

Genetic variation in *HSD17B13* reduces the risk of developing cirrhosis and hepatocellular carcinoma in alcohol misusers

Short title: Alcoholic cirrhosis, HCC, risk reduction and *HSD17B13*

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Key words: adiponutrin, candidate genes; fibrosis; gene-gene interactions; genetic risk association; genetic susceptibility; host genetics; lipotoxicity.

Word count: 6212

Tables: 3 (+7 Supplementary Tables)

Figures: 3 (+2 Supplementary Figures 1A-C, 2A-C)

Conflicting Interests

None of the authors has any conflict of interest in relation to the contents of this manuscript.

Funding

This work was supported by grants from the Swiss National Funds (SNF no. 310030_169196) and the Swiss Foundation for Alcohol Research (SSA) to FS, and the Liver Systems Medicine (LiSyM) Network funded by the German Federal Ministry for Education and Research (BmBF) to JH. HDN and US were supported by a grant from the Deutsche Krebshilfe (70112169).

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ABSTRACT

Background & Aims: Carriage of rs738409:G in *patatin-like phospholipase domain-containing 3* (*PNPLA3*) is associated with an increased risk of developing alcohol-related cirrhosis and hepatocellular carcinoma (HCC). Recently, rs72613567:TA, in *hydroxysteroid 17-beta dehydrogenase 13* (*HSD17B13*), was shown to be associated with a reduced risk of alcohol-related liver disease and to attenuate the risk associated with carriage of *PNPLA3* rs738409:G. This study aims to explore the risk-associations between these two genetic variants and the development of alcohol-related cirrhosis and HCC.

Methods: Variants in *HSD17B13* and *PNPLA3* were genotyped in 6,171 participants, including: 1,031 with alcohol-related cirrhosis and HCC; 1,653 with alcohol-related cirrhosis without HCC; 2,588 alcohol misusers with no liver disease; and 899 healthy controls. Genetic associations with the risks for developing alcohol-related cirrhosis and HCC were determined using logistic regression analysis.

Results: Carriage of *HSD17B13* rs72613567:TA was associated with a lower risk for developing cirrhosis (OR 0.79 [95% CI 0.72-0.88], $p=8.13\times 10^{-6}$) and HCC (OR 0.77 [95% CI 0.68-0.89], $p=2.27\times 10^{-4}$) while carriage of *PNPLA3* rs738409:G was associated with an increased risk for developing both cirrhosis (OR 1.70 [95% CI 1.54-1.88], $p=1.52\times 10^{-26}$) and HCC (OR 1.77 [95% CI 1.58-1.98], $p=2.31\times 10^{-23}$). These associations remained significant after adjusting for age, sex, body mass index, type II diabetes mellitus and country. Carriage of *HSD17B13* rs72613567:TA attenuated the risk for cirrhosis associated with *PNPLA3* rs738409:G in both men and women but the protective effect against the subsequent development of HCC was only observed in men ($p=1.72\times 10^{-4}$; OR_{allelic} , 0.75; 95% CI, 0.64–0.87).

Conclusions: Carriage of variants in *PNPLA3* and *HSD17B13* differentially affect the risk for developing advanced alcohol-related liver disease. A genotypic/phenotypic risk score might facilitate earlier diagnosis of HCC.

Word count: 273

LAY SUMMARY

Individuals misusing alcohol who carry a particular variant of the gene *PNPLA3* are at greater risk of developing alcohol-related cirrhosis and liver cancer, a condition with a poor chance of survival. Recently a variant in another gene, *HSD17B13*, has been shown to reduce the risk of developing alcohol-related cirrhosis, and, in men, of reducing the added risk of developing liver cancer. The variant in *HSD17B13* can off-set, to a degree, the risk of developing alcohol-related cirrhosis and HCC associated with the variant in *PNPLA3*. Knowing if someone carries these gene variants could influence the way in which they are managed.

HIGHLIGHTS

- Carriage of rs738409:G in *PNPLA3* increases the risk of developing alcohol-related cirrhosis and hepatocellular carcinoma (HCC)
- A splice variant in the gene coding for *hydroxysteroid 17-beta dehydrogenase 13* *HSD17B13* reduces the risk of developing alcohol-related cirrhosis in both men and women and the risk of developing HCC in men.
- Carriage of the splice variant in *HSD17B13* attenuated the population attributable fraction for developing alcohol-related cirrhosis associated with carriage of rs738409:G in *PNPLA3* from 27.2% to 16.5% and for developing HCC from 38.7% to 32.6%
- Genotypic -phenotypic risk scores could identify people at particular risk and facilitate their management.

INTRODUCTION

Alcoholic liver disease (ALD) has a global prevalence of 12% and accounts for half of all cirrhosis-associated deaths in Europe and the United States.¹⁻³ Alcohol-related cirrhosis is now the second most common indication for liver transplantation, accounting for approximately 40% of all primary liver transplants in Europe and approximately 25% in the United States.⁴

Chronic alcohol misuse is associated with the development of a broad spectrum of liver injury. Hepatic steatosis develops in most heavy alcohol users, but more substantial liver injury only develops with persistent alcohol misuse over time; inflammation and progressive fibrosis will develop in 10–35% of individuals, whereas cirrhosis is observed in only 10–15%.⁵⁻⁷ Between 5 to 15% of people with alcohol-related cirrhosis are at risk for developing hepatocellular carcinoma (HCC); the annual incidence is 2.5 to 3.0%,⁸⁻¹¹ with a 5-year cumulative risk of around 8%.¹² The global incidence of HCC is increasing; it is now the fifth most frequent cancer and third most frequent cause of cancer-related mortality world-wide; one third of cases develop on a background of alcohol-related cirrhosis.¹³

The susceptibility to develop significant ALD is determined by the interplay of a number of risk factors, including sex, ethnicity, the amount/pattern of alcohol drinking, coffee consumption, cigarette smoking, co-morbidities such as obesity, type 2 diabetes and hepatitis C infection and a number of host genetic factors.¹⁴ Carriage of the common missense variant rs738409:G in *patatin-like phospholipase domain-containing 3 (PNPLA3)* is the most robustly validated risk locus for the development of alcohol-related cirrhosis and HCC,¹⁵⁻²⁰ accounting for 26.6%¹⁵ and 43.5%¹⁹ of the variance, respectively. Two further gene variants, rs58542926 in *TM6SF2* and rs641738 in *MBOAT7*, are additional risk factors, albeit with much lower effect sizes.²⁰

Recently, Abul-Husn and colleagues²¹ identified a splice variant rs72613567 in *hydroxysteroid 17-beta dehydrogenase 13 (HSD17B13)*, which appeared to protect against the development

of chronic liver injury in people of European ancestry. *HSD17B13* encodes a hepatic lipid droplet protein; the insertion of an adenine adjacent to the donor splice site on exon 6 (rs72613567:TA allele) results in an unstable and truncated protein with reduced enzymatic activity, suggesting that it is a loss-of-function variant.²¹ In this study, carriage of *HSD17B13* rs72613567:TA was associated with reduced serum alanine and aspartate aminotransferase activities, a reduced risk for developing alcohol-related and non-alcohol-related fatty liver disease (NAFLD), and, more specifically, for developing both alcohol-related and NAFLD-related cirrhosis.²¹ The association with alcohol-related cirrhosis was the most compelling but the total number of cases was very small. Abul-Husn and colleagues²¹ also showed that carriage of this variant attenuated the risk of developing progressive liver injury derived from rs738409:G in *PNPLA3*.

Yang *et al.*²² recently confirmed that carriage of rs72613567:TA in *HSD17B13* was associated with a reduced risk for developing alcohol-, NAFLD- and hepatitis C-related liver disease, and more specifically the risk of developing cirrhosis. They also found protection against the development of HCC, but only in patients with a background of ALD. The numbers of patients included was small and those with HCC were more frequently male and older than their counterparts without HCC. Thus, in order to validate these findings much larger cohorts are needed to allow adjustments for these potential confounding factors.²² Interactions between variants in *HSD17B13* and *PNPLA3* were not addressed in this study.

The aims of the present study were to determine whether: (i) carriage of rs72613567:TA in *HSD17B13* protects against the development of alcohol related cirrhosis and alcohol-related HCC; and (ii) to explore possible risk interactions between rs72613567:TA in *HSD17B13* and rs738409:G in *PNPLA3*.

METHODS

Study cohorts

The study population comprised of 6,171 participants drawn from one of four study cohorts:

1. Alcohol related cirrhosis and HCC (**HCC**) (n=1,031)
2. Alcohol-related cirrhosis without HCC (**CIRR**) (n=1,653)
3. Alcohol misusers with no evident liver disease (**ALC**) (n=2,588)
4. Healthy controls with no history of alcohol misuse or liver disease (n = 899)

Participants in cohorts 1-3 were recruited from Hepatology units and Addiction centres across Europe and were of self-reported Swiss/German/Austrian/Italian/British ancestry. Ninety of the UK samples with cirrhosis and HCC were obtained from the Nottingham Digestive Diseases Centre Biomedical Research Unit Research Tissue Bank (Rec Ref: 14/WA/1234). Participants in cohort 4 were recruited from the UK.

The diagnosis of alcohol-related cirrhosis was established as described, in detail, previously;²⁰ briefly, the diagnosis was based on a history of prolonged, sustained alcohol intake of a minimum of 40g/day in women and 60g/day in men together with histological examination of liver tissue; or compatible historical, clinical, laboratory, radiological and endoscopic features. Patients were excluded if they had any other potential cause of liver injury, specifically if they were positive for hepatitis B surface antigen (HBsAg), anti-hepatitis C immunoglobulin G (anti-HCV IgG), anti-nuclear antibodies (ANA) (titre >1:80) or anti-mitochondrial antibodies (titre >1:40). Patients with elevated serum ferritin levels and a transferrin saturation of >50%, a serum caeruloplasmin of <20mg/dl (0.2 g/dl) or a serum alpha-1 antitrypsin of <70 mg/dL (13µmol/L) were further investigated and excluded, if necessary.

