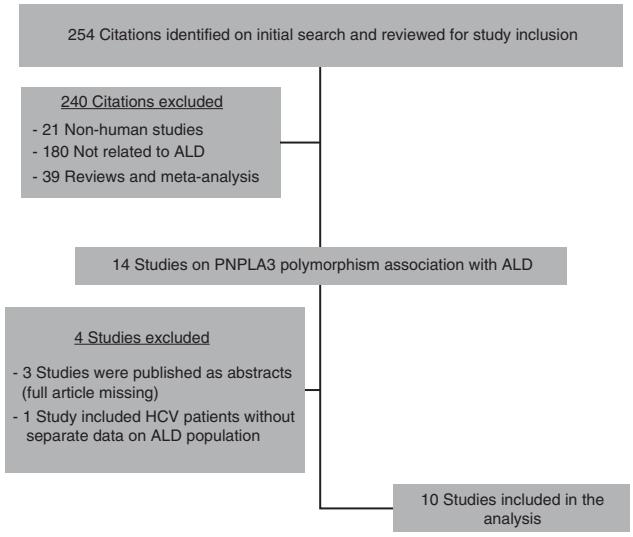

Figure 1. Attrition diagram for study inclusion. ALD, alcoholic liver disease; HCV, hepatitis C virus; PNPLA3, patatin-like phospholipase domain protein 3.

Table 1. Baseline characteristics of patients from studies included in the analysis

Author[a]		Controls					Cases						
		N	Males	BMI	Type	CC:CG:GG[b]	Case definition	*N*	Males	BMI	CC:CG:GG		
				—							AFL	ALI	AC	HCC
Seth *et al.* (18)[a]	P[c]	182	NR	—	HD	119:60:3	HD	266	NR	NR	—	—	173:170:23	—
Tian *et al.* (32)	P[c]	305	84%	—	HD	83:198:11	HD with <5 yrs or <200 g/wk	916	85	NR	—	91:266:305	59:264:371	—
Trepo *et al.* (33)	P	328	52%	—	ND	181:118:29	Histology-confirmed ALD and biochemical evidence of ALI	330	71	26	—	40:18:7	100:130:35	—
Nischalke *et al.* (34)	P	190	56%	—	ND	112:69:9	AC±HCC with >300 g ethanol/wk alcohol	160	86	25	—	—	32:38:10	17:40:23
Stickel *et al.* (35)	P	439	100%	—	HD and ND	264:153:22	Alcohol >80 g males and >60 g females for >10 yrs and DSM-IV ethanol dependence	MCS: 604	94	25.4	116:56:3	113:82:24	90:93:27	—
		269	95%	—		104:55:3		PBS: 269	95	29.4	46:16:2	107:81:17	—	—
Falleti *et al.* (36)	P[c]	428	73.4%	—	ND	218:175:35		198	73	25.4	—	—	38:64:30	15:26:25
Rosendahl *et al.* (37)	P	1,950	43.7%	—	ND	1,135:718:97	AC based on biopsy and/or clinical criteria	135	77	NR	—	—	51:59:25	—
Trepo *et al.* (38)[d]	P[c]	NA	NA	—	NA	NA	I: AC±HCC (imaging or biopsy)	571	75	27	—	—	190:191:45	39:58:48
Guyot *et al.* (39)	P	NA	NA	—	NA	NA	AC with ethanol >80 g per day males and >60 g per day females for >10 yrs and excluding other causes of liver disease	279	78	27.3	—	—	93:101:19	19:30:17
Burza *et al.* (40)	R	NA	NA	—	NA	NA	Liver disease with DSM-IV ethanol dependence and exclusion of other causes of liver disease	384	76	25.3	—	145:126:29	19:39:26	—

AC, alcoholic cirrhosis; AFL, alcoholic fatty liver; ALD, alcoholic liver disease; ALI, alcoholic liver injury; BMI, body mass index; DSM-IV, Diagnostic and Statistical Manual of Mental Disorder, 4th edition; HD, heavy drinkers; I, inclusion; MCS, multicenter sample; NA, not available; ND, nondrinker; NR, not reported; P, prospective; PBS, population-based sample; R, retrospective.

