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# Association Between the *PNPLA3* (rs738409 C>G) Variant and Hepatocellular Carcinoma: Evidence From a Meta-Analysis of Individual Participant Data

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**The incidence of hepatocellular carcinoma (HCC) is increasing in Western countries. Although several clinical factors have been identified, many individuals never develop HCC, suggesting a genetic susceptibility. However, to date, only a few single-nucleotide polymorphisms have been reproducibly shown to be linked to HCC onset. A variant (rs738409 C>G, encoding for p.I148M) in the *PNPLA3* gene is associated with liver damage in chronic liver diseases. Interestingly, several studies have reported that the minor rs738409[G] allele is more represented in HCC cases in chronic hepatitis C (CHC) and alcoholic liver disease (ALD). However, a significant association with HCC related to CHC has not been consistently observed, and the strength of the association between rs738409 and HCC remains unclear. We performed a meta-analysis of individual participant data including 2,503 European patients with cirrhosis to assess the association between rs738409 and HCC, particularly in ALD and CHC. We found that rs738409 was strongly associated with overall HCC (odds ratio [OR] per G allele, additive model = 1.77; 95% confidence interval [CI]: 1.42-2.19; $P = 2.78 \times 10^{-7}$). This association was more pronounced in ALD (OR = 2.20; 95% CI: 1.80-2.67; $P = 4.71 \times 10^{-15}$) than in CHC patients (OR = 1.55; 95% CI: 1.03-2.34; $P = 3.52 \times 10^{-2}$). After adjustment for age, sex, and body mass index, the variant remained strongly associated with HCC. *Conclusion:* Overall, these results suggest that rs738409 exerts a marked influence on hepatocarcinogenesis in patients with cirrhosis of European descent and provide a strong argument for performing further mechanistic studies to better understand the role of *PNPLA3* in HCC development. (HEPATOLOGY 2014;59:2170-2177)**

*Abbreviations: AASLD, American Association for the Study of Liver Diseases; AIC, Akaike information criterion; ALD, alcoholic liver disease; BMI, body mass index; CHB, chronic hepatitis B; CHC, chronic hepatitis C; CI, confidence interval; HCC, hepatocellular carcinoma; HWE, Hardy-Weinberg equilibrium; IPD, individual participant data; NAFLD, nonalcoholic fatty liver disease; OR, odds ratio; PNPLA3, patatin-like phospholipase domain-containing 3; SNPs, single nucleotide polymorphisms.*

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The incidence of hepatocellular carcinoma (HCC) is increasing in Western countries, and HCC has the fastest-growing rate of cancer-related death in the United States.[1] The risk of HCC is highly variable among individuals, but most frequently (80%-90%) develops in the context of cirrhosis.[1] In addition, several other clinical variables, including age, sex, and body mass index (BMI), have also been associated with HCC prevalence.[1,2] However, many individuals with these clinical risk factors never develop HCC, suggesting a genetic susceptibility.[2] Candidate gene and genome-wide association studies have reported on associations between single-nucleotide polymorphisms (SNPs) and HCC, but most of these studies suffered from various methodological drawbacks and, to date, only a few variants have been reproducibly shown to be linked to hepatocarcinogenesis.[3] Recently, an SNP located in the *patatin-like phospholipase domain-containing 3* (*PNPLA3*) gene (rs738409 C>G, encoding for p.I148M), initially associated with steatosis and nonalcoholic fatty liver disease (NAFLD),[4] was further shown to influence susceptibility toward inflammation and progression to severe fibrosis in this etiology,[5] but also alcoholic liver disease (ALD)[6-9] and chronic hepatitis C (CHC).[10,11] Interestingly, several studies have reported that the minor rs738409[G] allele, associated with higher risk of liver damage, is significantly more represented in HCC cases in CHC,[11-13] ALD,[13-17] and severe obesity.[18] However, a significant association with HCC related to CHC has not been consistently observed. This could be related to the limited sample size of some of the cohorts.[14,15,17] By increasing statistical power and decreasing random errors, meta-analyses are recommended by the Human Genome Epidemiology Network to confirm the strength of an association.[19] More specifically, some investigators have emphasized that a meta-analysis of individual participant data (IPD) is a useful tool that helps to clarify the role of candidate genes in complex human diseases.[20] In addition, the use of IPD for meta-analysis is considered to be the most reliable method for allowing adjustment for confounding factors at the patient level.[21]

In the present study, we sought to investigate the association between rs738409[G] and the prevalence of HCC in patients with cirrhosis. Thus, we performed a meta-analysis of IPD including all studies that assessed the rs738409 genotype in patients with cirrhosis with and without HCC. In addition, in two sensitivity analyses, we tested the association between this variant and HCC specifically in patients with ALD- and CHC-related cirrhosis, the two etiologies most represented in available studies.

