PNPLA3 polymorphism increases risk for and severity of chronic hepatitis C liver disease

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Abstract

AIM
To examine the association of PNPLA3 polymorphisms in chronic hepatitis C patients and development of liver disease spectrum.

METHODS
Literature was searched systematically from PubMed/ MEDLINE, EMBASE, and Cochrane search engines for full-length articles written in English that examined PNPLA3 polymorphism in chronic hepatitis C (CHC) patients. Studies evaluating the association of PNPLA3 polymorphism spectrum (fatty liver, steatohepatitis, cirrhosis, and hepatocellular carcinoma) of CHC were included. Pooled data are reported as OR with 95%CI. Our study endpoint was the risk of the entire liver disease spectrum including: Steatosis/fatty liver, cirrhosis, and hepatocellular carcinoma in CHC patients with PNPLA3 polymorphisms.

RESULTS
Of 380 studies identified, a total of 53 studies were included for full-text review. Nineteen on chronic he-
patitis C were eligible for analysis. Pooled ORs for rs738409 GG compared to CC and CG among patients with fatty liver was 2.124 (95%CI: 1.719-2.583). ORs among advanced fibrosis/cirrhosis were 1.762 (95%CI: 1.258-2.468). Similar odds ratios among hepatocellular carcinoma patients were 2.002 (95%CI: 1.519-2.639). Pooled ORs for rs738409 GG and CG compared to CC among patients with fatty liver were 1.750 (95%CI: 1.542-1.986). Pooled ORs for advanced fibrosis/cirrhosis patients were 1.613 (95%CI: 1.211-2.147). All analyses were homogeneous and without publication bias except one. The associations were maintained after adjusting for publication bias and heterogeneity.

CONCLUSION
PNPLA3 polymorphisms have strong association with increased risk and severity of the liver disease spectrum in CHC patients.

Key words: PNPLA3 polymorphism; Cirrhosis; rs738409; Hepatitis C virus; Hepatocellular carcinoma

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Core tip: PNPLA3 polymorphisms (rs738409 CG and GG) are associated with increased risk of steatosis, advanced fibrosis, cirrhosis, and hepatocellular carcinoma in chronic hepatitis C patients.

INTRODUCTION
Hepatitis C virus (HCV) infection is one of the most important causes of chronic liver disease in the United States[1]. About 27% of cases of cirrhosis and 25% of hepatocellular carcinoma (HCC) worldwide are secondary to HCV infection[2]. Multiple genetic factors identified within the past few years have been shown to be associated with the predisposition to chronic liver disease and the progression to cirrhosis and HCC[3,4]. The single nucleotide polymorphism (SNP) rs738409 C>G (isoleucine to methionine substitution at position 148, 1148M) in the PNPLA3 gene has been strongly linked to progression of liver disease in multiple studies, and this association was confirmed in meta-analyses of the spectrum of alcoholic liver disease (ALD)[5] as well as non-alcoholic fatty liver disease (NAFLD)[6,7].

The frequency of hepatic steatosis varies with ethnicity where it was reported as 45%, 33% and 24% in Hispanics, Whites and Blacks respectively[10]. At the same time the frequencies of the PNPLA3 rs738409[G] allele were 0.49, 0.23, and 0.17 in Hispanics, European Americans and African Americans[11]. In addition, the prevalence of the GG genotype in different races in fact correlates with the rate of NAFLD in each respective population, with nearly half of all Hispanics possessing the allele, who in turn are also most likely to have NAFLD. The same is true of the inverse, with less than one quarter of African Americans having the PNPLA3 rs738409[G] allele, and they are least likely to develop NAFLD compared to Hispanics and Caucasians[11,12].

Given that the association between PNPLA3 polymorphism and liver disease spectrum in chronic hepatitis C (CHC) patients has not been consistent, especially for HCC[12,13] and cirrhosis[14,15], we performed this meta-analysis to further examine the association of PNPLA3 polymorphisms with the predisposition to the entire spectrum of liver disease among patients with CHC.

MATERIALS AND METHODS

Literature search
Utilizing the Meta-analysis of Observational Studies in Epidemiology guidelines, literature was searched from PubMed/Medline, Embase, and Cochrane search engines for full-length articles written in English that examined PNPLA3 polymorphism in CHC patients[16]. The initial medical subject headings search terms were: “Hepatitis C, Chronic” and “adiponutrin, human”. The search was then expanded using the terms: “rs738409” and “patatin-like phospholipase domain-containing 3 protein”. All databases were searched from their inception date through March 2015. Meeting abstracts from major gastroenterology conferences over the past 3 years were also searched to identify studies that were potentially overlooked in our database search. Articles were selected for full text review based on title and abstract.