The diagnosis of HCC was based on histological examination of tumour tissue or evidence on imaging, preferably using two modalities, of lesions that were hypervascular in the arterial

phase with washout in the portal venous or delayed phases.²³ The severity of underlying cirrhosis was assessed using Pugh's modification of the Child's grading system.²⁴

The patients with alcohol misuse but no evidence of significant liver injury were recruited as described, in detail, previously;²⁰ in brief, they had a background of alcohol consumption of at least 60 g/d for women and 80 g/d for men for ≥ 10 years with or without features of alcohol dependence.²⁵ None had historical, clinical or laboratory evidence of liver disease and its absence was confirmed either by a liver stiffness measurement (Fibroscan, Echosens, Paris) of below 6 kPa (IQR<20%) or by the absence of histological liver damage.

Healthy controls were recruited from London branches of the National Health Service blood transfusion service, from General Practitioners' surgeries, from amongst university students, and from the general public. None currently drank alcohol above a weekly maximum of 112 g for women and 168 g for men, nor had they done so at any time in the past. None had a history or clinical evidence of liver disease

DNA preparation and genotyping

Genomic DNA was extracted from venous blood samples and quantified using standard procedures.²⁰ Genotyping of *PNPLA3* rs738409 (Assay ID: C_7241_10) and *HSD17B13* rs72613567 (primer and probe sets manufactured through custom TaqMan® Assay design) was performed using TaqMan® SNP Genotyping Assays and Chemistries (Applied Biosystems, Waltham, MA, USA) on an automated platform with TECAN Freedom EVO and 384well TEMO liquid handling robots (TECAN, Männedorf, Switzerland) as described previously.^{19,20}

All process data were logged and administered with a database-driven LIMS. Reactions were completed and read in a 7900 HT TaqMan sequence detector system (Applied Biosystems, Waltham, MA, USA). The amplification reaction was carried out with the TaqMan universal

master mix at cycling conditions of 1 cycle for 10 minutes at 95°C, followed by 45 cycles for 15 seconds at 95°C, and 45 cycles for 1 minute at 60°C.

Statistical analysis

Logistic regression and SNP*SNP interaction analyses were performed using SPSS v.25.0 (IBM Corp., Armonk, NY, USA). A three-way case control design was adopted: HCC vs. CIRR; CIRR vs. ALC; HCC vs. ALC. Genotypic and allelic tests of association were assessed by two logistic regression models:

Model A: univariate logistic regression;

Model B: adjustments for age, sex, body mass index (BMI), type 2 diabetes mellitus (T2DM) and country.

Results, expressed as odds ratios (OR) with their 95% confidence intervals (CI), were derived from beta coefficients and their standard deviations. Nominal two-sided asymptotic p-values are reported for all tests.

The interactions between *HSD17B13* and *PNPLA3* were examined by logistic regression for the univariate additive and genotypic regression models including both main SNP effects and the SNP*SNP interaction term.

Fixed-effect model meta-analysis using the inverse variance-weighted method to summarize effect sizes and forest plots were performed using the R package 'metafor' v.2.0-0.

Sex-specific *post-hoc* analyses for risk association of *HSD17B13* and *PNPLA3* were performed. The Mantel-Haenszel test for trend was applied for testing a linear trend in observed genotype proportions from contingency tables for (ALC < CIRR < HCC), by sex.

The population-attributable fraction (PAF) provides an epidemiological estimate of the proportion of the disorder that is attributable to a given risk factor. Thus, in this instance it is an estimate of how much lower the frequency of HCC would be in patients with alcohol-related cirrhosis, if the risk genotype(s) were eliminated from the population.

The PAF was estimated for heterozygous and homozygous carriage using the formula:²⁶

$$(x - 1)/x$$

where $x = (1 - p)^2 + 2p(1 - p)OR1 + p^2OR2$ (19); p is the allele frequency in the CIRR or ALC cohorts, and OR1 and OR2 are the ORs associated with hetero- and homozygosity.

Combined PAF estimates were calculated as $PAF = 1 - (1 - PAF1) (1 - PAF2) (1 - PAFn)$ based on the individual PAFs for each associated SNP, assuming no multiplicative interaction between them.

Ethics

The study protocol was approved by the ethics committees of the participating institutions; all included subjects provided written informed consent prior to inclusion into the study.

RESULTS

The patient cohorts were predominantly male and middle aged. Patients with cirrhosis were generally older than those misusing alcohol, more likely to be overweight and to be diabetic. The patients with cirrhosis and HCC were generally older than the patients with cirrhosis alone and proportionately more likely to be male, overweight, and diabetic (Table 1).

Genotyping was completed for both SNPs with call rates greater than 95% (Supplementary Table 1). All markers followed Hardy-Weinberg equilibrium (cutoff HWE $P > 0.05$).²⁷ In the healthy controls the minor allele frequencies (MAFs) for both *HSD17B13* rs72613567:TA (0.276) and *PNPLA3* rs738409 G (0.224) were comparable to those in public databases²⁸ and previous publications¹⁹⁻²¹ and did not differ significantly from the MAFs in the ALC group (Supplementary Table 1).

A progressive reduction in MAFs for *HSD17B13* rs72613567:TA was observed between the three groups: ALC (26.4%); CIRR (22.0%) and HCC (17.7%) ($P_{\text{trend}} = 1.09 \times 10^{-15}$) (Supplementary Table 1). This contrasted with the expected stepwise increase in the MAFs for *PNPLA3* rs738409:G in the same three groups *viz.* ALC (23.9%) CIRR (35.4%), HCC (49.8%) (Supplementary Table 1).

In the univariate model, allelic and genotypic associations for *HSD17B13* rs72613567:TA were highly significant for the comparisons HCC vs. CIRR ($P_{\text{allelic}} = 2.27 \times 10^{-4}$, $P_{\text{genotypic}} = 1.05 \times 10^{-3}$), CIRR vs. ALC ($P_{\text{allelic}} = 8.13 \times 10^{-6}$, $P_{\text{genotypic}} = 1.54 \times 10^{-6}$), and HCC vs ALC ($P_{\text{allelic}} = 1.69 \times 10^{-14}$, $P_{\text{genotypic}} = 3.22 \times 10^{-14}$) (Table 2). The protective effect associated with carriage of *HSD17B13* rs72613567:TA remained significant in the CIRR and HCC cohorts after correction for sex, age, BMI, T2DM and country (Table 2). The protective effect for HCC was greater with homozygous than heterozygous carriage of the *HSD17B13* rs72613567:TA allele (Figure 1).

Allelic and genotypic associations for *PNPLA3* rs738409:G allele were also significantly associated in the CIRR (OR_{allelic} 1.70 [1.54-1.88], P_{allelic} = 1.52×10⁻²⁶) and HCC (OR_{allelic} 1.77 [1.58-1.98], P_{allelic} = 2.31×10⁻²³) cohorts (Table 3). These associations were robust to corrections for sex, age, BMI, DMT2 and country.

Since patients were recruited from across Europe a meta-analysis of the *HSD17B13* and *PNPLA3* loci for association with cirrhosis and HCC was performed, by country (Supplementary Table 2 & 3). Associations were calculated consensually, but were not necessarily significant in each single European cohort. However, fixed effect meta-analysis on aggregate confirmed both the protective and risk-enhancing effects of *HSD17B13* and *PNPLA3* loci, respectively, in the total cohort. There was no evidence of heterogeneity (Supplementary Figures 1A-C and 2A-C).

The PAF for *PNPLA3* rs738409 for CIRR was 27.2% (20.7%-33.6%) and for *HSD17B13* rs72613567 -14.7% (-21.2% - -7.9%). The combined PAF for CIRR was 16.5%. The PAF% for *PNPLA3* rs738409 for HCC in was 38.7% (29.3%-47.2%) and for *HSD17B13* rs72613567 -10.0% (-16.7% - -2.7%). The combined PAF for HCC was 32.6%.

The potential modifying effect of *HSD17B13* rs72613567 on *PNPLA3* rs738409 was explored by calculating the proportion of the HCC, CIRR and ALC cohorts with different 'gene signatures' combining either the (high risk variant) *PNPLA3* rs739409 G with wild type *HSD17B13* rs72613567:T (lack of protection), or the (low risk variant) *PNPLA3* rs739409 C with *HSD17B13* rs72613567:TA (protection). A preponderance of patients in the CIRR and HCC cohorts were homozygous for *PNPLA3* rs739409 G and for *HSD17B13* rs72613567:T which is in line with the estimated risk contributions of each variant (Figure 2A & B).

Possible SNP-SNP interactions between *HSD17B13* and *PNPLA3* were explored by testing interaction terms in the logistic regression models for the risk for developing CIRR and the

further risk for developing HCC. The genotypic interactions for the risk association with CIRR was not significant in the total study population ($p = 0.598$) (Supplementary Table 4) nor in the men only cohort ($p = 0.689$) (Supplementary Table 5). None of the *HSD17B13* by *PNPLA3* genotype interaction pair combinations was significant. The interactions in the additive CIRR and HCC risk models were not significant either. Thus, there does not appear to be an *HSD17B13* rs72613567:TA \times *PNPLA3* rs739409:G interaction.

In a sex-specific post hoc analysis of the *HSD17B13* locus, an association with HCC disease risk was observed in men ($P = 1.72 \times 10^{-4}$; $OR_{\text{allelic}}, 0.75$; 95% CI, 0.64–0.87) but not in women ($P = 0.772$; $OR_{\text{allelic}}, 1.07$; 95% CI, 0.73–1.58), while the protective effect for cirrhosis risk associated with carriage of *HSD17B13* rs72613567:TA was observed in both sexes (Supplementary Table 6; Figures 3A-C). The association between carriage of *PNPLA3* rs739409:G and cirrhosis and HCC disease risk showed no sex differential (Supplementary Table 7).