## Material and Methods

***Literature Search.*** Medline, Cancerlit, Embase and manual searches were combined.[22] Data abstraction was done independently by two investigators (E.T. and C.M.) using standardized data collection forms. Search terms were *PNPLA3*, rs738409, adiponutrin, and hepatocellular carcinoma. General reviews and references from published trials were used. The two investigators also extensively screened all abstracts presented in English at liver and gastroenterology congresses over the last 3 years.

***Criteria for Inclusion and Exclusion of Studies.*** Inclusion and exclusion criteria were defined before commencement of the literature search. For inclusion, a study had to (1) include patients with cirrhosis, (2) assess the presence of the variant (rs738409 C>G) in the *PNPLA3* gene in patients with and without HCC, and (3) have been published as a full-length article or presented as an abstract at an international congress using English as the official language (American Association for the Study of Liver Diseases [AASLD] Annual Meeting, European Association for the Study of the Liver Annual Meeting, Digestive Disease Week

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Annual Meeting, or United European Gastroenterology Week Annual Meeting) in 2010, 2011, or 2012.

We excluded (1) studies without information on the cirrhotic status of patients, not enabling comparison of rs738409 between patients with cirrhosis with (cases) and without HCC (controls)[18] and (2) studies that did not provide a control group with cirrhosis without HCC.[23,24] When several publications existed concerning the same study population,[15,25] only the most recent was taken into account[15] (Fig. 1).

***Study Characteristics.*** Seven studies involving 2,503 patients with cirrhosis were included in the analysis (Table 1). The underlying diseases causing cirrhosis varied among the selected studies. One study only included patients with alcoholic-related cirrhosis,[16] two were composed only of patients with CHC-related cirrhosis,[11,12] and two included patients with both etiologies.[14,17] In addition, three studies also included patients with chronic hepatitis B (CHB),[13-15] and two patients with NAFLD.[13,15] The diagnosis of HCC was performed according to the Barcelona criteria[26] in studies[13,15-17] and/or the AASLD practice guidelines.[11,12,14-16]

Genotyping of rs738409 was carried out using Taqman assay in five studies,[11-13,16,17] allele-specific oligonucleotides in one,[14] and restriction fragment length polymorphism in another.[15]

***Endpoints and Criteria for Combinability.*** Endpoints were defined before the beginning of the meta-analysis. Our primary endpoint was to investigate whether rs738409 could increase the risk of HCC among patients with cirrhosis. In addition, we aimed to evaluate whether the strength of the association between rs738409 and HCC onset was similar in ALD- and CHC-related cirrhosis. As a first step, an overall meta-analysis was performed. This analysis combined studies that included patients with cirrhosis, with and without HCC, regardless of etiology. In a second step, sensitivity analyses that separately considered patients with ALD- or CHC-related cirrhosis were performed. Sensitivity analyses were only performed when at least three studies could be analyzed.

***Data Extraction.*** All investigators of the selected studies were contacted directly and asked to provide their individual patient data, including the genotype distributions for rs738409 (CC, CG, and GG) according to the underlying diseases (ALD- or CHC-related cirrhosis or other causes). In addition, to ensure that the association between the SNP and HCC was studied in homogeneous populations of patients with ALD or CHC, investigators were asked for potential other coexisting causes of chronic liver disease, such as daily alcohol consumption. Concerning the sensitivity analysis in HCC related to CHC, patients who had significant alcohol consumption (>30 g/day) were excluded. Finally, to adjust for potential confounders, the individual database included information on mean age, proportion of males, and mean BMI for the study cohort. Discrepancies in data collection (in comparison with those already published) and interpretation were resolved by discussion, rereview of the studies, and

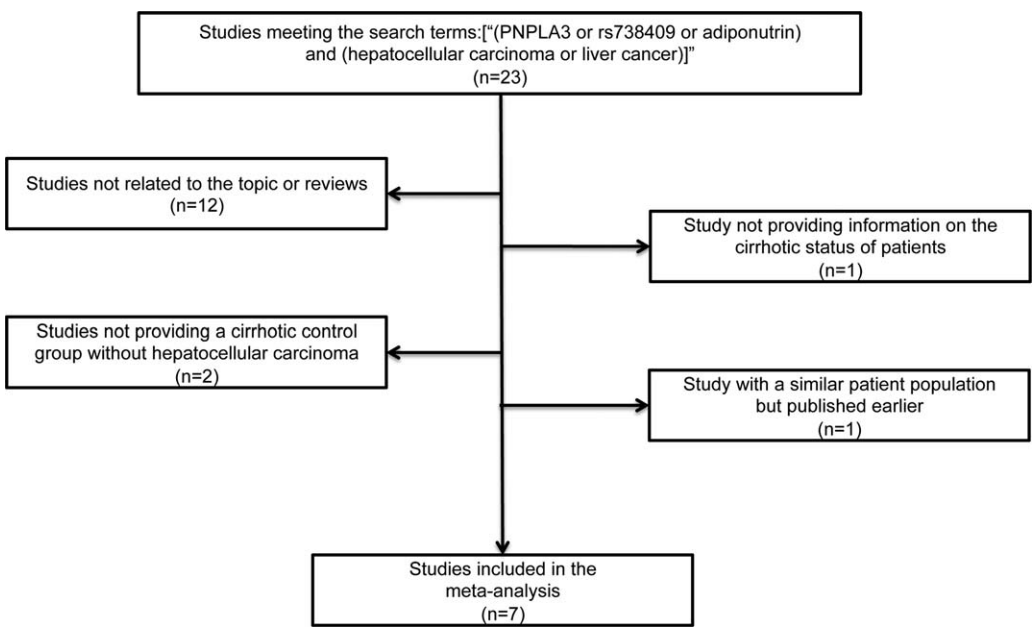


Fig. 1. Flow chart illustrating the selection of studies included in the meta-analysis.