Study selection
Three independent investigators (Masadeh M, Al Hanayneh M and Maslonka M) manually search the retrieved publications to ensure all appropriate articles were discovered and included. Two authors (HS and AKS) reviewed articles in question for possible inclusion. The following inclusion criteria were set for inclusion in this meta-analysis: (1) studies published as full-length articles which reported association of the PNPLA3 variant (rs738409 C>G) among CHC patients; and (2) studies which analyzed patients with other liver diseases and reported separate data on PNPLA3 polymorphisms for HC.

The following exclusion criteria were set: (1) studies without available gene frequency data for analysis; and (2) studies including subjects with other liver diseases without separate data on CHC patients.

Definitions
HCV infection was diagnosed with both positive serum
anti-HCV antibodies and serum HCV ribonucleic acid (RNA). The disease spectrum was defined as the following: steatosis = fatty liver (FL) on imaging without evidence of cirrhosis or HCC; advanced fibrosis and cirrhosis = biopsy-proven bridging fibrosis, or clinical evaluation supported by hematological, biochemical, and radiologic imaging findings; and HCC = diagnostic imaging findings on triple phase magnetic resonance imaging or computed tomography, or using histological confirmation from liver tissue. Healthy controls were defined as subjects without liver disease and without HCV infection.

**Data extraction**

After determining eligibility for inclusion, two reviewers (Masadeh M and Al Hanayneh M) independently extracted data for (1) study characteristics: Author and year of publication, and study design (population based or not, using controls or not); (2) study population: Liver disease spectrum and sample size; (3) frequencies of PNPLA3 polymorphism genotypes (rs738409 CC, CG, and GG); and (4) OR: For association of PNPLA3 polymorphism and the spectrum of liver disease and for severity of liver disease. Any discrepancies amongst the reviewers were resolved by jointly reviewing the study in question. Among studies comparing diseased population with healthy controls, similar data were also extracted on healthy controls.

**Endpoints and outcomes**

Our study endpoint was the risk of the entire liver disease spectrum including: Steatosis/fatty liver, cirrhosis, and HCC in CHC patients with PNPLA3 polymorphisms.

**Quality assessment**

The quality of included studies was assessed independently by three authors (Masadeh M, Al Hanayneh M and Maslonka M) using the Newcastle–Ottawa Quality Assessment Scale for case-control studies\(^{[17]}\). This scale has one instrument for assessing case-control studies and another one for cohort studies. Each of these instruments includes measures of quality in selection, comparability, and exposure domains. While one point is granted for each of the areas measured within the selection and exposure domains, a maximum of two points can be assigned within the comparability domain with highest possible total score of nine. Previous studies have reported that a score of seven or greater denotes a high-quality study\(^{[18]}\). Any discrepancies between the three coauthors were addressed by a joint reevaluation of the original article.

**Statistical analysis**

The strength of the association between rs738409 and CHC liver disease spectrum prevalence was expressed by OR and their corresponding 95% CI. The Random effects model was used for analyzing pooled data for all the analyses\(^{[19]}\). Heterogeneity was measured using \(I^2\) statistics for inter-study variance, with the \(\chi^2\) test used for statistical analysis. Heterogeneity was defined with \(I^2 \geq 50\%\) or \(\chi^2 P < 0.10\)^\(^{[20]}\). At least two studies are needed to examine and report heterogeneity. To examine the heterogeneous data and source of heterogeneity, sensitivity analyses were performed in a stepwise fashion for (1) study quality; and (2) excluding studies with the highest and lowest OR. Publication bias was assessed using Egger regression and the Begg–Mazumdar rank correlation tests\(^{[21–23]}\). Egger test is a regression method checking for association between effect sizes and standard error and uses actual effect size for each study\(^{[23]}\). Begg–Mazumdar is a rank correlation test examining the potential association between effect estimates (taken as a rank and not exact effect size) and sampling variance (or standard error)\(^{[22]}\). At least three studies are needed for examining and reporting publication bias. For analyses with publication bias, the analyses were repeated either by performing sensitivity analysis or using the Duval and Tweedie Trim and Fill method, a nonparametric (rank-based) data augmentation technique\(^{[24]}\). The method can be used to estimate the number of studies missing from a meta-analysis resulting in a skew of the data due to the suppression of the most extreme results on one side of the funnel plot. The method then amplifies the observed data so that the funnel plot is more symmetric and re-computes the summary estimate based on the comprehensive data. The method should not be regarded as a way of yielding a more “valid” estimate of the overall effect or outcome, but as a means of examining the sensitivity of the results to one particular selection mechanism\(^{[25]}\). All statistical analyses were performed using R (Foundation for Statistical Computing) utilizing the metaphor package, or Comprehensive Meta-analysis (Biostat, Englewood, NJ). Singal AK from University of Alabama, Birmingham, reviewed the statistical methods of this study.