[a]Caucasian subjects in all studies except Tian *et al.* (32) with Mestizio subjects, people from Mexico with mixed European and Native American ancestry.

[b]Genotype counts were reported as ratios (CC wild genotype: CG heterozygote genotype: GG homozygote genotype).

[c]Population-based studies.

[d]Data not reported in the original study but extracted from a recent meta-analysis (45).

with AFL (1 study), ALI (3 studies), AC (7 studies), and HCC (2 studies).

Alcoholic fatty liver. The OR of the rs738409 CG and GG genotypes as compared with CC genotype in AFL was similar to controls when data were pooled from population-based and multicenter samples in one study (35): 0.74 (0.54–1.03) and 0.62 (0.13–2.93), respectively (**Figures 2a** and **3a**). The data were homogeneous for both analyses with respective values of I^2=0%, P=0.69 and I^2=45.98%, P=0.17.

Alcoholic liver injury. The OR of rs738409 CG genotype compared with CC genotype was 1.45 (1.24–1.69) on comparing patients with ALI with healthy controls (**Figure 2b**). Data were homogeneous (I^2=0%, P=0.995) without any publication bias as assessed by Egger's test (P=0.569) or Begg and Mazumdar's test (P=0.5). Similar OR for the rs738409 GG genotype was 2.22 (1.50–3.28) as compared with healthy controls (**Figure 3b**). Data were homogeneous (I^2=0%, P=0.524) without publication bias as assessed by Egger's test (P=0.182) or Begg and Mazumdar's test (P=0.120).

Alcoholic cirrhosis. The OR of rs738409 CG genotype compared with CC genotype was 2.09 (1.79–2.44) on comparing patients with AC with healthy controls (**Figure 2c**). Data were homogeneous (I^2=0%, P=0.989) without publication bias as assessed by Egger's test (P=0.17) or Begg and Mazumdar's test (P=1.0). Similar OR for the rs738409 GG genotype was 3.37 (2.49–4.575; **Figure 3c**). Data were homogeneous (I^2=0%, P=0.42) without publication bias as assessed by Egger's test (P=0.46) or Begg and Mazumdar's test (P=0.31).

Hepatocellular carcinoma. The OR of rs738409 CG genotype compared with CC genotype was 2.87 (1.61–5.10) on comparing HCC patients with healthy controls (**Figure 2d**). Data were homogeneous (I^2=35.43%, P=0.21). Similar OR for the rs738409 GG