**Table 1. Patient Characteristics in the Selected Studies Evaluating the Association Between rs7380409 and HCC**

| | | | | | rs738409 Genotype Count | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Overall (n = 2,503) | | | ALD (n = 1,374) | | | CHC (n = 945) | | |
| Study | | Age (Years) | Male Sex n (%) | BMI (kg/m$^2$) | CC | CG | GG | CC | CG | GG | CC | CG | GG |
| Corradini et al., 2011[12] | Cases | 60.2 ± 8.7 | 64 (71.1) | -* | 29 | 41 | 20 | - | - | - | 29 | 41 | 20 |
| | Controls | 56.7 ± 12.1 | 77 (58.8) | -* | 60 | 57 | 14 | - | - | - | 60 | 57 | 14 |
| Falleti et al., 2011[15] | Cases | 60.1 ± 9.2 | 121 (85.8) | 26.3 ± 4.2 | 43 | 60 | 38 | 15 | 26 | 25 | 17 | 25 | 10 |
| | Controls | 54.2 ± 9.8 | 223 (65.2) | 24.6 ± 3.4 | 125 | 160 | 57 | 38 | 64 | 30 | 53 | 66 | 18 |
| Guyot et al., 2013[17] | Cases | 65.8 ± 10.8 | 122 (75.8) | 31.4 ± 5.4 | 73 | 57 | 31 | 19 | 31 | 18 | 54 | 26 | 13 |
| | Controls | 55.2 ± 11.5 | 232 (62.6) | 27.0 ± 5.8 | 179 | 149 | 43 | 93 | 100 | 18 | 86 | 49 | 25 |
| Hamza et al., 2012[13] | Cases | 64.6 ± 8.9 | 114 (88.4) | 27.8 ± 5.3 | 49 | 51 | 29 | 27 | 36 | 23 | 7 | 5 | 0 |
| | Controls | 61.4 ± 10.0 | 113 (86.9) | 26.4 ± 5.1 | 61 | 50 | 19 | 37 | 33 | 15 | 7 | 2 | 0 |
| Nischalke et al., 2011[14] | Cases | 57.0 ± 10.0 | 115 (71.4) | 26.0 ± 5.2 | 57 | 73 | 31 | 15 | 40 | 22 | 40 | 33 | 8 |
| | Controls | 57.1 ± 9.9 | 115 (71.4) | 26.8 ± 5.9 | 77 | 69 | 15 | 32 | 36 | 10 | 45 | 30 | 5 |
| Trépo et al., 2012[16] | Cases | 65.3 ± 9.4 | 129 (89.0) | 27.9 ± 4.8 | 39 | 58 | 48 | 39 | 58 | 48 | - | - | - |
| | Controls | 56.8 ± 9.9 | 301 (70.7) | 26.7 ± 5.1 | 190 | 191 | 45 | 190 | 191 | 45 | - | - | - |
| Valenti et al., 2011[11] | Cases | 67.5 ± 9.7 | 35 (70.0) | 25.8 ± 3.1 | 12 | 21 | 17 | - | - | - | 9 | 19 | 16 |
| | Controls | 63.5 ± 11.8 | 32 (49.2) | 25.8 ± 3.4 | 28 | 30 | 7 | - | - | - | 26 | 24 | 6 |

Continuous variables are expressed with their mean ± standard deviation. Cases refer to patients with cirrhosis with HCC and controls refer to patients with cirrhosis without HCC.

*Data not available.

consultation with one other investigator (P.D.), when necessary.

***Statistical Analysis.*** Because of the lack of current knowledge regarding the genetic model of inheritance that might explain the effect of rs738409 on HCC occurrence, we avoided choosing *a priori* between an additive, dominant or recessive model as recommended.[27] Therefore, we investigated which model was the most appropriate by calculating pseudo R.[28] This value, which evaluates the goodness of fit of logistic regressions, was calculated for each study and for the related meta-analysis using the three possible genetic models. In addition, we also calculated Akaike information criterion (AIC) values.[29] The best model was defined as the one with the highest pseudo R and the smallest AIC (Supporting Table 1). The additive model was tested using the Cochran-Armitage test for trend, and the related chi-squared test was transformed into an odds ratio (OR) according to a standard procedure of effect-size conversion.[30] The strength of the association between SNP and HCC prevalence was expressed by ORs and their corresponding 95% confidence interval (CI). For each meta-analysis, we estimated a pooled OR by inverse-variance weighting using a random-effects model.[31] This model was chosen because it takes into account the possibility of heterogeneity between studies. For all three meta-analyses, *a priori* power calculations were performed. In each individual study, Hardy-Weinberg equilibrium (HWE) was assessed in control groups (patients without HCC) as recommended.[32] If a study showed a significant deviation of HWE, which might indicate genotyping errors or other bias, we performed sensitivity analyses including and excluding the HWE-deviating study(ies) as recommended.[33]