**RESULTS**

**Baseline study characteristics**

A total of 380 citations were retrieved on initial search. After reviewing article titles and abstracts, a total of 53 studies were included for full-text review (Figure 1). Of these, twenty articles were excluded because they did not have sufficient data for our analysis. Nine studies were excluded for including subjects with liver disease not caused by HCV\(^{[11,26–33]}\), and three studies were excluded for including subjects co-infected with human immunodeficiency virus and/or hepatitis B virus infection\(^{[34–36]}\). One duplicate study\(^{[27]}\) and one study that looked at treatment response\(^{[38]}\) were excluded. Nineteen studies evaluating 9093 patients (57.6% males, mean body mass index 25.1 kg/m\(^2\)) on association of PNPLA3 polymorphisms in CHC\(^{[12,15,39–53]}\) were included for the analysis. Data on study design, ethnicity and genotype frequency are summarized in Table 1.
Table 1 Baseline characteristics of patients from studies included in the analysis

| Ref. | Study design | Controls (n) | Ethnicity | M% | Mean age | Mean BMI | rs 738409 genotype count (CC:CG:GG) | FL | Hepatitis | Cirrhosis | HCC |
|------|--------------|--------------|-----------|-----|----------|----------|---------------------------------|----|-----------|----------|-----|
| Cai et al[40](2011) | R | - | 626 | C | 61.8 | 44.7 | 23.7 | 62.288 | - | - | - |
| Valenti et al[41](2011) | R | 179 | 819 | C + NA | 56.4 | 57.4 | 24.8 | 269:219:73 | - | 119:172:229 | 17:21:12 |
| Trépo et al[42](2011) | R | - | 537 | C | 63 | 49.4 | 25.5 | 136:106:31 | - | 108:85:23 | - |
| Corradini et al[43](2011) | P[5] | - | 221 | C | 63 | 58 | - | - | - | - | - |
| Ninchakule et al[44](2011) | P | 190 | 162 | C | 37 | 56 | 28.4 | - | - | 45:31:05 | 40:33:08 |
| Valenti et al[45](2012) | P | - | 567 | NS | - | - | - | - | - | - | - |
| Valenti et al[46](2012) | P[6] | - | 602 | NS | 51 | 51 | 25.1 | 364:42 | - | 158:21 | - |
| Guyot et al[47](2013) | P | - | 253 | NS | 54.2 | 56.7 | 27.3 | - | - | 140:75:38 | 54:26:13 |
| Ezikouki et al[48](2013) | P | 132 | 230 | NA | 45.2 | 63.63 | - | - | 47:71:11 | - | 43:35:23 |
| Stättermayer et al[49](2014) | R | - | 478 | NS | 65.7 | 44.9 | 25.6 | 190:23 | - | 101:57 | - |
| Amperso et al[50](2014) | P | - | 474 | M | 64.8 | 43.4 | 25.7 | 94:126 | - | - | - |
| Sato et al[51](2014) | R | - | 358 | A | 55.9 | 69.76 | - | - | 41:26 | - | 112:37 | 100:176:82 |
| Yasui et al[52](2014) | P | - | 276 | A | 40.6 | 58.2 | 23 | 23:75:39 | 45:66:38 | 20:31:21 |
| Petta et al[53](2015) | P | - | 434 | C | 53.9 | 51.7 | - | - | 40:35:12 | 71:36:13 | - |
| Nakaoa et al[54](2015) | P | - | 231 | A | 44.6 | 62.9 | 22.5 | - | - | 90:25 | 12:22 | 14 |
| Tamaki et al[55](2015) | R | - | 176 | A | 39.8 | 56.5 | 22.9 | - | - | 52:87:37 | - |
| Huang et al[56](2015) | R | - | 1018 | A | 56.6 | 51.8 | 24.9 | 175:205:75 | - | - | - |
| Petta et al[57](2016) | P | - | 694 | C | 53 | 54 | 26.5 | 151:151:45 | - | - | - |
| Ali et al[58](2016) | P | - | 937 | M | 70.1 | 49.5 | - | - | - | - | 172:21 |
| Summary | 501 | 9093 | 57.6 | 52.7 | 25.1 | - | - | - | - | - |