DISCUSSION

The genetic contribution to the development of significant alcohol-related liver injury is gradually being unraveled. Three genetic loci have been identified which are associated with an increased risk for developing cirrhosis at genome-wide significance, namely *PNPLA3*, *TM6SF2*, *MBOAT7*,²⁰ and at least two of these variants, *PNPLA3* and *TM6SF2*, are associated with an increased risk for developing HCC on this background.^{16,17,19}

There is little published evidence for genetic variants conferring protection against the development of significant alcohol-related liver injury. Indirect protection is afforded in individuals, primarily of East Asian ancestry, who carry rs1229984 in alcohol dehydrogenase IB (*ADH1B*)²⁹ and/or rs671 in aldehyde dehydrogenase 2 (*ALDH2*)³⁰ and, in consequence, develop an abreaction to alcohol and so tend to avoid it.

Recently, however, Abul-Husn and colleagues²¹ identified a splice variant, rs72613567, in *HSD17B13*, which appeared to protect against the development of chronic liver injury in people of European ancestry. Specifically, this SNP appeared to confer protection against the development of alcohol-related and NAFLD-related cirrhosis.²¹ The association with alcohol-related cirrhosis was the more compelling even though the total number of cases was very small amounting to only 124 in the discovery cohort and 215 in the validation cohort.

Yang and coworkers,²² in a recent French/Belgian collaboration confirmed that rs72613567 in *HSD17B13* confers protection against the development of alcohol-, NAFLD- and HCV-related liver disease. They further showed that this SNP confers protection against the development of HCC but only in people with alcohol-related liver disease. The data were adjusted for age, sex and degree of hepatic fibrosis but not for other important confounders such as BMI and diabetes. Approximately half of the patients with alcohol-related HCC in their study had

cirrhosis so the best estimate of the numbers of cases and controls they included, comparable to those in the present study, would be 217 with alcohol-related cirrhosis and HCC and 1243 with alcohol-related cirrhosis without HCC. The patients with alcohol-related cirrhosis and HCC were more frequently male and were older than those without HCC. Adjustments were not made for these possible confounders leading the authors to conclude that larger cohorts were needed to validate their findings.

The cohorts included in the present study were large comprising of 1,031 patients with alcoholic cirrhosis and HCC and 1653 with alcohol-cirrhosis without HCC. In addition, 2,588 alcohol misusers, with no evidence of liver disease, were included to exclude the possibility that any observed genetic effect might relate to the susceptibility to develop alcohol dependence. The cohort of 1196 healthy controls allowed the population MAF for the SNPs of interest to be directly established. All the cases and controls were of European ancestry.

The results of the present study robustly establish that carriage of *HSD17B13*:rs72613567:TA protects against the development of alcohol-related cirrhosis and its subsequent evolution to HCC. The results also confirm that carriage of *PNPLA3* rs738409 is associated with a significantly increased risk of developing alcohol-related cirrhosis and HCC. However, the magnitude of these two effects is of a different order. Thus, while carriage of the *HSD17B13*:rs72613567:TA allele *reduces* the PAF for cirrhosis by 14.7% and for HCC by 10%, carriage of the *PNPLA3* rs738409:G allele *increases* the risks by 27.2% and 38.7% respectively. Thus, the combined PAF for cirrhosis was 16.5% and for HCC 32.5%.

It would, therefore, appear that the risk of carriage of *PNPLA3* rs738409:G is attenuated, to a degree, by co-carriage of *HSD17B13*:rs72613567:TA. Abul-Husn and colleagues²¹ explored the possibility of interactions between these two SNPs and observed nominally significant interactions in association analyses with serum transaminase activities, primarily in the obese,

but not apparently with chronic liver disease. They also showed, using RNA sequencing-based expression analysis that *HSD17B13* rs72613567:TA was associated with decreased *PNPLA3* messenger RNA expression in an allele dose-dependent manner. However, exploration of SNP-SNP interaction, in the present study, showed no evidence of a significant interaction. Yang and coworkers²² did not report data on possible SNP-SNP interaction.

Male sex is a significant risk factor for the development of HCC in people with alcohol-related cirrhosis.^{13,14} The reasons for this are unknown. Men are more likely to drink at harmful levels and hence are more likely to develop alcohol-related cirrhosis but they are proportionately over represented amongst those developing HCC. Malignant transformation is more likely to occur in individuals with alcohol-related cirrhosis who stop drinking;³¹ hence the suggestion that the difference in sex-specific HCC rates may reflect the generally lower rates of abstinence amongst women. The possibility of sex-genetic variant interactions should also be considered. The comparative survival advantage in women with alcohol-related cirrhosis is well-documented,³²⁻³⁴ and it has recently been shown that this may relate, at least in part, to a sex-variant interaction with rs738409:G in *PNPLA3*.^{35,36}

The possibility of sex-variant interactions was explored in the present study. The progressive increase in the risk of developing alcohol-related cirrhosis and HCC associated with carriage of *PNPLA3* rs738409:G HCC was observed in both men and women. Likewise, the protective effect for cirrhosis risk associated with carriage of *HSD17B13* rs72613567:TA was observed in both sexes. However, the protective association of *HSD17B13* rs72613567:TA and HCC was only found in men. There was no significant sex difference in the MAFs for *HSD17B13* rs72613567:TA in the alcohol misusers (26.3% vs. 26.6%) or in the patients with alcohol related cirrhosis (22.4% vs. 20.5%). However, whereas the frequency further reduced in men with HCC (17.8%) the trend partially reversed in women (21.7%). These findings are

counterintuitive and are without explanation. The number of women with HCC was relatively small so this finding needs further exploration in a larger cohort.

Neither of the previous studies explored sex-differences in the effects of *HSD17B13* rs72613567:TA.^{21,22} However, Ferenci and colleagues,³⁷ did find sex-related phenotypic variation associated with carriage of *HSD17B13* rs72613567:TA in patients with Wilson's disease. Thus, the MAF for this variant in men who developed fulminant Wilson's disease was 0% compared to a frequency of 13.6% in their female counterparts. Clearly, sex-gene variant interaction should be considered in any further genetic studies in the field of liver disease.

HSD17B13 belongs to the family of pluripotent 17-hydroxysteroid dehydrogenase enzymes which members convert 17-keto- and 17-hydroxysteroids, regulate the biological activity of sex hormones, participate in fatty acid and cholesterol metabolism, and contribute to bile acid synthesis.³⁸ The function of *HSD17B13* is incompletely understood, but it is located on the surface of lipid droplets and is mainly expressed in the liver. Su and colleagues³⁹ have demonstrated that transcriptional regulation of *HSD17B13* expression is likely liver X receptor (LXR)-mediated via a sterol-regulatory-element binding protein 1c (SREBP-1c)-dependent mechanism; they also found evidence for a SREBP-1c response element (SRE) in the promoter region of the *HSD17B13* gene located on chromosome 4q22.1. *HSD17B13* expression is increased in patients with NAFLD^{40,41} and in murine models of fatty liver disease,⁴²⁻⁴⁴ and its overexpression in Huh-7 and HepG2 hepatoma cell lines result in accumulation of *HSD17B13* on the surface of lipid droplets.^{41,45}

Ma and coworkers⁴⁶ have recently shown that *HSD17B13* is a hepatic retinol dehydrogenase. The vitamin A metabolites retinal, retinoic acid and retinol binding protein have been implicated in the pathogenesis of hepatic steatosis, fibrosis, adipogenesis, and insulin resistance.⁴⁷ Thus, *HSD17B13* may be involved in the complex nuclear receptor interaction in

non-alcoholic fatty liver disease *via* activation of the retinoic acid receptor.⁴⁸ Of greater importance, in the context of the present study, is the possibility that *HSD17B13*, functioning as a retinol dehydrogenase, may contribute to the depletion of hepatic retinoic acid observed in individuals chronically misusing alcohol. Retinoic acid depletion results in a functional down regulation of liver retinoic acid receptors and a marked increase in the expression of the activator protein-1 (c-jun and c-fos) transcriptional complex which is associated with hepatic cell hyperproliferation, a decrease in apoptosis and stimulated hepatic carcinogenesis.^{49,50} The retinol dehydrogenase activity of the rs72613567:TA variant is likely reduced or absent and this may explain, at least in part, its protective effect against HCC in the context of alcohol-related cirrhosis. Accordingly, repletion of hepatic retinoic acid concentrations experimentally or in patients with HCC may have a therapeutic role.^{51, 52}

There is considerable interest in the possibility of pharmaceutical inhibition of *HSD17B13* would counteract the steatogenic effect of over expressed *HSD17B13*, and mimic the loss-of-function derived from the *HSD17B13* rs72613567:TA variant. For example, fenofibrate which is an agonist of the peroxisome proliferator-activated receptor- α (PPAR α) and is approved for use in humans, effectively suppresses hepatic *HSD17B13* expression in mouse liver,⁵³ a finding supported by the high expression of *HSD17B13* observed in *ppar α* knock-out mice.⁴⁵

This study has a number of strengths. The study cohorts were large and well-characterized and specifically selected to look at the genetic risks associated with development of alcohol-related cirrhosis and HCC in Caucasians of European origin. Controls were exercised for a number of factors known to affect the risk for developing cirrhosis and HCC such as age, sex, BMI and diabetes. Similar effect sizes were observed across all individual geographical cohorts excluding the possibility of heterogeneity and, thus, broadening the applicability of the

findings. The specificity of the genetic associations was explored by including a large control cohort of alcohol misusers who had no evidence of liver disease.

This study also has some limitations; first, it was cross-sectional and undertaken retrospectively, meaning that phenotypic data sets were not always complete. Second, it is likely that some of the patients with alcohol-related cirrhosis will develop HCC over time and equally likely that some of the individuals misusing alcohol might develop cirrhosis in the future, particularly if they continue to drink. However, the group differences were significant and robust to adjustment for confounders and these shifts would, if anything, serve to strengthen the findings. Finally, the findings can not be used to define the genetic risks associated with the development of cirrhosis and HCC in people with liver disease of different aetiologies or from outside of Europe.