Heterogeneity was assessed by Cochran's Q test,[34] and its magnitude was measured by the between-study variance using the $I^2$ statistic.[35] These statistics were calculated as previously described using fix-effect weights and then applied to the random-effects model.[30] In case of substantial heterogeneity ($P$ value of Q statistic's test below 0.10 and/or $I^2$ higher than 25%),[32] we proceeded as follows. First, the methodological section of each study was rereviewed to determine whether any discrepancies could be identified. Second, we stratified the studies by underlying liver disease causing cirrhosis. To test whether the link between rs738409 and HCC was independent of potential clinical confounders, we performed additional analyses adjusted for age, sex, and BMI. We used a two-step approach using IPD, as previously described.[21] First, the association between rs738409 and HCC was analyzed independently in each individual study using a multivariable logistic regression, including age, sex, and BMI, and adjusted ORs with 95% CIs for this association was calculated. Then, the aggregate OR and 95% CI values were synthesized in a second step using a random-effects model.[31] To assess the extent of publication bias, Egger's and the Begg and Mazumdar's tests were used on unadjusted analyses.[34,36] A $P$ value less than 0.05 was considered statistically significant. All statistical analyses were performed using *R* (*R* Foundation for Statistical Computing) using the metaphor package, or Comprehensive Meta-analysis (Biostat, Englewood, NJ).

## Results

***Choice of the Genetic Model and HWE Calculations.*** The additive and recessive models were similarly able to adequately address the association between rs738409 and HCC, regardless of etiology (Supporting Table 1). Concerning HCC related to ALD or CHC, the additive model was the most appropriate (Supporting Table 1). Thus, all results are presented using the additive model (see Supporting Table 2 for the results of meta-analyses using dominant or recessive models). The available sample size was sufficient to achieve a power of 80% with a type I error of 0.05 to detect OR per rs738409[G] allele of 1.36, 1.32, and 1.80 for the overall, ALD, and CHC analyses, respectively (Supporting Fig. 1). We found only one study showing a significant deviation from HWE with respect to the sensitivity analysis related to CHC patients ($P = 3.39 \times 10^{-4}$; Supporting Table 2).[17]

***Association Between rs738409 and HCC Regardless of Etiology.*** Seven studies involving 2,503 patients with cirrhosis were included in the overall analysis (Table 1). There were 877 patients with HCC (cases) and 1,626 (controls). The rs738409[G] allele was associated with HCC (OR = 1.77; 95% CI: 1.42-2.19; $P = 2.78 \times 10^{-7}$; Fig. 2). There was a significant heterogeneity among the studies ($P = 4.26 \times 10^{-2}$; $I^2 = 52.2\%$). After adjustment for age, sex, and BMI, the variant remained significantly linked to HCC (OR = 1.55; 95% CI: 1.22-1.98; $P = 3.93 \times 10^{-4}$) with significant heterogeneity ($P = 5.11 \times 10^{-2}$; $I^2 = 53.2\%$). We did not find any evidence of methodological drawbacks that would warrant exclusion of a study. Therefore, we stratified the studies by underlying liver disease causing cirrhosis. No publication bias was detected by Egger's test ($P = 0.48$) or by Begg and Mazumdar's test ($P = 0.29$).

***Association Between rs738409 and HCC Related to ALD.*** Five studies involving 1,374 patients (442 cases and 932 controls) were included in the sensitivity analysis restricted to patients with ALD-related cirrhosis (Table 1).[13-17] The rs738409[G] allele was associated with HCC (OR = 2.20; 95% CI: 1.80-2.67; $P = 4.71 \times 10^{-15}$; Fig. 3) without any evidence of heterogeneity ($P = 0.50$; $I^2 = 0\%$). After adjustment for age, sex, and BMI, the variant remained significantly linked to HCC (OR = 2.13; 95% CI: 1.73-2.61; $P = 5.52 \times 10^{-13}$) with no statistical heterogeneity ($P = 0.63$; $I^2 = 0\%$). No publication bias was detected by Egger's test ($P = 0.35$) or by Begg and Mazumdar's test ($P = 0.81$).