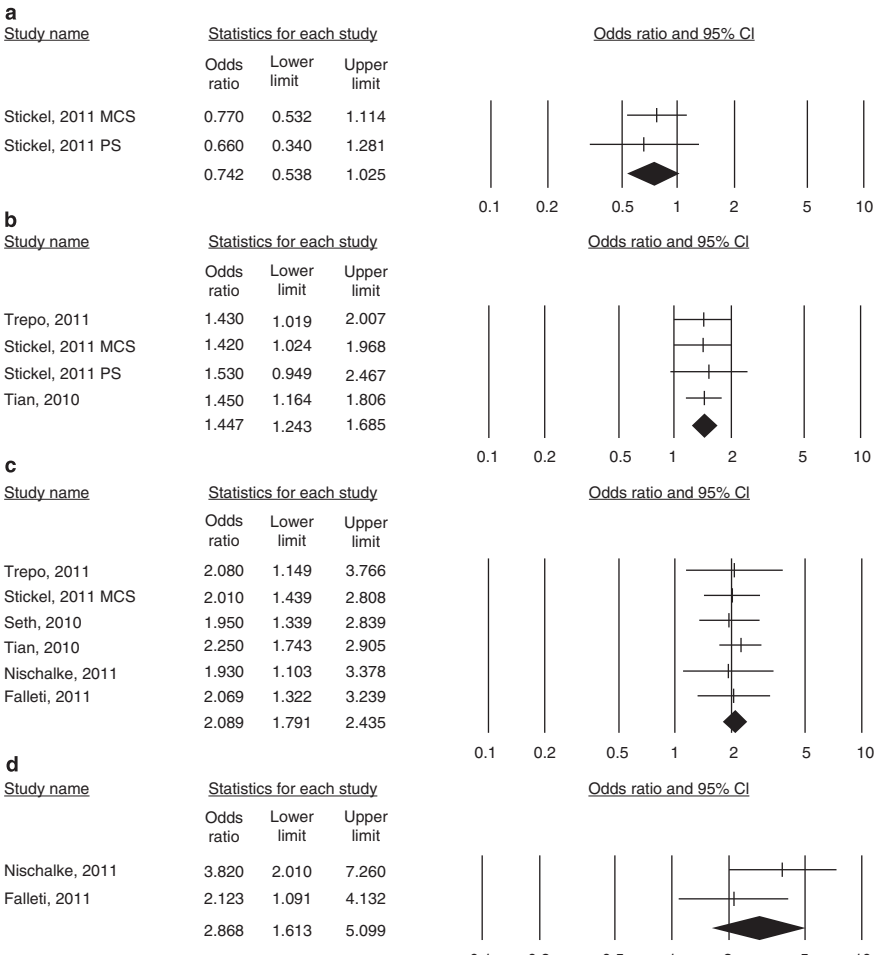

Figure 2. Forest plots showing the effect size with (95% confidence interval (CI)) on the pooled data for the association between PNPLA3 (patatin-like phospholipase domain protein 3) polymorphism (CG vs. CC) and alcoholic liver disease (ALD). (**a**) Fatty liver, (**b**) alcoholic liver injury, (**c**) alcoholic cirrhosis, and (**d**) hepatocellular carcinoma vs. disease-free individuals. The bottom line in the statistics for each study heading is the pooled effect size analyzed using the random effects model. The odds ratio (OR) of >1 denotes risk for the respective outcome or positive association and OR <1 indicates protective effect or negative association. The 95% CI not crossing 1 indicates a significant association. MCS, multicenter sample; PS, population-based sample.

genotype was 12.41 (6.99–22.03) as compared with controls with homogeneous data (I^2=0%, P=0.41; **Figure 3d**).

PNPLA3 polymorphism (rs738409) and severity of alcoholic liver disease

A total of 8 studies involving 3,711 ALD patients were pooled for these analyses (**Figure 4a–e**).

AC vs. AFL. The OR of CG and GG genotypes among AC patients compared with AFL was 2.62 (1.73–3.97) and 8.45 (2.52–28.37), respectively, in one study (35) (data not shown in the forest plots).

AC vs. ALI. The OR of rs738409 CG genotype compared with CC genotype was 1.98 (1.24–3.17; **Figure 4a**). There was significant heterogeneity among the studies (I^2=67.04, P=0.048). No publication bias was detected by Egger's test (P=0.11) or Begg and Mazumdar's test (P=0.12). Sensitivity analysis after excluding the study with the Mestizio subjects (32) showed similar effect 2.59 (1.69–3.98) without any heterogeneity (I^2=0, P=0.66). The OR (95% CI) of developing AC compared with ALI was 3.86 (1.18–12.60) in the presence of rs738409 GG compared with CC genotype (**Figure 4b**). The data were heterogeneous (I^2=76%, P=0.039). Differences on proportion of patients with AC in the two studies included in this analysis, ~80% in one study (33) and only 22% in the other study (40), probably explains this heterogeneity. Publication bias could not be done with only two studies in this analysis. In addition, OR of rs738409 GG genotype compared with CG genotype was 2.90 (1.53–5.49) in one study (40).

