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Modulation of the effect of *PNPLA3* I148M mutation on steatosis and liver damage by alcohol intake in patients with chronic hepatitis C

To the Editor:

The Patatin-like phospholipase domain-containing-3 (*PNPLA3*) rs738409 polymorphism, encoding for the I148M protein variant, is a strong genetic determinant of hepatic fat accumulation and progressive alcoholic as well as nonalcoholic steatohepatitis in adults and children [1–4], explaining almost a quarter of cirrhosis variability in alcoholic liver disease [5]. We and others [6,7] have demonstrated that in patients with chronic hepatitis C (CHC), this genetic factor influences steatosis development in nongenotype 3 patients, the progression of liver damage, and susceptibility to develop cirrhosis and its complications [8] (Table 1).

Muller *et al.* have very recently replicated the association of the *PNPLA3* 148M polymorphism with steatosis and cirrhosis in an independent cross-sectional study in German patients with CHC [9]. Interestingly, there was a significantly higher prevalence of subjects who consumed a moderate, but potentially harmful, intake of alcohol in this series than in that described in our paper, and a distinct effect of the 148M polymorphism on steatosis and liver damage according to the amount of daily alcohol intake could be observed, in that homozygosity for the 148M was associated with steatosis only in abstainers, whereas it was associated with cirrhosis only in at risk drinkers. These data led the authors to hypothesize that *PNPLA3* genotype may exert a differential effect on liver damage according to alcohol consumption.

As the mechanisms underpinning the detrimental effect of the 148M *PNPLA3* variant in a rapidly widening range of liver diseases is still unknown, to contribute elucidating this issue we have now replicated the same analyses in our series of patients subdivided according to alcohol intake, and provide a pooled estimate of the effect of the 148M on the risk of steatosis and cirrhosis in the two combined studies.

Although the effect of *PNPLA3* genotype might be more evident when considering more severe steatosis [2,7], pooled results confirm that in CHC the 148M polymorphism is associated with the presence of steatosis only in "abstainers" (<30 g alcohol/day), possibly because of the confounding effect of alcohol in moderate drinkers, but, on the contrary, is significantly associated with cirrhosis both in abstainers and in moderate drinkers, and the effect seems more marked in those who consume more than 30 g alcohol/day, as suggested by Muller *et al.* [9]. Thus, as previously suggested [2], the detrimental effect of the 148M *PNPLA3* variant on the progression of liver damage might not be limited to predisposition to steatosis development.

The discrepant results obtained in our series, as reflected by the significant heterogeneity observed between the two studies for the effect of at risk drinking on cirrhosis, may be due to the relatively small number of drinkers in Italian patients, or to other unmeasured confounders, such as additional genetic [10] and environmental factors, and possibly the prevalence of occult HBV infection.

Nevertheless, these results indicate that additional studies are warranted to analyze the interaction between even moderate amounts of alcohol and *PNPLA3* genotype in liver diseases.

Table 1. Logistic regression analysis and meta-analysis results for the association of *PNPLA3* rs738409 SNP (I148M), under a recessive inheritance model, with the presence of steatosis and cirrhosis according to daily alcohol intake in 819 previously described Italian patients [7] and 494 German patients [9] with CHC.

	Italian CHC	German CHC	Pooled data
Steatosis	OR (95% CI)	OR (95% CI)	OR (95% CI)
Total	1.82 (1.33-2.58) n = 819	5.53 (1.55-19.8) n = 442	1.95 (1.43-2.67) n = 1261
Abstainers	1.84 (1.33-2.65) n = 724	12.6 (1.48-108) n = 235	1.93 (1.38-2.69) n = 959
At risk drinkers	1.30 (0.49-5.83) n = 95	2.61 (0.50-13.5) n = 207	1.67 (0.63-2.41) n = 302
Cirrhosis	OR (95% CI)	OR (95% CI)	OR (95% CI)
Total	1.30 (1.01-1.66) n = 819	2.76 (1.22-6.25) n = 605	1.38 (1.10-1.75) n = 1424
Abstainers	1.39 (1.06-1.81) n = 724	1.65 (0.54-5.03) n = 335	1.40 (1.09-1.81) n = 1059
At risk drinkers	0.89 (0.37-1.94) n = 95	4.77 (1.39-6.38) n = 270	2.20 (1.28-3.80)* n = 365

OR: odds ratio for the presence of homozygosity for the 148M *PNPLA3* variant, adjusted for age, sex, BMI, and, only for steatosis, viral genotype 3, as in [9]. c.i.: Confidence interval. Steatosis: grade 1–3 *vs.* grade 0. Cirrhosis: Ishak stage 5–6 *vs.* 0–4. Abstainers: <30 g/day; at risk drinkers: ⩾20 g/day, as in [9].
Pooled estimates (OR, 95% c.i.) were calculated by the inverse variance method (fixed effect model) by the Review Manager v.5 software (cochrane collaboration). *p <0.05 for heterogeneity between studies.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

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Reply to: "Modulation of the effect of *PNPLA3* I148M mutation on steatosis and liver damage by alcohol intake in patients with chronic hepatitis C"

***PNPLA3* rs738409 and fibrosis progression in chronic hepatitis C – There is more to it than just fat!**

To the Editor:

The Hepatology community has seen a substantial rise in publications reporting data on genetic risk factors conferring risk to fibrosis progression in non-alcoholic, alcoholic, and chronic hepatitis C-related liver diseases. For long, hypothesis-driven, often single-centre genetic case control studies have reported associations of certain candidate genes with fibrosis/cirrhosis which could not be replicated. With genome-wide association studies (GWAS) becoming a standard tool in translational research, novel candidate genes enter the stage which have been identified by systematic screening experiments [1]. GWAS make use of a hypothesis-free, or rather – hypothesis-generating approach, and have the potential to uncover genetic risk factors which had not been considered based on our previous pathophysiological understanding. A striking example for such revelation is the gene coding for patatin-like phospholipase domain containing-3 (*PNPLA3*; adiponutrin) of which a polymorphic variant (rs738409 G >C) was found associated with liver fat content in a landmark GWAS [2], and subsequently with progression of non-alcoholic fatty liver disease (NAFLD) [3], and alcoholic liver disease (ALD) [4,5] by means of candidate case control studies. The role of *PNPLA3* rs738409 as a risk factor for progressive fibrosis can now be extended to chronic hepatitis C. Only in 2011, four studies presented data on the role of *PNPLA3* rs738409 G which unanimously confirm that carriage of at least one allele increases the risk of advanced fibrosis and cirrhosis [6–9] (Table 1). Our own data indicate that this association is particularly evident in hepatitis C virus-infected individuals who regularly drink alcohol as opposed to abstainers [6]. Regarding the latter, *PNPLA3* rs738409 G appears to modulate fat storage in the liver by a yet unknown mechanism, possibly by interaction of *PNPLA3* with viral epitopes. Indeed, from the data presented in published studies, it becomes clear that *PNPLA3* rs738409 G neither aggravates steatosis nor fibrosis in patients with genotype 3, supporting the hypothesis that mostly genotype 1-specific interactions with *PNPLA3* are involved.

In this issue of the *Journal*, Valenti and coworkers have undertaken a pooled analysis of our [6] and their data [7] after stratifying their patients into "at-risk" drinkers (daily alcohol consumption $\geqslant$20 g) and abstainers [10]. Hereby, they confirm that carriage of *PNPLA3* rs738409 G is a risk factor for steatosis in abstainers, but not in at risk drinkers, possibly due to a confounding effect on steatosis by concomitant alcohol consumption. In addition, by increasing the numbers of total cases and controls,