***Association Between rs738409 and HCC Related to CHC.*** Six studies involving 945 patients (372 cases and 573 controls) were included in the sensitivity analysis restricted to patients with CHC-related cirrhosis.[11-15,17] The rs738409[G] allele was associated with HCC (OR = 1.55; 95% CI: 1.03-2.34; $P = 3.52 \times 10^{-2}$; Fig. 4), but with significant heterogeneity ($P = 2.91 \times 10^{-2}$; $I^2 = 62.1\%$). We performed a sensitivity analysis that excluded one study deviating from HWE.[17] The variant was still linked to HCC (OR = 1.78; 95% CI: 1.24-2.54; $P = 1.67 \times 10^{-3}$) with less heterogeneity ($P = 0.20$; $I^2 = 33.4\%$). After adjustment for age, sex, and BMI in the studies that

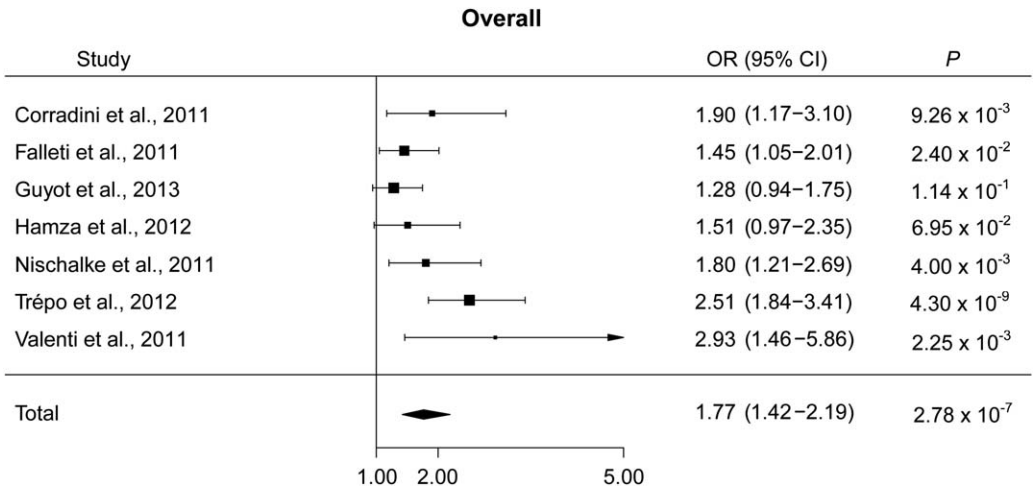


Fig. 2. Forest plot of the ORs and 95% CIs of studies of the association between *PNPLA3* rs738409[G] using an additive model of inheritance and HCC, regardless of etiology. ORs are per G-allele. The size of the black square corresponding to each study is proportional to the sample size. The combined estimate is based on a random-effects model shown by the diamond. The vertical line represents the null result.

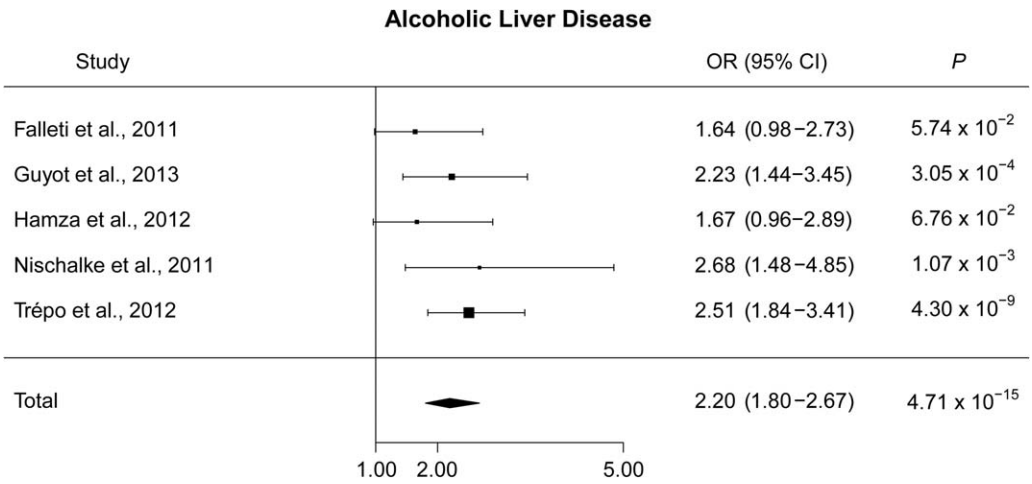


Fig. 3. Forest plot of the ORs and 95% CIs of studies of the association between *PNPLA3* rs738409[G] using an additive model of inheritance and HCC related to ALD. ORs are per G-allele. The size of the black square corresponding to each study is proportional to the sample size. The combined estimate is based on a random-effects model shown by the diamond. The vertical line represents the null result.

did not deviate from HWE, the SNP was still significantly linked to HCC (OR = 1.56; 95% CI: 1.03-2.36; $P = 3.44 \times 10^{-2}$) with modest heterogeneity ($P = 0.35$, $I^2 = 18.6\%$). We did not observe evidence of publication bias ($P = 0.30$ and $P = 0.26$ for Egger's and Begg and Mazumdar's tests, respectively).