In conclusion: *HSD17B13* rs72613567:TA protects against the development of alcohol-related cirrhosis and, at least in men, the subsequent development of HCC. Its carriage attenuates the increased risk associated with carriage of *PNPLA3*: *PNPLA3* rs738409:G. Combining phenotypic and genetic signatures to score risk could facilitate management of patients with alcohol-related liver disease. Further evaluation of the function of *HSD17B13* and the rs72613567 variant may identify suitable drug targets.

Acknowledgements

The authors thank the Clinical Research Support Service of the CHUV-UNIL, Lausanne, Switzerland for providing the infrastructure for patient recruitment and collecting phenotypic data.

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Legends to figures**Figure 1**

Forrest plot showing genotypic ORs for heterozygous and homozygous carriage of the *HSD17B13* rs72613567:TA allele. Homozygous carriage of *HSD17B13* rs72613567:T wild type is set as a reference.

Figure 2

A: Relative proportions of patients with alcohol-related cirrhosis (CIRR) according to gene signature comprised of *HSD17B13* rs72613567 and *PNPLA3* rs739409 genotypes in relation to alcohol misusers without liver disease (ALC).

B: Relative proportions of patients with HCC according to gene signature comprised of *HSD17B13* rs72613567 and *PNPLA3* rs739409 genotypes in relation to patients with alcohol-related cirrhosis (CIRR).

C: Proportions of HCC patients according to gene signature comprised of *HSD17B13* rs72613567 and *PNPLA3* rs739409 genotypes in relation to alcohol misusers with no liver disease (ALC).

Figure 3

A: Test for trend on the proportion of *HSD17B13* rs72613567 genotypes in the three cohorts showing a gradual decrease in TA allele carriage with escalating severity of liver disease.

B: Test for trend on the proportion of *HSD17B13* rs72613567 genotypes in women showing a decrease in TA carriage in patients with cirrhosis but not in patients with HCC.

C: Test for trend on the proportion of *HSD17B13* rs72613567 genotypes in men showing a gradual decrease of TA allele carriage with escalating severity of liver disease.

Supplementary Figure 1

A: Forrest plot showing unadjusted allelic ORs for carriage of the *HSD17B13* rs72613567:TA allele for each geographical cohort comparing patients with alcohol-related cirrhosis with HCC (HCC) with patient with alcohol-related cirrhosis without HCC (CIRR).

B: Forrest plot showing unadjusted allelic ORs for carriage of the *HSD17B13* rs72613567:TA allele stratified for each geographical cohort comparing patients with alcohol-related cirrhosis (CIRR) with alcohol misusers with no liver disease (ALC).

C: Forrest plot showing unadjusted allelic ORs for carriage of the *HSD17B13* rs72613567:TA allele stratified for each geographical cohort comparing patients with alcohol-related cirrhosis and HCC (HCC) with alcohol misusers with no liver disease (ALC).

Supplementary Figure 2

A: Forrest plot showing unadjusted allelic ORs for carriage of the *PNPLA3* rs738409:G allele stratified for each geographical cohort comparing patients with alcohol-related cirrhosis with HCC (HCC) with patient with alcohol-related cirrhosis without HCC (CIRR).

B: Forrest plot showing unadjusted allelic ORs for carriage of the *PNPLA3* rs738409:G allele stratified for each geographical cohort comparing patients with alcohol-related cirrhosis (CIRR) with alcohol misusers with no liver disease (ALC).

C: Forrest plot showing unadjusted allelic ORs for carriage of the *PNPLA3* rs738409:G allele stratified for each geographical cohort comparing patients with alcohol-related cirrhosis and HCC (HCC) with alcohol misusers with no liver disease (ALC).

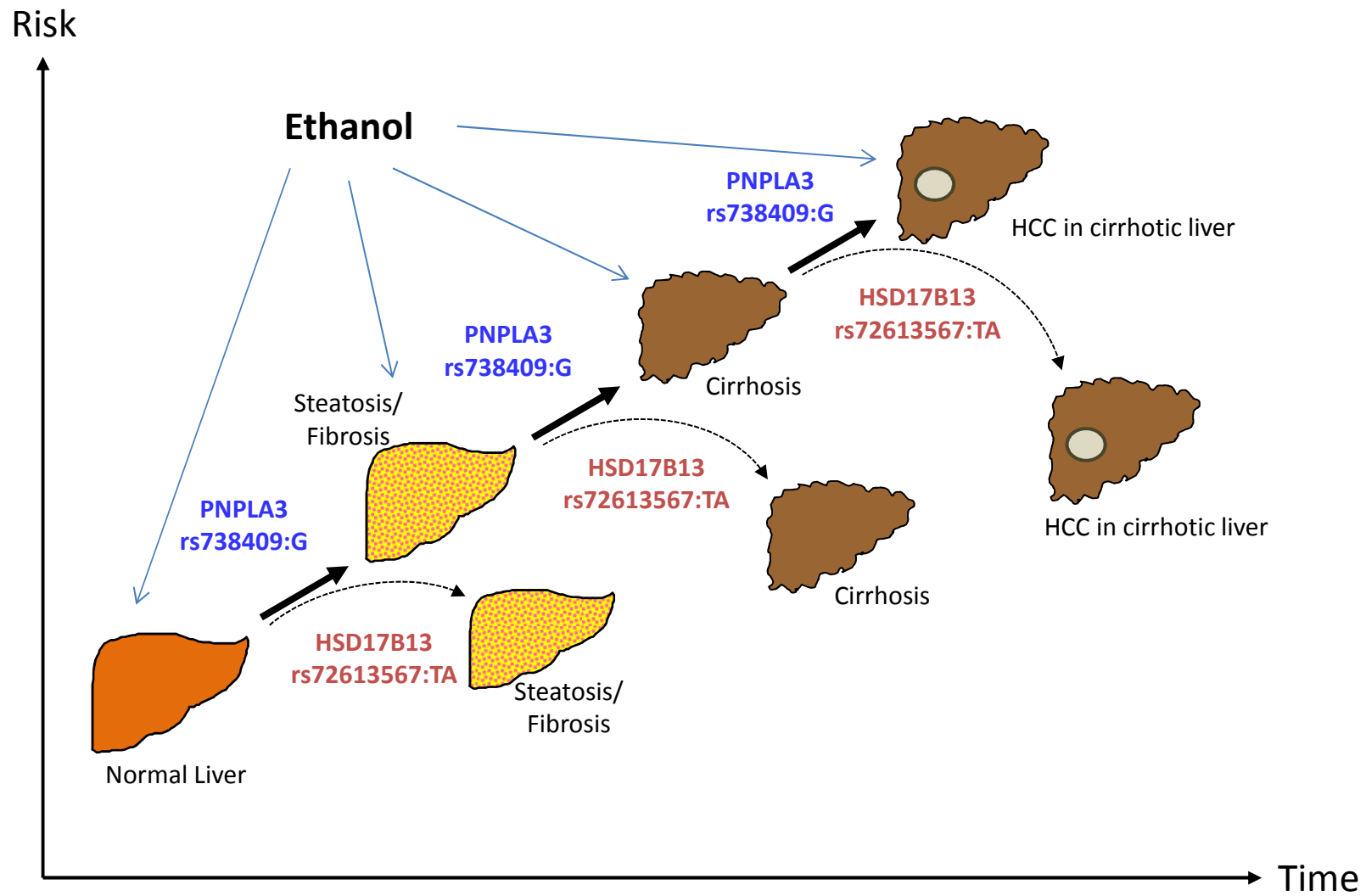


Table 1: Demographic characteristics of the study cohorts

Cohorts	Number	Age [SD]	Men (%)	BMI [SD]	T2DM
Alcohol-related cirrhosis with HCC (HCC)	1031	62 [10]*	91*	27.8 [4.8] *	45%*
Germany	778	61 [10]	91	27.8 [4.8]	45%
Switzerland	115	61 [11]	85	27.9 [5.0]	53%
United Kingdom [♦]	65	65 [9]	93	25.6 [3.1]	0%
Italy	73	72 [8]	92	N/A	47%
Alcohol-related cirrhosis without HCC (CIRR)	1653	55 [10][†]	72[†]	25.9 [4.9][†]	16%[†]
Germany	1050	56 [10]	72	26.2 [5.2]	22%
Switzerland	192	56 [10]	73	26.2 [5.8]	29%
United Kingdom*	376	53 [11]	68	24.6 [2.6]	N/A
Italy	35	54 [9]	86	N/A	24%
Alcohol misusers (ALC)	2588	48 [10]	84	24.7 [4.1]	4%
Germany	1827	48 [9]	88	24.8 [4.3]	4%
Switzerland	417	45 [12]	74	24.4 [4.0]	14%
United Kingdom	344	49 [10]	76	24.7 [2.3]	2%
Italy	N/A	N/A	N/A	N/A	N/A

BMI, body mass index; DMT2, type II diabetes mellitus; N/A, not available;

[♦] People with a BMI > 30 and/or with type II diabetes were excluded *a priori*;

Significance of the difference between patients with alcohol-related cirrhosis with and without HCC: *: $p < 0.001$;

Significance of the difference between patients with alcohol-related cirrhosis and alcohol misusers: [†] $p < 0.001$

Table 2: Univariate and multivariate analyses for association of *HSD17B13* rs72613567 with alcohol-related cirrhosis and HCC in the total European cohort

Cohorts	<i>HSD17B13</i> (rs72613567)	Comparative groups		Genotypic OR (95% CI) regression model: A	Genotypic OR (95% CI) regression model: B	Regression model	Allelic OR (95% CI)	Significance (P)	Cases/controls (n)
		HCC	CIRR						
Alcoholic-related cirrhosis and HCC (HCC)	T T	705	1027						
	T TA	287	524	0.80 (0.67-0.95)	0.92 (0.69-1.22)	A	0.77 (0.68-0.89)	2.27×10 ⁻⁴	1031/1653
	TA TA	39	102	0.56 (0.38-0.82)	0.43 (0.23-0.80)	B	0.79 (0.63-0.99)	0.037	604/679
	MAF	0.177	0.220	P = 1.05×10 ⁻³	P = 0.028				
Alcohol-related cirrhosis without HCC (CIRR)	T T	1027	1401						
	T TA	524	1009	0.71 (0.62-0.81)	0.79 (0.63-0.98)	A	0.79 (0.72-0.88)	8.13×10 ⁻⁶	1653/2588
	TA TA	102	178	0.78 (0.61-1.01)	0.82 (0.54-1.23)	B	0.85 (0.72-0.99)	0.048	679/1483
	MAF	0.220	0.264	P = 1.54×10 ⁻⁶	P = 0.085				
Alcohol misusers (ALC)	T T	705	1401						
	T TA	287	1009	0.57 (0.48-0.66)	0.68 (0.52-0.89)	A	0.60 (0.53-0.69)	1.69×10 ⁻¹⁴	1031/2588
	TA TA	39	178	0.44 (0.30-0.62)	0.39 (0.21-0.72)	B	0.66 (0.53-0.81)	1.14×10 ⁻⁴	604/1483
	MAF	0.177	0.264	P = 3.32×10 ⁻¹⁴	P = 6.00×10 ⁻⁴				

CI, confidence intervals; MAF, minor allele frequency; OR, odds ratio

Genotypic and allelic odds ratios were assessed by logistic regression models: model A - univariate logistic regression; model B –multivariate logistic regression adjusted for age, sex, body mass index, type 2 diabetes mellitus and country.