HCC vs. AC. The OR of rs738409 CG compared with CC genotype was 1.43 (0.76–2.72) (**Figure 4c**). The data were homogeneous (I^2=33, P=0.22). Similarly, OR of rs738409 GG genotype vs. CC genotype was 2.81 (1.57–5.01; **Figure 4d**). However, there was significant heterogeneity for this analysis (I^2=70.38%, P=0.018). No publication bias was detected by Egger's test (P=0.36) or Begg and Mazumdar's test (P=0.0.490). Sensitivity analysis performed after excluding the study with the lowest (39) and highest OR showed similar effect: 2.91 (1.44–5.86) with no

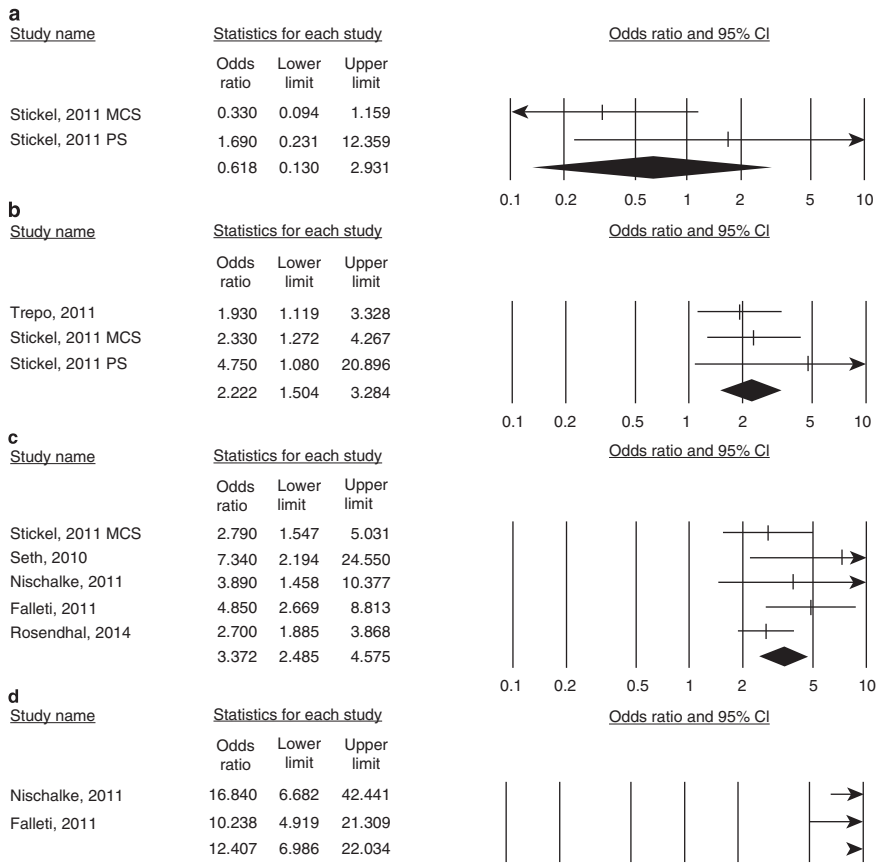

Figure 3. Forest plots showing the effect size with odds ratio (95% confidence interval (CI)) on the pooled data for the association between PNPLA3 (patatin-like phospholipase domain protein 3) polymorphism (GG vs. CC) and alcoholic liver disease (ALD). (**a**) Fatty liver, (**b**) alcoholic liver injury, (**c**) alcoholic cirrhosis, and (**d**) hepatocellular carcinoma vs. disease-free individuals. The bottom line in the statistics for each study heading is the pooled effect size analyzed using the random effects model. The odds ratio (OR) of >1 denotes risk for the respective outcome or positive association and OR <1 indicates protective effect or negative association. The 95% CI not crossing 1 indicates a significant association. MCS, multicenter sample; PS, population-based sample.

heterogeneity (I^2=20.37%, P=0.26). OR of rs738409 GG vs. CG genotype was 2.10 (1.22–3.63) with homogeneous data (I^2=0%, P=0.912; **Figure 4e**).