## Discussion

This is the first meta-analysis of IPD to assess the association between rs738409 and the presence of HCC in patients with cirrhosis. The main finding of this study is the strong, independent association between this variant and HCC in individuals with cirrhosis. This collaborative work involved all of the researchers that have been studying the link between rs738409 and HCC in patients with cirrhosis preceding and through the first quarter of 2013. The strength of the association between rs738409[G] and HCC was particularly evident in patients with ALD-related cirrhosis, but was milder in patients with CHC-related cirrhosis. Overall, these results suggest that rs738409[G] exerts a marked influence on hepatocarcinogenesis in patients with cirrhosis of European descent.

First, each study investigator was involved in this project and provided IPD data, allowing reliable subgroup and multivariable analyses. Moreover, although meta-analysis of IPD requires a much greater commitment of

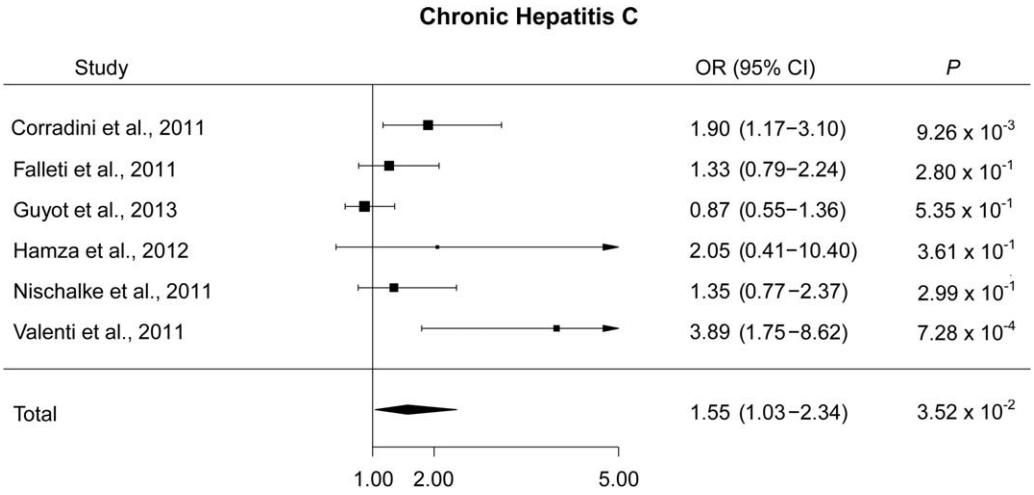


Fig. 4. Forest plot of the ORs and 95% CIs of studies of the association between *PNPLA3* rs738409[G] using an additive model of inheritance and HCC related to CHC. ORs are per G-allele. The size of the black square corresponding to each study is proportional to the sample size. The combined estimate is based on a random-effects model shown by the diamond. The vertical line represents the null result.

time and resources to collect primary data, it is an exhaustive approach that avoids patient duplication and is the most reliable method for adjusting for confounding factors at the patient level.[20,21] Indeed, the Human Genome Epidemiology Network recommends that this type of quantitative synthesis be done whenever possible.[37] Second, in contrast to the vast majority of studies that evaluated the association between SNPs and HCC,[3] we carefully selected studies that incorporated the appropriate control group in this clinical setting, namely, patients with cirrhosis. A potential limitation of this study, that is inherent to any meta-analysis, is the possible presence of publication bias. In particular, this includes the failure to identify negative studies, which are less likely to be published and may ultimately result in an overestimate of the true effect size.[38] To minimize this risk, we combined searches from a number of databases, including Medline, Cancerlit, and Embase, with manual searches.[22] We also extensively screened all abstracts presented in English at liver and gastroenterology congresses over the last 3 years. In addition, we used statistical methods (Egger's and Begg and Mazumdar's tests) to test for the presence of publication bias, and no evidence of publication bias was found. However, although we used procedures in agreement with current guidelines, we cannot formally rule out the possibility that we missed studies that were not accessible.[38]

*PNPLA3* encodes a protein that is highly expressed in the liver and becomes tightly attached to intracellular membranes. Although the exact function of the enzyme is unclear, the rs738409[G] allele results in an isoleucine (I) to methionine (M) substitution at the amino acidic position 148 (p.I148M), which promotes intracellular triglyceride retention.[39] The link between this mutation and liver damage or HCC onset remains to be established. Interestingly, the association between rs738409 C>G variant and liver disease progression has been reported to be independent of the severity of liver fat accumulation.[10,11,40] This may suggest that this mutation may directly or indirectly regulate the release of molecules involved in inflammation and fibrogenesis.[40] Indeed, rs738409 has been linked to increasing circulating levels of the proinflammatory mediator, intercellular adhesion molecule 1,[41] and decreased levels of adiponectin,[42] which has anti-inflammatory, antifibrotic, and oncosuppressive properties.[43]

The effect of the rs738409[G] allele on liver damage has been reported to be higher in fatty liver diseases (NAFLD and ALD),[5-9] compared to CHC,[10,11] where viral factors may dilute the genetic association. Therefore, it is not surprising that the strength of the association between rs738409 and HCC was milder in patients with CHC-related cirrhosis. Moreover, ALD and CHC may both promote oncogenesis by acting through common proteins, but through different signaling pathways.[44] Furthermore, studies concerning the association between CHC-related HCC and rs738409 are warranted. In addition, the association of this SNP with HCC in CHB, the leading cause of HCC worldwide, and in NAFLD, characterized by frequent development outside cirrhosis, still need to be evaluated.