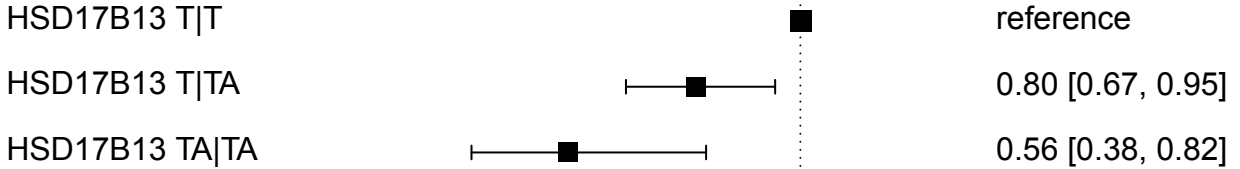
Table 3: Univariate and multivariate analyses for association of *PNPLA3* rs738409 with alcohol-related cirrhosis and HCC in the total European cohort

Cohorts	<i>PNPLA3</i> (rs738409)	Comparative groups		Genotypic OR (95% CI) regression model: A	Genotypic OR (95% CI) regression model: B	Regression model	Allelic OR (95% CI)	Significance (P)	Cases/controls (n)
		HCC	CIRR						
Alcohol-related cirrhosis with HCC (HCC)									
	C C	269	697						
	C G	497	714	1.80 (1.50-2.16)	1.75 (1.30-2.36)	A	1.77 (1.58-1.98)	2.31×10 ⁻²³	1030/1631
	G G	264	220	3.11 (2.48-3.90)	2.75 (1.88-4.01)	B	1.67 (1.39-2.01)	7.19×10 ⁻⁸	603/660
	MAF	0.498	0.354	P = 2.98×10 ⁻²²	P = 4.37×10 ⁻⁷				
Alcohol-related cirrhosis without HCC (CIRR)		CIRR	ALC						
	C C	697	1364						
	C G	714	802	1.74 (1.52-2.00)	1.64 (1.31-2.06)	A	1.70 (1.54-1.88)	1.52×10 ⁻²⁶	1631/2319
	G G	220	153	2.81 (2.25-3.53)	2.65 (1.85-3.78)	B	1.63 (1.39-1.91)	1.67×10 ⁻⁹	660/1469
	MAF	0.354	0.239	P = 1.61×10 ⁻²⁵	P = 1.31×10 ⁻⁸				
Alcohol misuse (ALC)		HCC	ALC						
	C C	269	1364						
	C G	497	802	3.14 (2.65-3.73)	2.75 (2.06-3.66)	A	3.00 (2.67-3.36)	2.17×10 ⁻⁷⁹	1030/2319
	G G	264	153	8.75 (6.90-11.10)	6.76 (4.60-9.95)	B	2.63 (2.18-3.17)	3.89×10 ⁻²⁴	603/1469
	MAF	0.498	0.239	P = 4.14×10 ⁻⁷⁸	P = 5.50×10 ⁻²³				

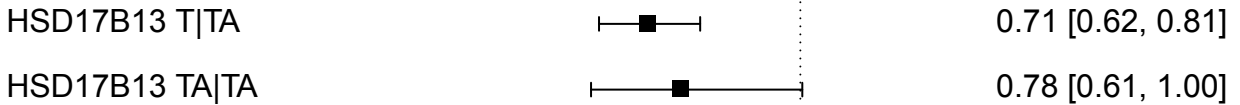
CI, confidence intervals; MAF, minor allele frequency; OR, odds ratio

Genotypic and allelic odds ratios were assessed by logistic regression models: model A - univariate logistic regression; model B –multivariate logistic regression adjusted for age, sex, body mass index, type 2 diabetes mellitus and country

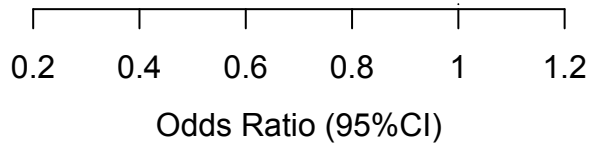
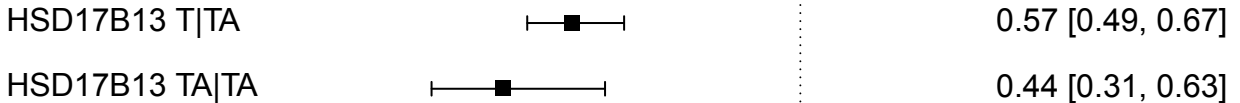
HCC vs. CIRR



CIRR vs. ALC

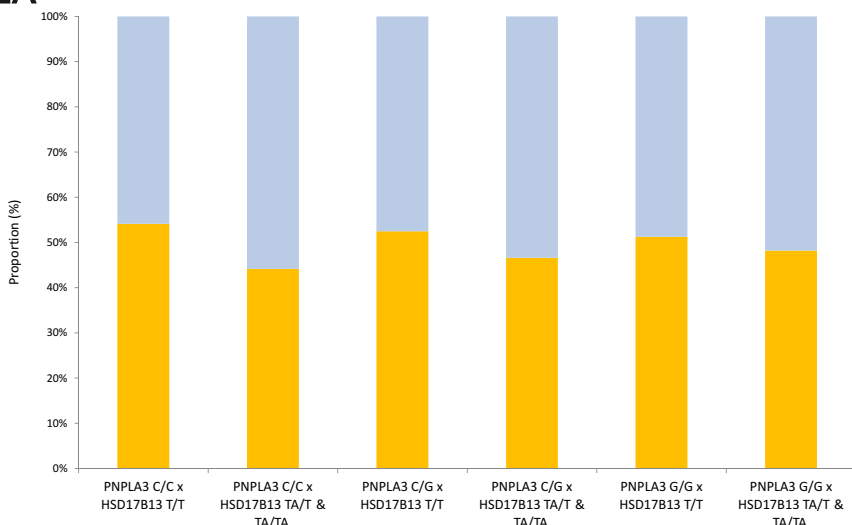


HCC vs. ALC

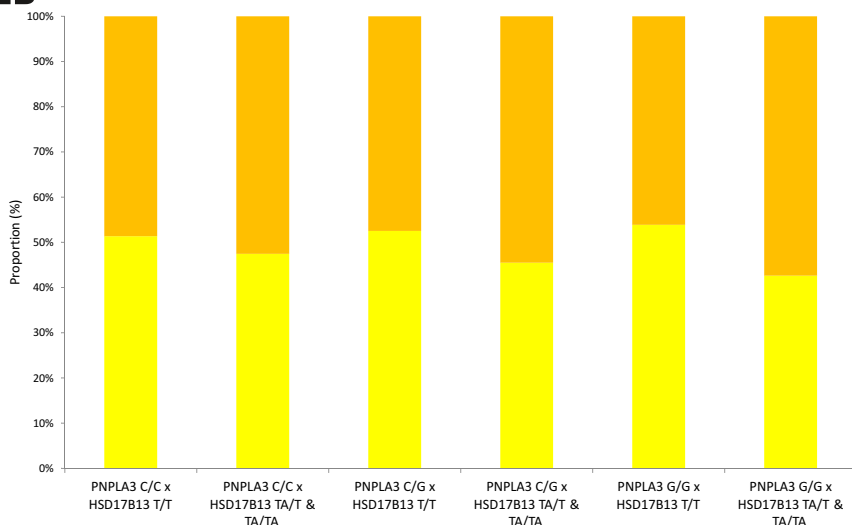


2A

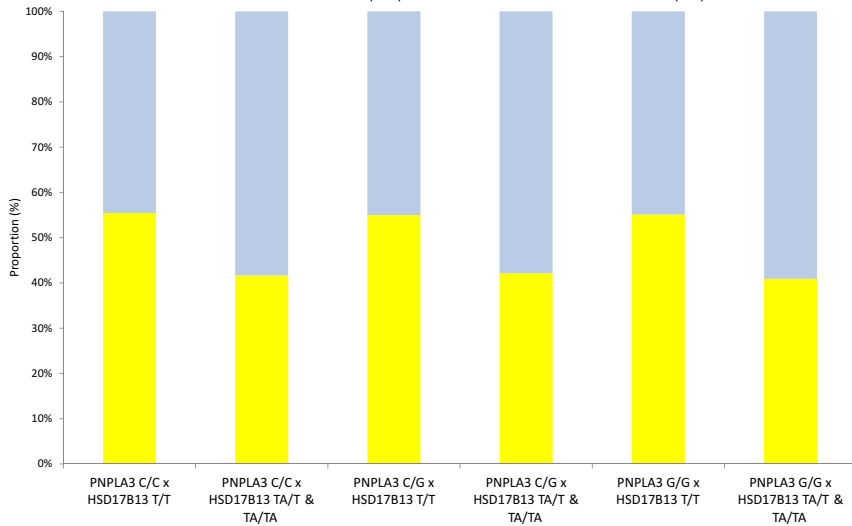
■ Alcohol-related cirrhosis (CIRR) ■ Alcohol misusers with no liver disease (ALC)