Subgroup analyses

Ethnicity analysis. After excluding the study recruiting Mestizo population (32), pooled effect size remained in the same direction on the outcomes including this study (**Figures 2b,c and 4a**)—CG genotype compared with CC genotype for ALI vs. healthy controls: 1.45 (1.17–1.78); CG genotype compared with CC genotype for AC vs. healthy controls: 1.99 (1.61–2.46); and CG genotype compared with CC genotype for AC vs. ALI: 2.59 (1.69–3.98).

Analysis of good-quality studies: on analyzing five good-quality studies, data remain unchanged as reported in **Supplementary Table S2**.

Individual participant data analyses. Individual participant data were obtained on 2,033 ALD patients (734 ALI; 1,153 AC without HCC; and 146 AC with HCC) from 5 studies (**Table 2**). Data on 487 healthy controls (drinkers with normal blood tests)

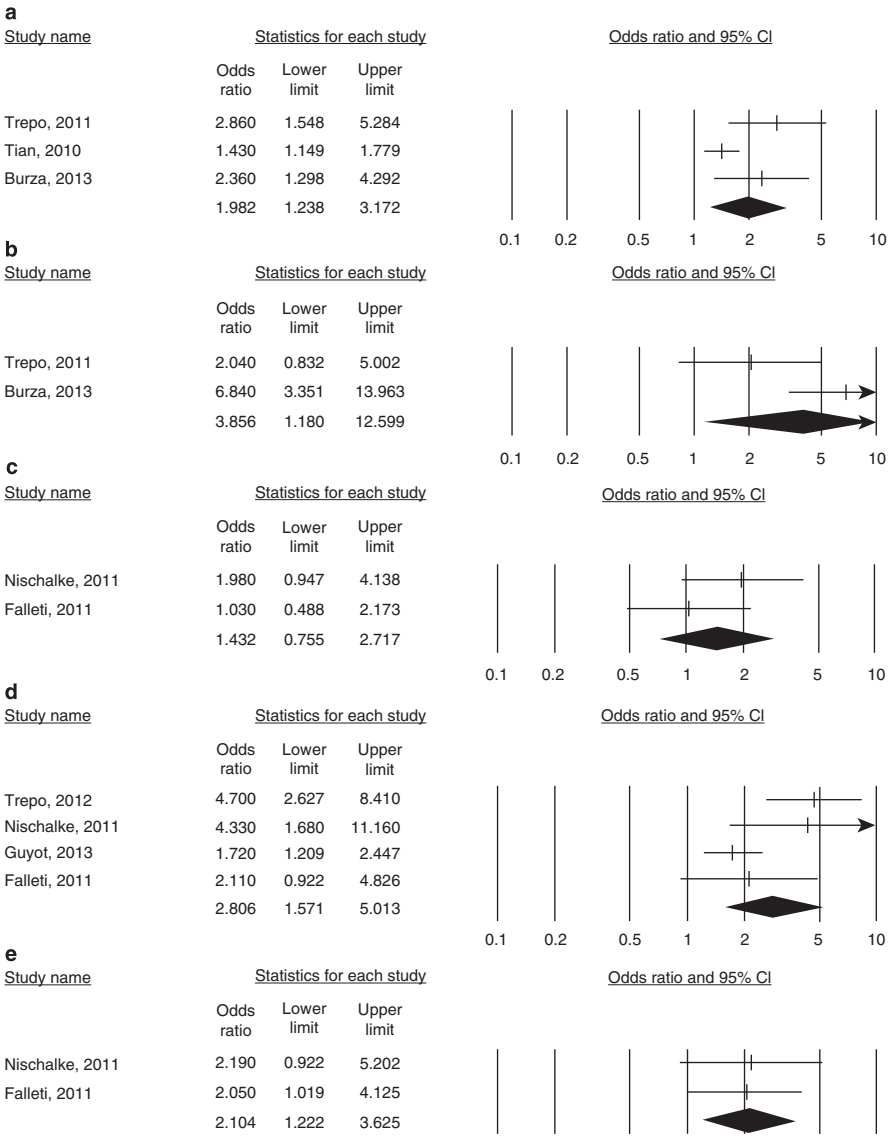

Figure 4. Forest plots showing the effect size with odds ratio (95% confidence interval (CI)) on the pooled data for the association of PNPLA3 (patatin-like phospholipase domain protein 3) polymorphism among alcoholic liver disease (ALD) spectrum patients. (**a**) Alcoholic cirrhosis compared with alcoholic liver injury (CG vs. CC), (**b**) alcoholic cirrhosis compared with alcoholic liver injury (GG vs. CC), (**c**) hepatocellular carcinoma compared with alcoholic cirrhosis (CG vs. CC), (**d**) hepatocellular carcinoma compared with alcoholic cirrhosis (GG vs. CC), and (**e**) hepatocellular carcinoma compared with alcoholic cirrhosis (GG vs. CG). The bottom line in the statistics for each study heading is the pooled effect size analyzed using the random effects model. The odds ratio (OR) of >1 denotes risk for the respective outcome or positive association and OR <1 indicates protective effect or negative association. The 95% CI not crossing 1 indicates a significant association.