Overall, our results provide a strong argument for performing further mechanistic studies to better understand the role of *PNPLA3* in HCC development. Whether the rs738409 variant promotes the inflammation/cirrhosis/carcinogenesis sequence by favoring liver fat accumulation or by affecting specific biological pathways requires further investigation.

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## References

1. El-Serag HB. Hepatocellular carcinoma. N Engl J Med 2011;365:1118-1127.
2. El-Serag HB, Rudolph KL. Hepatocellular carcinoma: epidemiology and molecular carcinogenesis. Gastroenterology 2007;132:2557-2576.
3. Nahon P, Zucman-Rossi J. Single nucleotide polymorphisms and risk of hepatocellular carcinoma in cirrhosis. J Hepatol 2012;57:663-674.
4. 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.
5. Sookoian S, Pirola CJ. Meta-analysis of the influence of I148M variant of patatin-like phospholipase domain containing 3 gene (PNPLA3) on the susceptibility and histological severity of nonalcoholic fatty liver disease. Hepatology 2011;53:1883-1894.
6. 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.
7. Seth D, Daly AK, Haber PS, Day CP. Patatin-like phospholipase domain containing 3: a case in point linking genetic susceptibility for alcoholic and nonalcoholic liver disease. Hepatology 2010;51:1463-1465.
8. Stickel F, Buch S, Lau K, zu Schwabedissen HM, 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.
9. Trépo E, Gustot T, Degre D, Lemmers A, Verset L, Demetter P, et al. Common polymorphism in the PNPLA3/adiponutrin gene confers higher risk of cirrhosis and liver damage in alcoholic liver disease. J Hepatol 2011;55:906-912.
10. Trépo E, Pradat P, Potthoff A, Momozawa Y, Quertinmont E, Gustot T, et al. Impact of patatin-like phospholipase-3 (rs738409 C>G) polymorphism on fibrosis progression and steatosis in chronic hepatitis C. Hepatology 2011;54:60-69.
11. Valenti L, Rumi M, Galmozzi E, Aghemo A, Del Menico B, De Nicola S, et al. Patatin-Like phospholipase domain-containing 3 I148M polymorphism, steatosis, and liver damage in chronic hepatitis C. Hepatology 2011;53:791-799.