**2B**

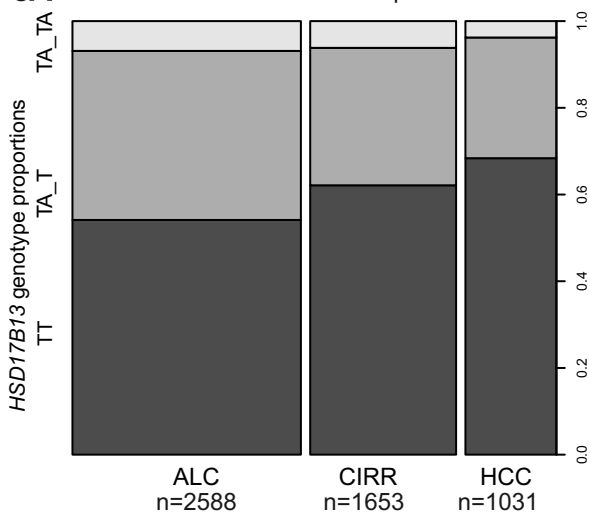
■ Alcoholic cirrhosis with HCC (HCC) ■ Alcohol-related cirrhosis (CIRR)

**2C**

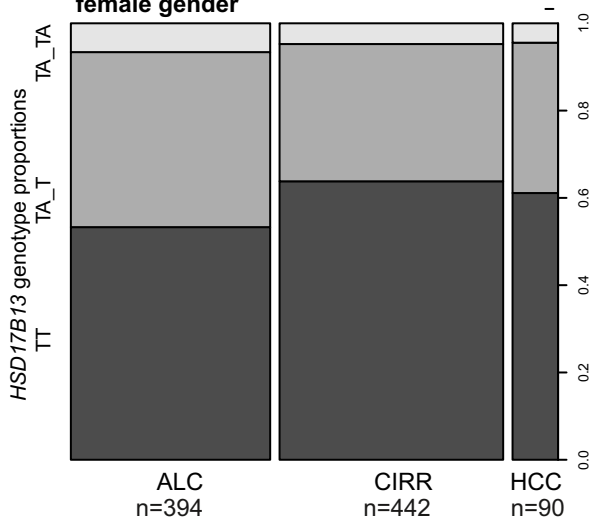
■ Alcoholic cirrhosis with HCC (HCC) ■ Alcohol misusers with no liver disease (ALC)



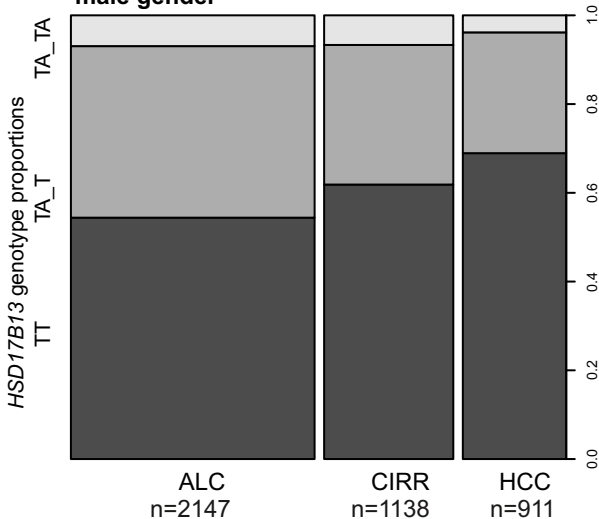
3A Mantel-Haenszel test of trend $p = 1.09 \times 10^{-15}$



3B Mantel-Haenszel test of trend $p = 0.011$
female gender



3C Mantel-Haenszel test of trend $p = 1.61 \times 10^{-13}$
male gender



Supplementary Table 1: Genotype counts and allelic frequencies of the *HSD17B13* and *PNPLA3* loci

Cohorts	<i>HSD17B13</i> (rs72613567)					<i>PNPLA3</i> (rs738409)				
	Number	T T	T TA	TA TA	MAF	Number	C C	C G	G G	MAF
Alcohol-related cirrhosis with HCC (HCC)	1031	705	287	39	0.177	1030	269	497	264	0.498
Germany	778	510	240	28	0.190	779	193	382	204	0.507
Switzerland	115	83	28	4	0.157	114	35	53	26	0.461
United Kingdom	65	54	8	3	0.108	63	28	21	14	0.389
Italy	73	58	11	4	0.130	74	13	41	20	0.547
Alcohol-related cirrhosis without HCC (CIRR)	1653	1027	524	102	0.220	1631	697	714	220	0.354
Germany	1050	655	330	65	0.219	1044	397	482	165	0.389
Switzerland	192	126	55	11	0.201	191	79	89	23	0.353
United Kingdom	376	224	126	26	0.237	361	208	128	25	0.247
Italy	35	22	13	0	0.186	35	13	15	7	0.414
Alcohol misusers (ALC)	2588	1401	1009	178	0.264	2319	1364	802	153	0.239
Germany	1827	974	721	132	0.270	1551	860	569	122	0.262
Switzerland	417	244	150	23	0.235	418	242	149	27	0.243
United Kingdom	344	183	138	23	0.267	350	262	84	4	0.131
Italy	N/A									
Healthy controls (HC)	1196	627	479	90	0.276*	1196	720	417	59	0.224[†]
United Kingdom	899	466	359	74	0.282	899	544	313	42	0.221
EUR 1000 Genomes (CEU, GBR, TSI) [♦]	297	161	120	16	0.256	297	176	104	17	0.232

MAF, minor allele frequency; N/A, not available

[♦]CEU: Utah residents with Northern and Western European ancestry; GBR: British in England and Scotland; TSI: Toskani in Italy²⁸

* Significance of the difference in MAF for *HSD17B13* rs72613567 between ALC and HC: $P_{\text{genotypic}} = 0.600$

[†] Significance of the difference in MAF for *PNPLA3* rs738409 between ALC and HC: $P_{\text{genotypic}} = 0.166$

Supplementary Table 2: Meta-analyses of the univariate analyses of the *HSD17B13* locus for association with alcohol-related cirrhosis and HCC in individual European cohorts

<i>HSD17B13</i> (rs72613567)		Univariate – all		Univariate – men		Univariate – women	
Alcohol-related cirrhosis with HCC (HCC)	vs CIRR	Allelic OR (95% CI)	Significance (P)	Allelic OR (95% CI)	Significance (P)	Allelic OR (95% CI)	Significance (P)
Fixed effect meta-analysis		0.79 (0.68-0.91)	8.47×10⁻⁴	0.76 (0.65-0.90)	8.94×10⁻⁴	1.21 (0.80-1.81)	0.366
		Heterogeneity	0.083	Heterogeneity	0.116	Heterogeneity	0.354
Germany		0.84 (0.72-0.99)	0.038	0.80 (0.67-0.96)	0.014	1.17 (0.75-1.83)	0.481
Switzerland		0.76 (0.50-1.15)	0.196	0.75 (0.48-1.17)	0.201	0.78 (0.22-2.76)	0.704
United Kingdom		0.39 (0.22-0.69)	0.001	0.30 (0.14-0.66)	0.003	3.24 (0.70-14.9)	0.133
Italy		0.66 (0.30-1.42)	0.282	0.85 (0.39-1.84)	0.676	low n	
Alcohol-related cirrhosis without HCC (CIRR)	vs ALC						
Fixed effect meta-analysis		0.79 (0.71-0.88)	6.75×10⁻⁶	0.82 (0.73-0.92)	8.35×10⁻⁴	0.70 (0.56-0.89)	2.78×10⁻³
		Heterogeneity	0.749	Heterogeneity	0.627	Heterogeneity	0.958
Germany		0.77 (0.68-0.87)	2.89×10 ⁻⁵	0.79 (0.69-0.91)	0.001	0.72 (0.54-0.97)	0.031
Switzerland		0.82 (0.61-1.10)	0.188	0.88 (0.63-1.24)	0.471	0.66 (0.36-1.21)	0.179
United Kingdom		0.85 (0.67-1.08)	0.179	0.91 (0.68-1.22)	0.511	0.68 (0.41-1.09)	0.110
Alcohol-related cirrhosis with HCC (HCC)	vs ALC						
Fixed effect meta-analysis		0.61 (0.54-0.70)	1.35×10⁻¹²	0.61 (0.52-0.70)	2.11×10⁻¹¹	0.85 (0.56-1.28)	0.433
		Heterogeneity	0.097	Heterogeneity	0.094	Heterogeneity	0.284
Germany		0.64 (0.55-0.74)	1.70×10 ⁻⁹	0.62 (0.53-0.73)	2.36×10 ⁻⁹	0.84 (0.54-1.32)	0.448
Switzerland		0.61 (0.41-0.90)	0.012	0.65 (0.43-0.98)	0.040	0.48 (0.13-1.75)	0.263
United Kingdom		0.33 (0.19-0.59)	9.62×10 ⁻⁵	0.25 (0.11-0.56)	0.001	2.79 (0.50-15.6)	0.243

ALC, alcohol misusers; CI, confidence intervals; OR, odds ratio.

Allelic odds ratios were assessed by univariate logistic regression.