Table 2. Baseline characteristics and genotype frequencies on individual participant data from 5 studies involving 2,520 patients and 487 healthy controls

Study	*N*	Age	*N* (%) M	BMI	CC:CG:GG
Burza *et al.* (40)	300 ALD without AC	44.6±11	230 (77)	25.5±4.4	145:126:29
	84 ALD with AC	54.2±11*	63 (75)	26.7±4.7	19:39:26
Nischalke *et al.* (34)	80 AC without HCC	57±10.2	69 (86)	26.7±6.8*	32:38:10
	80 AC with HCC	56.7±10.1	69 (86)	24.9±3.9	17:40:23
Falleti *et al.* (36)	132 AC without HCC	54±9.4	82 (62)	24.4±4.1	38:64:30
	66 AC with HCC	60.6±8.4*	62 (94)*	27.3±3.9*	15:26:25
Seth *et al.* (cases) (18)	375 ALD with AC	49±9.7*	271 (72)	NA	173:170:32
Seth *et al.* (controls[a]) (18)	182	43.4±9.8	133 (73)	NA	119:60:3
Tian *et al.* (cases) (32)	434 ALD without AC	41±12.4	367 (85)	NA	63:185:186
	482 ALD with AC	52±11.6	411 (85)	NA	39:179:261
Tian *et al.* (controls[a]) (32)	305	39±12.7	257 (84)	NA	59:155:89

AC, alcoholic cirrhosis; ALD, alcoholic liver disease; BMI, body mass index; HCC, hepatocellular carcinoma; NA, not available.
*$P<0.05$.
[a]Drinkers with normal blood tests.

were obtained from two studies (**Table 2**). Pooled data comparing the OR (for AC vs. healthy controls) of CG vs. CC, GG vs. CC, and GG vs. CG were 1.84 (1.47–2.31), 4.40 (2.98–6.50), and 2.40 (1.62–3.55), respectively. Similar OR for HCC vs. healthy controls was 2.96 (1.88–4.66), 13.30 (7.61–23.27), and 4.50 (2.71–7.46), respectively.