1 C allele: G allele; ‘CC + CG: GG; ‘CC: CC + GG; ‘GG: genotype counts were reported as ratios (CC wild genotype: CG heterozygote genotype: GG homozygote genotype) unless indicated by star(s); ‘Calculated from percentages in the original article; ‘Population based studies. A: Asian; BME: Body mass index; C: Caucasian; FL: Fatty liver; HCC: Hepatocellular carcinoma; M: Mixed (Caucasians and non-Caucasians genotype) unless indicated by star(s); ‘NS: Not specified in the original manuscripts (although all 4 studies included European referral centers only).

380 citations identified on intial search and reviewed for study inclusion
327 citations excluded
242 not related to HCV
48 non-human studies
37 reviews and meta-analysis
53 studies for full text review
34 studies excluded
22 with no sufficient data (no ORs or CIs), duplicate or treatment response 9 etiology other than HCV 3 patients co-infected with HIV/HBV
19 studies included in the analysis

Figure 1 Attrition on literature search and study inclusion. HCV: Hepatitis C virus; HBV: Hepatitis B virus; HIV: Human immunodeficiency virus.

Study quality assessment
Based on the Newcastle-Ottawa Scale, nine studies were of “high quality” with a score of seven or more, and the remaining ten studies had a score of six or below (Table 2).

Association between PNPLA3 polymorphism and liver disease spectrum (GG vs CG and CC analysis)

Association of PNPLA3 polymorphisms with FL in CHC patients: Among six studies on 3310 patients, the pooled OR for rs738409 GG genotype compared to CC and GG genotypes in CHC was 2.214 (95%CI: 1.719-2.853) (Figure 2A). The data was homogeneous ($I^2 = 9.4\%$, $P = 0.36$) and without publication bias as assessed by Egger test ($P = 0.08$) and Begg-Mazumdar test ($P = 0.14$).

Association of PNPLA3 polymorphisms with advanced fibrosis and cirrhosis in CHC patients: Among seven studies on 3377 patients, the pooled OR for rs738409 GG genotype compared to CC and CG genotypes in CHC was 1.762 (CI: 1.258-2.469) (Figure 2B). The data was heterogeneous ($I^2 = 65.9\%$, $P = 0.08$), with evidence of publication bias as assessed by Begg-Mazumdar test ($P = 0.036$) and tendency for publication bias as assessed by Egger test ($P = 0.059$). Sensitivity analysis after excluding studies with lowest[14] and highest[40] OR revealed similar effect size: 1.82 (95%CI: 1.41-2.34) with $I^2 = 18.8\%$, $P = 0.30$. Additionally, when Dual and Tweedie trim and fall test was used to assess publication bias, 3 studies were trimmed with no change in effect size (OR = 1.39, 95%CI: 1.01-1.92).

Association of PNPLA3 polymorphisms and HCC in CHC patients: Among seven studies on 2274 patients, the pooled OR for rs738409 GG genotype compared to CC and CG genotypes in CHC was 2.002 (95%CI: 1.392-2.853) (Figure 2A). The data was homogeneous ($I^2 = 9.4\%$, $P = 0.36$) and without publication bias as assessed by Egger test ($P = 0.08$) and Begg-Mazumdar test ($P = 0.14$).
Association between PNPLA3 polymorphism and liver disease spectrum (GG and CG vs CC analysis)

Association of PNPLA3 polymorphisms with FL in CHC patients: Among three studies on 1794 patients, the pooled OR for rs738409 GG and CG genotypes compared to CC genotype in CHC was 1.750 (95%CI: 1.542-1.986) (Figure 3A). The data was homogeneous ($I^2 = 0.0\%$, $P = 0.84$), without publication bias as assessed by Egger test ($P = 0.57$) and Begg-Mazumdar test ($P = 0.99$).