Supplementary Table 3: Meta-analyses of the univariate analyses of the *PNPLA3* locus for association with alcohol-related cirrhosis and HCC in individual European cohorts

<i>PNPLA3</i> (rs738409)		Univariate – all		Univariate – men		Univariate – women	
		Allelic OR (95% CI)	Significance (P)	Allelic OR (95% CI)	Significance (P)	Allelic OR (95% CI)	Significance (P)
Alcoholic-related cirrhosis with HCC (HCC)	vs CIRR						
		1.64 (1.46-1.84)	5.99×10⁻¹⁷	1.62 (1.42-1.84)	2.51×10⁻¹³	1.58 (1.13-2.21)	0.007
Fixed effect meta-analysis		Heterogeneity	0.476	Heterogeneity	0.971	Heterogeneity	0.480
	Germany	1.60 (1.40-1.83)	4.63×10 ⁻¹²	1.62 (1.40-1.88)	1.97×10 ⁻¹⁰	1.60 (1.10-2.30)	0.013
	Switzerland	1.55 (1.11-2.17)	0.01	1.65 (1.13-2.39)	0.009	1.13 (0.45-2.80)	0.798
	United Kingdom	2.24 (1.49-3.35)	7.61×10 ⁻⁵	1.48 (0.91-2.39)	0.113	3.47 (0.71-16.9)	0.123
	Italy	1.67 (0.94-2.98)	0.080	1.79 (0.95-3.37)	0.070	low n	
Alcohol-related cirrhosis without HCC (CIRR)	vs ALC						
Fixed effect meta-analysis		1.79 (1.62-1.98)	1.29×10⁻²⁹	1.74 (1.55-1.95)	9.52×10⁻²¹	1.87 (1.48-2.37)	2.14×10⁻⁷
		Heterogeneity	0.419	Heterogeneity	0.771	Heterogeneity	0.876
	Germany	1.75 (1.56-1.97)	2.03×10 ⁻²⁰	1.74 (1.52-1.99)	9.63×10 ⁻¹⁶	1.79 (1.34-2.40)	8.35×10 ⁻⁵
	Switzerland	1.69 (1.30-2.21)	9.61×10 ⁻⁵	1.60 (1.18-2.17)	2.28×10 ⁻³	1.99 (1.16-3.43)	0.012
	United Kingdom	2.12 (1.61-2.80)	7.38×10 ⁻⁸	1.89 (1.35-2.65)	2.12×10 ⁻⁴	2.09 (1.14-3.85)	0.018
Alcoholic-related cirrhosis with HCC (HCC)	vs ALC						
Fixed effect meta-analysis		2.86 (2.54-3.22)	9.60×10⁻⁶⁹	2.74 (2.41-3.11)	5.19×10⁻⁵⁵	2.98 (2.02-4.40)	4.27×10⁻⁸
		Heterogeneity	0.0502	Heterogeneity	0.788	Heterogeneity	0.423
	Germany	2.78 (2.43-3.17)	8.34×10 ⁻⁵²	2.78 (2.41-3.20)	6.21×10 ⁻⁴⁶	2.93 (1.90-4.51)	1.05×10 ⁻⁶
	Switzerland	2.56 (1.87-3.48)	2.98×10 ⁻⁹	2.47 (1.78-3.44)	7.36×10 ⁻⁸	2.45 (0.90-6.64)	0.079
	United Kingdom	4.74 (3.09-7.28)	3.92×10 ⁻¹⁴	2.92 (1.72-4.96)	7.54×10 ⁻⁵	12.4 (1.33-115.4)	0.027

ALC, alcohol misusers; CI, confidence intervals; OR, odds ratio.

Allelic odds ratios were assessed by univariate logistic regression.

Supplementary Table 5: Interaction analysis of *PNPLA3* rs738409 and *HSD17B13* rs72613567 for risk association with alcohol-related cirrhosis (CIRR vs. ALC) in the total European cohort.

	Genes	Additive model		Genes	Additive model	
		P value	OR (95% CI)		P value	OR (95% CI)
Regression model A - without interaction term				Regression model A - with interaction term		
Main effect	<i>HSD17B13</i>	5.05×10 ⁻⁵	0.80 (0.72-0.89)	<i>HSD17B13</i>	4.15×10 ⁻⁵	0.74 (0.64-0.85)
Main effect	<i>PNPLA3</i>	2.97×10 ⁻²⁶	1.70 (1.54-1.87)	<i>PNPLA3</i>	1.77×10 ⁻¹³	1.59 (1.41-1.80)
Interaction term	N/A			<i>HSD17B13</i>×<i>PNPLA3</i>	0.087	1.15 (0.980-1.35)
	Genes	Genotypic model		Genes	Genotypic model	
		P value	OR (95% CI)		P value	OR (95% CI)
Regression model A - without interaction term				Regression model A - with interaction term		
Main effect term	<i>HSD17B1</i>	2.81×10 ⁻⁵		<i>HSD17B1</i>	1.61×10 ⁻⁴	
	<i>HSD17B13</i> (T/TA)	8.26×10 ⁻⁶	0.67 (0.55-0.82)	<i>HSD17B13</i> (T/TA)	8.15×10 ⁻⁵	0.67 (0.55-0.82)
	<i>HSD17B13</i> (TA/TA)	0.06	0.66 (0.44-0.98)	<i>HSD17B13</i> (TA/TA)	0.038	0.66 (0.44-0.98)
Main effect term	<i>PNPLA3</i>	4.75×10 ⁻²⁵		<i>PNPLA3</i>	4.76×10 ⁻¹²	
	<i>PNPLA3</i> (C/G)	2.04×10 ⁻¹⁵	1.74 (1.52-1.99)	<i>PNPLA3</i> (C/G)	7.86×10 ⁻⁸	1.63 (1.36-1.94)
	<i>PNPLA3</i> (G/G)	5.91×10 ⁻¹⁹	2.79 (2.22-3.50)	<i>PNPLA3</i> (G/G)	1.15×10 ⁻⁹	2.49 (1.86-3.35)
Interaction term	N/A			<i>HSD17B13</i>×<i>PNPLA3</i>	0.598	
Genotype interaction				<i>HSD17B13</i> (T/TA) by <i>PNPLA3</i> (C/G)	0.313	1.16 (0.87-1.56)
Genotype interaction				<i>HSD17B13</i> (T/TA) by <i>PNPLA3</i> (G/G)	0.397	1.23 (0.76-2.01)
Genotype interaction				<i>HSD17B13</i> (TA/TA) by <i>PNPLA3</i> (C/G)	0.415	1.27 (0.72-2.25)
Genotype interaction				<i>HSD17B13</i> (TA/TA) by <i>PNPLA3</i> (G/G)	0.199	1.82 (0.73-4.51)

OR, odds ratio. *P* values, genotypic and allelic odds ratios were assessed by logistic regression models: model A - univariate logistic regression.

Supplementary Table 4: Interaction analysis of *PNPLA3* rs738409 and *HSD17B13* rs72613567 for risk association with alcohol-related HCC (HCC vs. CIRR) in the male European cohort.

	Genes	Additive model		Genes	Additive model		
		P value	OR (95% CI)		P value	OR (95% CI)	
Regression model A - without interaction term				Regression model A - with interaction term			
Main effect	<i>HSD17B13</i>	1.98×10 ⁻⁴	0.75 (0.64-0.87)	<i>HSD17B13</i>	0.171	0.84 (0.66-1.08)	
Main effect	<i>PNPLA3</i>	1.84×10 ⁻¹⁸	1.77 (1.56-2.01)	<i>PNPLA3</i>	1.74×10 ⁻¹⁵	1.87 (1.64-2.16)	
Interaction term	N/A			<i>HSD17B13</i>×<i>PNPLA3</i>	0.204	0.87 (0.70-1.08)	
		Genotypic model			Genotypic model		
Regression model A - without interaction term		P value	OR (95% CI)	Regression model A with interaction term		P value	OR (95% CI)
Main effect term	<i>HSD17B1</i>	0.001		<i>HSD17B1</i>	0.374		
	<i>HSD17B13</i> (T/TA)	0.013	0.78 (0.64-0.95)	<i>HSD17B13</i> (T/TA)	0.327	0.84 (0.53-1.58)	
	<i>HSD17B13</i> (TA/TA)	0.002	0.51 (0.33-0.77)	<i>HSD17B13</i> (TA/TA)	0.258	0.65 (0.11-1.27)	
Main effect term	<i>PNPLA3</i>	1.80×10 ⁻¹⁷		<i>PNPLA3</i>	1.72×10 ⁻¹³		
	<i>PNPLA3</i> (C/G)	5.59×10 ⁻⁹	1.83 (1.49-2.25)	<i>PNPLA3</i> (C/G)	5.70×10 ⁻⁷	1.95 (1.32-2.89)	
	<i>PNPLA3</i> (G/G)	1.42×10 ⁻¹⁷	3.09 (2.38-4.00)	<i>PNPLA3</i> (G/G)	8.85×10 ⁻¹⁴	3.57 (2.11-6.03)	
Interaction term	N/A			<i>HSD17B13</i>×<i>PNPLA3</i>	0.689		
Genotype interaction				<i>HSD17B13</i> (T/TA) by <i>PNPLA3</i> (C/G)	0.680	0.91 (0.58-1.43)	
Genotype interaction				<i>HSD17B13</i> (T/TA) by <i>PNPLA3</i> (G/G)	0.581	0.85 (0.48-1.52)	
Genotype interaction				<i>HSD17B13</i> (TA/TA) by <i>PNPLA3</i> (C/G)	0.759	0.86 (0.33-2.24)	
Genotype interaction				<i>HSD17B13</i> (TA/TA) by <i>PNPLA3</i> (G/G)	0.167	0.43 (0.13-1.43)	

OR, odds ratio. *P* values, genotypic and allelic odds ratios were assessed by logistic regression models: model A - univariate logistic regression.