Generalized linear mixed model analysis on the individual participant data showed unadjusted OR for AC vs. ALI comparing CG with CC, GG with CC, and GG with CG to be 1.9 (1.3–2.9), 3.0 (2.0–4.6), and 1.6 (1.1–2.3), respectively. After adding age and gender into the model in a stepwise manner, the significance of the OR remained unchanged and in the same direction (**Table 3**). Participant age but not the gender independently predicted development of cirrhosis (**Table 3**). Similar unadjusted OR on the development of HCC among AC patients was 1.5 (0.5–2.6), 2.9 (0.8–11.1), and 2.1 (0.1–68.1), respectively. The data for HCC remained significant with wide confidence interval probably because of small sample size, with data on HCC available on only 358 patients from two studies.

DISCUSSION

The current meta-analysis demonstrates several important findings on the association of the PNPLA3 gene polymorphisms with ALD. Compared with controls, prevalence of PNPLA3 polymorphisms was higher among patients with ALI, AC, and HCC in AC. Furthermore, the prevalence of these polymorphisms was higher among patients with AC compared with those with ALI or AFL and among AC patients with HCC compared with those without HCC. Data on prevalence of PNPLA3 polymorphisms were similar when drinkers with FL were compared with controls. Individual participant data also confirmed these observations.

The disease spectrum in both alcoholic and nonalcoholic fatty liver disease is similar and starts with fatty liver change followed by inflammation, fibrosis, and finally cirrhosis. Hepatic steatosis is seen in 90% of heavy alcohol drinkers and is usually macrovesicular (41–43). Fatty infiltration develops rapidly within 2 weeks and resolves with abstinence (44,45). The similar polymorphism prevalence in drinkers with AFL and controls, as found in the current meta-analysis, blends well with the fact that AFL is a universal phenomenon in alcoholics and is reversible on abstinence. However, this is not true for ALI, AC, and HCC.

Stickel *et al.* (7) have reviewed previous studies on alcoholic dehydrogenase gene polymorphism in alcoholic patients and showed that results were inconsistent. The available data do not provide clear evidence that demonstrates a contribution of alcohol metabolism (alcohol dehydrogenase), antioxidant enzymes (CYP2E1, GSTM1, MnSOD), or cytokines (interleukin and tumor necrosis factors) genotype polymorphisms to the development of ALD (7). PNPLA3 is the first genetic marker that was confirmed by repeated studies to predispose to ALD and is associated with worse severity as we showed in the current meta-analysis.

Two recent meta-analyses have shown association of PNPLA3 with advanced fibrosis (46,47). Heterogeneous data due to inclusion of patients with various liver disease etiologies was a limitation in one study (46). Furthermore, this study included only three studies on alcoholic liver disease (29,33,35) in comparison with five studies in our current analysis (18,32–35). Our meta-analysis provides pooled data comparing ALD patients with healthy controls on the association of PNPLA3 polymorphisms with HCC in two studies (34,36) and AC in four studies (34,36,38,39). Furthermore, we also examine the association of the remaining spectrum of ALD and PNPLA3 polymorphisms including FL and ALI. Our study findings on the risk of fibrosis and AC are similar to the

Table 3. Generalized linear regression model for odds of cirrhosis and of HCC from individual participant data

	Alcoholic cirrhosis vs. alcoholic liver injury (2,033 patients in 5 studies)					HCC vs. no HCC in alcoholic cirrhosis (358 patients in 2 studies)				
	CG vs. CC	GG vs. CC	GG vs. CG	Age	M vs. F	CG vs. CC	GG vs. CC	GG vs. CG	Age	M vs. F
Model 1	1.9 (1.3–2.9)	3.0 (2.0–4.6)	1.6 (1.1–2.3)			1.5 (0.5–2.6)	2.9 (0.8–11.1	2.1 (0.1–68.1)		
Model 2	1.7 (1.1–2.7)	2.8 (1.7–4.4)	1.7 (1.1–2.5)	1.07 (1.06–1.08)		1.5 (0.5–5.0)	3.1 (0.8–12.1)	2.1 (0.1–73.3)	1.04 (1.02–1.07)	
Model 3	1.7 (1.1–2.7)	2.8 (1.7–4.4)	1.7 (1.1–2.5)	1.07 (1.06–1.08)	1.1 (0.8–1.6)	1.5 (0.4–4.9)	2.9 (0.7–11.7)	2.1 (0.1–77.3)	1.04 (1.02–1.06)	2.9 (0.1–174.2)