Association of PNPLA3 polymorphisms with advanced fibrosis, and cirrhosis in CHC patients: Among three studies on 2131 patients, the pooled OR
We have previously described that PNPLA3 polymorphism is a modifier in the natural history of ALD[5] and NAFLD[6-8]. In this meta-analysis, we found a clear association between PNPLA3 polymorphisms and the entire spectrum (steatosis/fatty liver, cirrhosis, and HCC) of liver disease in CHC patients.

It was previously reported that PNPLA3 polymorphisms were an independent predictor of more rapid fibrosis progression in patients with chronic hepatitis C[50]. The mechanism whereby rs738409 influences the development of fatty liver likely involves a decreased ability of the 148M PNPLA3 variant to regulate hepatic lipid metabolism[59]. It is not known whether the rs738409 SNP influences the steatogenic effect of HCV and the progression of CHC. However, if steatosis causes fibrosis progression in CHC, then it may be assumed the rs738409 SNP should also be associated with advanced fibrosis and HCC[60].

Like any other meta-analysis, our study had to face the possibility of publication bias. In order to minimize this possibility, and the subsequent overestimation of the true effect size due to negative study identification failure[55], 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 overlooked studies that were not accessible[55].

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 to randomized trials, and they are more inherent to confounding factors. In contrary to the previous meta-analysis on PNPLA3 polymorphisms in alcoholic and non-alcoholic liver diseases that compared different genotypes[6-8], our current analysis used the recessive model when comparing GG genotype vs CC and CG genotypes, and the dominant model when comparing GG and CG genotypes related to the CC genotype. Finally, no pooled data were provided on steatohepatitis in chronic HCV patients as only one study had reported such an association[14], while the studies by Ezzikouri et al[51] and Yasui et al[40] either did not have biopsies performed or reported "necroinflammatory changes". Lack of standard definition amongst these studies prevented pooling them together.

The PNPLA3 GG genotype was negatively associated with sustained virological response and early viral kinetics in patients receiving peginterferon and ribavirin[15]. Also, in patients with chronic hepatitis C who failed to achieve sustained virologic response following interferon-based therapy, IL28B and PNPLA3 were independent predictors of rapid fibrosis progression[50]. Tamaki et al[50] developed a fibrosis progression-score by combining IL28B and PNPLA3 genotypes and ALT values, which stratified patients into low, intermediate, and high-risk groups for fibrosis progression. However, this fibrosis progression score needs external validation. In the era of direct-acting antiviral therapy, the question that remains unanswered is whether or not PNPLA3 polymorphisms identify high-risk CHC patients that are responsive to new treatment regimens. In summary, this meta-analysis provides strong evidence for the association of PNPLA3 polymorphisms and the spectrum of liver disease in patients with CHC, beginning with fatty liver disease and extending as far as cirrhosis and even HCC in patients with CHC. Further studies on treatment response are needed in this group of patients who carry a higher risk for more rapidly progressive liver disease.

### COMMENTS

**Background**

Hepatitis C virus (HCV) infection is one of the most important causes of chronic liver disease in the United States. About 27% of cases of cirrhosis and 25% of hepatocellular carcinoma (HCC) worldwide are secondary to HCV infection.

**Research frontiers**

Given that the association between PNPLA3 polymorphism and liver disease...
spectrum in chronic hepatitis C (CHC) patients has not been consistent, especially for HCC and cirrhosis, the authors performed this meta-analysis to further examine the association of PNPLA3 polymorphisms with the predisposition to the entire spectrum of liver disease among patients with CHC.

**Innovations and breakthroughs**

In this meta-analysis, they found a clear association between PNPLA3 polymorphisms and the entire spectrum (steatosis/fatty liver, cirrhosis, and HCC) of liver disease in CHC patients.

**Peer-review**

This manuscript is very well designed; the authors did a great effort in selecting the articles to be included in the meta-analysis with a proper quality scoring of selected articles.

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The document contains a review of studies on the interaction between different genetic factors and liver disease, with a focus on the role of PNPLA3 and other genetic markers. Key points include:

- Genetic factors are involved in the progression of liver disease.
- PNPLA3 polymorphism is associated with the risk of developing severe liver disease.
- Genetic variants such as PPARG, MTTP, and IL28B play a role in disease severity.
- The impact of viral genotype on disease progression is discussed.
- The association between PNPLA3 polymorphism and HCV-related liver cancer is highlighted.
- Genetic markers can be used to predict disease progression and identify patients who require urgent treatment.

These findings suggest that genetic testing could be a useful tool in managing liver disease, particularly in identifying patients at high risk for severe outcomes.
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