Supplementary Table 6: Univariate and multivariate analyses for association of *HSD17B13* rs72613567 with alcohol-related cirrhosis and HCC in the total European cohort by gender

Cohorts	Gender	HSD17B13 (rs72613567)	Comparative groups		Genotypic OR (95% CI) regression model: A	Genotypic OR (95% CI) regression model: B	Regression model	Allelic OR (95% CI)	P value	N cases / controls
			HCC	CIRR						
Alcoholic-related cirrhosis and HCC (HCC)	male	T T	628	704						
		T TA	248	358	0.78 (0.64-0.94)	0.85 (0.63-1.16)	A	0.75 (0.64-0.87)	1.72×10 ⁻⁴	911/1138
		TA TA	35	76	0.52 (0.34-0.78)	0.37 (0.19-0.71)	B	0.73 (0.57-0.92)	8.44×10 ⁻³	558/495
		MAF	0.175	0.224	P value =0.001	P value =0.009				
	female	T T	55	282						
		T TA	31	139	1.14 (0.70-1.86)	1.36 (0.64-2.90)	A	1.07 (0.73-1.58)	0.722	90/442
		TA TA	4	21	0.98 (0.32-2.96)	1.26 (0.18-8.78)	B	1.27 (0.67-2.39)	0.459	46/184
		MAF	0.217	0.205	P value =0.856	P value =0.719				
Alcohol-related cirrhosis without HCC (CIRR)	male		CIRR	ALC						
		T T	704	1168						
		T TA	358	830	0.72 (0.61-0.84)	0.79 (0.61-1.02)	A	0.82 (0.73-0.92)	0.001	1138/2147
		TA TA	76	149	0.85 (0.63-1.13)	0.90 (0.56-1.43)	B	0.87 (0.72-1.06)	0.170	495/1255
	MAF	0.224	0.263	P _{genotypic} =1.27×10 ⁻⁴	P value =0.204					
	female	T T	282	210						
		T TA	139	158	0.66 (0.49-0.88)	0.80 (0.51-1.25)	A	0.71 (0.57-0.89)	0.003	442/394
		TA TA	21	26	0.60 (0.33-1.10)	0.53 (0.21-1.34)	B	0.76 (0.54-1.08)	0.127	184/228
MAF		0.205	0.266	P value =0.009	P value =0.302					

ALC, Alcohol misuse; BMI, body mass index; CIRR, cirrhosis; OR, odds ratio; MAF minor allele frequency. P values, genotypic and allelic odds ratios were assessed by logistic regression models: model A - univariate logistic regression; model B – fully adjusted model: with adjustments for age, BMI, T2DM and cohort.

Supplementary Table 7: Univariate and multivariate analyses for association of *PNPLA3* rs738409 with alcohol-related cirrhosis and HCC in the total European cohort by gender

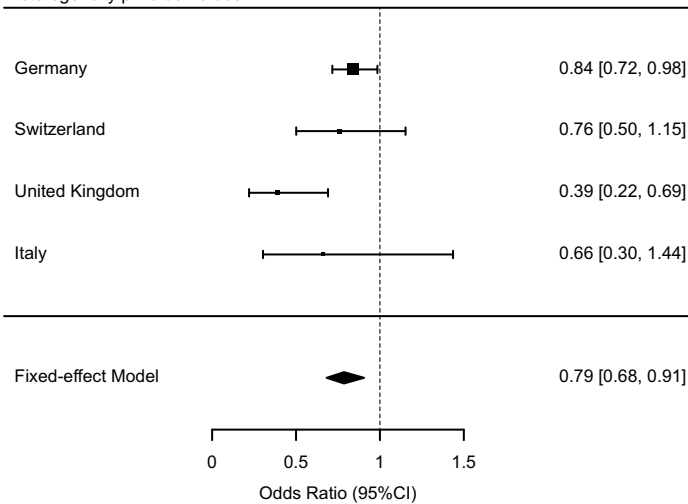
Cohorts	Gender	PNPLA3 (rs738409)	Comparative groups		Regression model	Allelic OR (95% CI)	P value	N cases / controls
			HCC	CIRR				
Alcoholic-related cirrhosis and HCC (HCC)	male		HCC	CIRR				
		C C	238	476	A	1.75 (1.55-1.99)	2.81×10 ⁻¹⁸	910/1119
		C G	440	491	B	1.76 (1.44-2.16)	3.80×10 ⁻⁸	557/479
		G G	232	152				
		MAF	0.497	0.355				
	female		HCC	CIRR				
		C C	23	190	A	1.75 (1.27-2.40)	0.001	90/439
		C G	44	186	B	1.14 (0.69-1.91)	0.606	46/181
		G G	23	63				
		MAF	0.500	0.355				
Alcohol-related cirrhosis without HCC (CIRR)	male		CIRR	ALC				
		C C	476	1120	A	1.69 (1.51-1.89)	8.50×10 ⁻²⁰	1119/1910
		C G	491	659	B	1.55 (1.29-1.87)	2.89×10 ⁻⁶	479/1287
		G G	152	131				
		MAF	0.355	0.241				
	female		CIRR	ALC				
		C C	190	221	A	1.91 (1.53-2.39)	1.31×10 ⁻⁸	439/363
		C G	186	125	B	1.80 (1.30-2.50)	4.39×10 ⁻⁴	195/232
		G G	63	17				
		MAF	0.355	0.219				

ALC, Alcohol misuse; BMI, body mass index; CIRR, cirrhosis; OR, odds ratio; MAF minor allele frequency. *P* values, genotypic and allelic odds ratios were assessed by logistic regression models: model A - univariate logistic regression; model B - with adjustments for age, country; model C – fully adjusted model: with adjustments for age, BMI, T2DM and country.

A *HSD17B13* rs72613567:TA HCC vs. CIRR

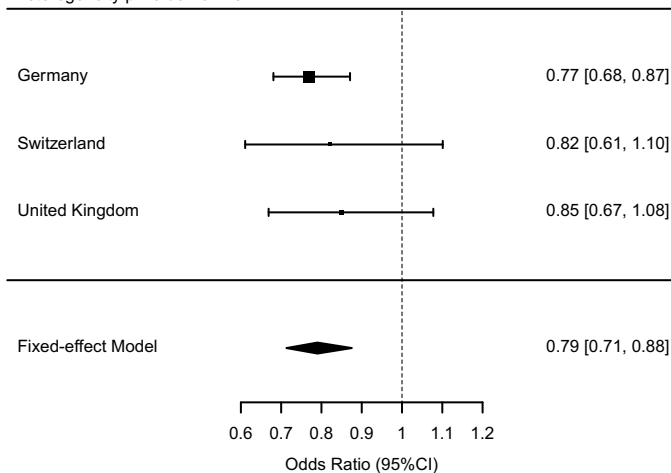
Association p-value= 0.000847

Heterogeneity p-value= 0.0834

**B** *HSD17B13* rs72613567:TA CIRR vs. ALC

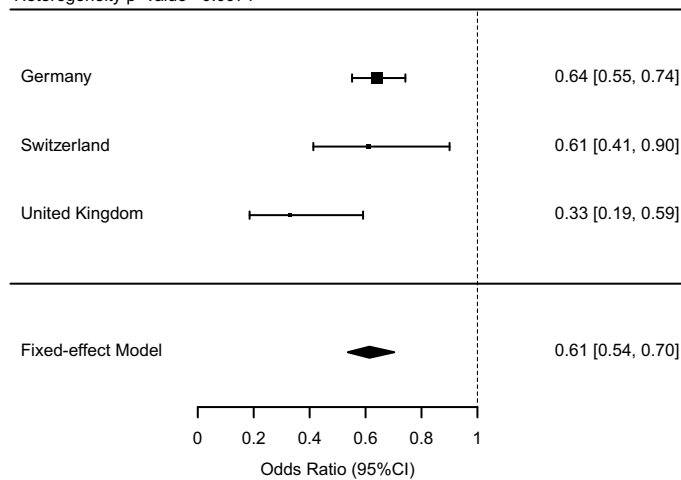
Association p-value= 6.754e-06

Heterogeneity p-value= 0.748

**C** *HSD17B13* rs72613567:TA HCC vs. ALC

Association p-value= 1.352e-12

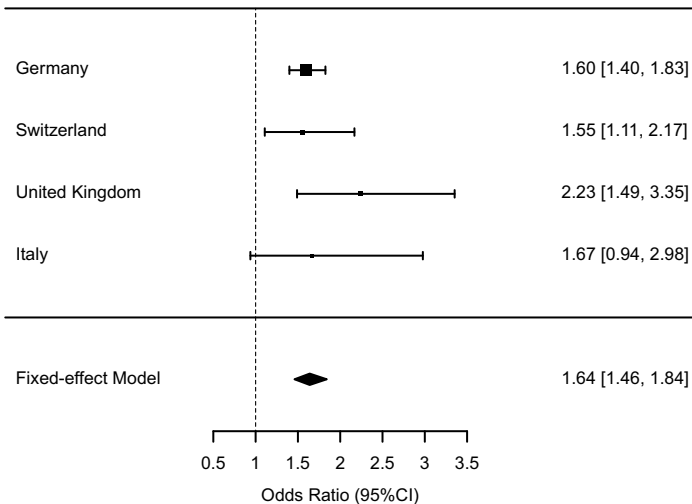
Heterogeneity p-value= 0.0971



A*PNPLA3* rs738409:G HCC vs. CIRR

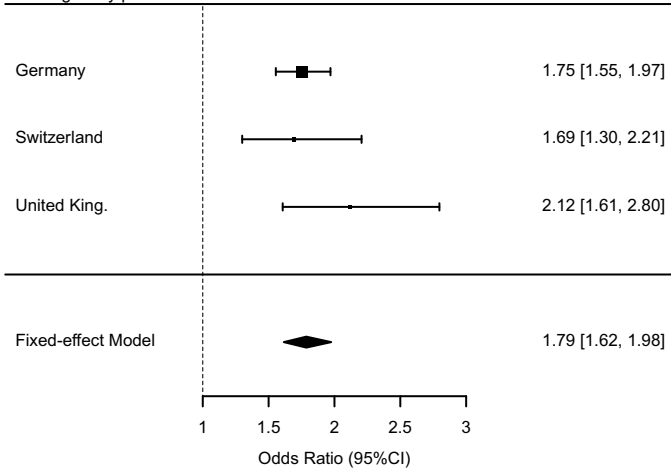
Association p-value= 5.988-17

Heterogeneity p-value= 0.4758

**B***PNPLA3* rs738409:G CIRR vs. ALC

Association p-value= 1.293e-29

Heterogeneity p-value= 0.4186

**C***PNPLA3* rs738409:G HCC vs. ALC

Association p-value= 9.602e-69

Heterogeneity p-value= 0.0502