F, female; HCC, hepatocellular carcinoma; M, male.
Model 1: unadjusted for age and gender; model 2: adjusted for age; and model 3: adjusted for age and gender.

second meta-analysis except for lower OR for the GG genotype. This may be as this analysis included three citations published as abstracts (29–31) that were not included in our current analysis. In another meta-analysis, PNPLA3 polymorphisms among cirrhotics have been associated with HCC (48). Our meta-analysis in addition to confirming the findings from this study also examines the HCC risk in alcoholics compared with healthy controls. Furthermore, we also examined the association of PNPLA3 polymorphisms with the other spectrum of ALD.

Our analysis may be limited by the possibility of publication bias. In order to minimize this possibility and subsequently overestimation of the true effect size because of negative study identification failure (49), we combined searches from PubMed/Medline, Embase, and Cochrane with manual searches. Although we used procedures in agreement with current guidelines, we cannot formally rule out the possibility that we missed studies that were not accessible (49). Another limitation of this meta-analysis is the inclusion of case–control studies in which the potential for biases (e.g., selection and reporting) is higher when compared with randomized trials and they are more inherent to confounding factors. In the current analysis, the rs738409 genotype frequencies in the control group that followed the HWE is a strength of this study as sampling bias or coincidental gene association because of population stratification could be excluded (39), but some authors have shown that HWE testing is not a reliable way of detecting genotyping error and can certainly not exclude population stratification (21,50). Finally, we were not able to retrieve all the individual participant data (only 46% of all individual participant data were obtained) from the included studies, especially the largest one by Stickel *et al.* (35) (1,419 patients). Therefore, we used the individual participant data for only the subgroup analyses and not our main outcome analyses.

In conclusion, the PNPLA3 rs738409 polymorphism is associated with increased risk for the entire spectrum of ALD among drinkers, and increased propensity for AC and HCC in ALI patients. Studies are needed to clarify the association of PNPLA3 and steatosis in alcohol drinkers. The *PNPLA3* gene may potentially be a therapeutic target in ALD and in the selection of donor organs for transplantation of patients with ALD.

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CONFLICT OF INTEREST

Guarantor of the article: Ashwani K. Singal, MD, MS.

Specific author contributions: H.S.: study design, data collection, data analysis and interpretation, and writing the paper; E.R: data collection and writing the paper; A.E.: performing the HWE analysis and reviewing the manuscript; D.S., H.D.N, E.F., M.A.B., J.L., S.R., A.M., S.G.C., P.T., S.U., A.D., and C.P.D.: provided individual participant data and reviewed the paper; A.K.S.: study design, data collection, data analysis and interpretation, reviewing the paper, and study supervision.

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Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ The *patatin-like phospholipase domain protein 3* (*PNPLA3*) gene polymorphism confers risk of steatosis.
- ✓ This is also associated with cirrhosis and hepatocellular carcinoma (HCC) in alcoholic liver disease (ALD).

WHAT IS NEW HERE

- ✓ PNPLA3 polymorphism increases risk for entire spectrum of ALD.
- ✓ The magnitude of this association increases in parallel to increasing severity of liver disease from alcoholic liver injury to alcoholic cirrhosis to hepatocellular carcinoma.
- ✓ Data on association of PNPLA3 polymorphisms with alcoholic fatty liver are inconsistent.
- ✓ Data remained unchanged for separate analyses (i) on individual patient data from five studies and (ii) on five good-quality studies.

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