12. Corradini SG, Burza MA, Molinaro A, Romeo S. Patatin-like phospholipase domain containing 3 sequence variant and hepatocellular carcinoma. HEPATOLOGY 2011;53:1776; author reply, 1777.
13. Hamza S, Petit JM, Masson D, Jooste V, Binquet C, Sgro C, et al. PNPLA3 rs738409 GG homozygote status is associated with increased risk of hepatocellular carcinoma in cirrhotic patients. J Hepatol 2012; 56:S281-S282.
14. Nischalke HD, Berger C, Luda C, Berg T, Müller T, Grünhage F, et al. The PNPLA3 rs738409 148M/M genotype is a risk factor for liver cancer in alcoholic cirrhosis but shows no or weak association in hepatitis C cirrhosis. PloS One 2011;6:e27087.
15. Falleti E, Fabris C, Cmet S, Cussigh A, Bitetto D, Fontanini E, et al. PNPLA3 rs738409C/G polymorphism in cirrhosis: relationship with the aetiology of liver disease and hepatocellular carcinoma occurrence. Liver Int 2011;31:1137-1143.
16. Trépo E, Guyot E, Ganne-Carrie N, Degre D, Gustot T, Franchimont D, et al. PNPLA3 (rs738409 C>G) is a common risk variant associated with hepatocellular carcinoma in alcoholic cirrhosis. HEPATOLOGY 2012;55:1307-1308.
17. Guyot E, Sutton A, Rufat P, Laguillier C, Mansouri A, Moreau R, et al. PNPLA3 rs738409, hepatocellular carcinoma occurrence and risk model prediction in patients with cirrhosis. J Hepatol 2013;58:312-318.
18. Burza MA, Pirazzi C, Maglio C, Sjoholm K, Mancina RM, Svensson PA, et al. PNPLA3 I148M (rs738409) genetic variant is associated with hepatocellular carcinoma in obese individuals. Dig Liver Dis 2012;44:1037-1041.
19. Ioannidis JP, Boffetta P, Little J, O'Brien TR, Uitterlinden AG, Vineis P, et al. Assessment of cumulative evidence on genetic associations: interim guidelines. Int J Epidemiol 2008;37:120-132.
20. Ioannidis JP, Rosenberg PS, Goedert JJ, O'Brien TR; International Meta-analysis of HIVHG. Commentary: meta-analysis of individual participants' data in genetic epidemiology. Am J Epidemiol 2002;156: 204-210.
21. Riley RD, Lambert PC, Abo-Zaid G. Meta-analysis of individual participant data: rationale, conduct, and reporting. BMJ 2010;340:c221.
22. Poynard T, Conn HO. The retrieval of randomized clinical trials in liver disease from the medical literature. A comparison of MEDLARS and manual methods. Control Clin Trials 1985;6:271-279.
23. Takeuchi Y, Ikeda F, Moritou Y, Hagihara H, Yasunaka T, Kuwaki K, et al. The impact of patatin-like phospholipase domain-containing protein 3 polymorphism on hepatocellular carcinoma prognosis. J Gastroenterol 2013;48:405-412.
24. Valenti L, Motta B, Soardo G, Bertelli C, Sangiovanni A, Rametta R, et al. I148M PNPLA3 polymorphism is associated with clinical features in patients with hepatocellular carcinoma. J Hepatol 2012;56:S129.
25. Fornasiere E, Cmet S, Cussigh A, Bitetto D, Furnolo E, Bignulin S, et al. PNPLA3 rs738409 C/G polymorphism in liver cirrhosis: relationship with the etiology of liver disease and HCC occurence. J Hepatol 2011;54:S244-S245.
26. Bruix J, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, et al. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. J Hepatol 2001;35:421-430.
27. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. The choice of a genetic model in the meta-analysis of molecular association studies. Int J Epidemiol 2005;34:1319-1328.
28. McFadden D. Conditional logit analysis of qualitative choice behavior. In: Zarembka P, ed. Frontiers in Econometrics. New York: Academic; 1973:105-142.
29. Akaike H. Fitting autoregressive models for prediction. Ann Inst Stat Math 1969;21:243-247.
30. Borenstein M. Introduction to Meta-Analysis. Chichester, UK: John Wiley & Sons; 2009:xxviii.
31. DerSimonian R, Laird N. Meta-analysis in clinical trials. Control Clin Trials 1986;7:177-188.
32. Minelli C, Thompson JR, Abrams KR, Thakkinstian A, Attia J. How should we use information about HWE in the meta-analyses of genetic association studies? Int J Epidemiol 2008;37:136-146.
33. Thakkinstian A, McElduff P, D'Este C, Duffy D, Attia J. A method for meta-analysis of molecular association studies. Stat Med 2005;24: 1291-1306.
34. Deeks JJ, Altman DG, Bradburn MJ: Statistical methods for examining heterogeneity and combining results from several studies in meta-analysis. In: Egger M, Davey Smith G, Altman DG, eds. Systematic Reviews in Health Care—Meta-Analysis in Context. London: BMJ Books; 2005:285-312.
35. Higgins JP, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. BMJ 2003;327:557-560.
36. Begg CB, Mazumdar M. Operating characteristics of a rank correlation test for publication bias. Biometrics 1994;50:1088-1101.
37. Little J, Higgins JPT (eds). The HuGENet™ HuGE Review Handbook, version 1.0. Atlanta, GA; Centers for Disease Control and Prevention; 2006.
38. Thornton A, Lee P. Publication bias in meta-analysis: its causes and consequences. J Clin Epidemiol 2000;53:207-216.
39. Pirazzi C, Adiels M, Burza MA, Mancina RM, Levin M, Stahlman M, et al. Patatin-like phospholipase domain-containing 3 (PNPLA3) I148M (rs738409) affects hepatic VLDL secretion in humans and in vitro. J Hepatol 2012;57:1276-1282.
40. Valenti L, Al-Serri A, Daly AK, Galmozzi E, Rametta R, Dongiovanni P, et al. Homozygosity for the patatin-like phospholipase-3/adiponutrin I148M polymorphism influences liver fibrosis in patients with nonalcoholic fatty liver disease. HEPATOLOGY 2010;51:1209-1217.
41. Pare G, Ridker PM, Rose L, Barbalic M, Dupuis J, Dehghan A, et al. Genome-wide association analysis of soluble ICAM-1 concentration reveals novel associations at the NFKBIK, PNPLA3, RELA, and SH2B3 loci. PLoS Genet 2011;7:e1001374.
42. Valenti L, Rametta R, Ruscica M, Dongiovanni P, Steffani L, Motta BM, et al. The I148M PNPLA3 polymorphism influences serum adiponectin in patients with fatty liver and healthy controls. BMC Gastroenterol 2012;12:111.
43. Marra F, Bertolani C. Adipokines in liver diseases. HEPATOLOGY 2009; 50:957-969.
44. Alisi A, Ghidinelli M, Zerbini A, Missale G, Balsano C. Hepatitis C virus and alcohol: same mitotic targets but different signaling pathways. J Hepatol 2011;54:956-963.