Impact of patatin-like phospholipase domain containing 3 rs738409 G/G genotype on hepatic decompensation and mortality in patients with portal hypertension

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Summary

Background: The rs738409 C>G p.I148M variant in the patatin-like phospholipase domain containing 3 (PNPLA3)-gene promotes triglyceride accumulation in hepatocytes and hepatic stellate cell activation and has previously been linked to hepatic steatosis/liver fibrosis.
Aim: To investigate its impact on hepatic decompensation and (liver-related) mortality in patients who had already developed portal hypertension. Moreover, we assessed its link with hepatic steatosis as evaluated by controlled attenuation parameter.
Methods: We performed a retrospective analysis in prospectively characterised patients with viral hepatitis/fatty liver disease-induced portal hypertension (hepatic venous pressure gradient [HVPG] ≥ 6 mm Hg) diagnosed at the Medical University of Vienna who underwent HVPG measurement (until 2013; n = 372; longitudinal study) or simultaneous HVPG and controlled attenuation parameter measurement (2014-2017; n = 153; cross-sectional study).
Results: While survival was similar between PNPLA3-C/C and -C/G patients, we observed substantially increased mortality in PNPLA3-G/G patients. PNPLA3-G/G had no impact on mortality in the subgroup of patients with viral hepatitis; however, we observed a strong independent association between PNPLA3-G/G and hepatic decompensation (adjusted sub-distribution hazard ratio [aSHR]: 2.1, 95% confidence interval [95% CI]: 1.1-4; P = 0.024) as well as mortality (overall: aSHR: 2.2, 95% CI: 1.22-3.98; P = 0.009; liver-related: aSHR: 2.2, 95% CI: 1.08-4.46; P = 0.029) in patients with fatty liver disease. Interestingly, even in the subgroup of patients who had already progressed to clinically significant portal hypertension (HVPG ≥ 10 mm Hg), PNPLA3-G/G substantially increased mortality (aSHR: 2.33, 95% CI: 1.27-4.29; P = 0.006). PNPLA3-genotype had no influence on controlled attenuation parameter or the prevalence of values ≥248 dB/m.
Conclusion: PNPLA3-G/G-genotype seems to double the risks of hepatic decompensation and (liver-related) mortality in patients with portal hypertension due to fatty liver disease. Further studies are warranted to investigate potential underlying pathophysiological mechanisms unrelated to hepatic steatosis.
1 | INTRODUCTION

The patatin-like phospholipase domain containing 3 (PNPLA3) protein is a lipase with activity towards triglycerides in hepatocytes as well as retinyl esters in hepatic stellate cells and experimental studies have shown that p.I148M substitution leads to a loss of function.

The PNPLA3 rs738409 G allele encoding the I148M variant (Human Genom Variation Society Nomenclature: NC_000022.10:g.44324727C>G) has been linked to non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) and disease severity, alcoholic liver disease (ALD)-induced cirrhosis and outcome after severe alcoholic hepatitis. Moreover, it has been linked to hepatic steatosis/liver fibrosis in hepatitis C virus-monoinfected and HIV/hepatitis C virus-coinfected patients, although its effects in viral hepatitis were less consistent when compared to patients with fatty liver disease. Finally, the PNPLA3 G allele has repeatedly been found to be associated with hepatocellular carcinoma (HCC) development.

Recent data indicates that the PNPLA3 G allele potentiates the proinflammatory and profibrogenic features of hepatic stellate cells. Hepatic stellate cells are not only a key player in liver fibrogenesis but also play an essential role in the perpetuation of portal hypertension. Due to its impact on hepatic stellate cell activation, the PNPLA3 G allele might promote liver disease progression, even beyond the initiation of liver fibrosis/portal hypertension, and thus, have prognostic implications in advanced chronic liver disease (ie in patients who have already developed portal hypertension).

We aimed to investigate the impact of the PNPLA3 G allele on (liver-related) mortality in our thoroughly characterised cohort of 372 patients who had already developed portal hypertension due to ALD/NAFLD or viral hepatitis (longitudinal study). To examine the underlying mechanism, we assessed whether PNPLA3 genotype is linked to hepatic steatosis, as evaluated by controlled attenuation parameter in a similar cohort of 153 consecutive patients (cross-sectional study).

2 | PATIENTS AND METHODS

2.1 | Study population

To investigate the impact of the PNPLA3 G allele on mortality, we performed a retrospective analysis in 372 prospectively characterised patients with portal hypertension (hepatic venous pressure gradient [HVPG] ≥ 6 mm Hg) diagnosed at the Medical University of Vienna through 2013 (cohort I; longitudinal study). Since the effect of PNPLA3 might vary throughout different aetiologies, only patients with ALD/NAFLD or viral hepatitis-induced portal hypertension were included. Aetiology was determined based on medical history at the time of HVPG measurement.

The impact of PNPLA3 genotype on hepatic steatosis was evaluated in a cohort of 153 prospectively characterised patients who underwent both HVPG and controlled attenuation parameter measurement between 2014 and 2017 (similar inclusion criteria; cohort II; cross-sectional study).

2.2 | PNPLA3 genotyping

PNPLA3 rs738409 genotyping was performed by a StepOnePlus Real-Time PCR System and a TaqMan SNP Genotyping Assay (Applied Biosystems, Foster City, CA, USA), using published sequences from the NCBI Entrez SNP Database (http://www.ncbi.nlm.nih.gov/sites/entrez): 5’-AAGAGGGATAAGGCCACTGTA-3’ as forward and 5’-CTTTCACAGGCCCTTGTATGTT-3’ as reverse primer.

2.3 | Hepatic venous pressure gradient measurement

The Vienna Hepatic Hemodynamic Laboratory at the Medical University of Vienna performed the HVPG measurements according to a standardised operating procedure. HVPG measurements were performed in the absence of nonselective beta blockers and nitrates. Clinically significant portal hypertension was defined by HVPG values ≥ 10 mm Hg.

2.4 | Definition of hepatic decompensation

Patients’ medical records were reviewed for the following events which defined (further) hepatic decompensation: Requirement of paracentesis, admission for grade 3/4 hepatic encephalopathy, variceal bleeding and liver-related death.

2.5 | Liver stiffness and controlled attenuation parameter measurement

Measurement of controlled attenuation parameter was performed after an overnight fast by transient elastography using a FibroScan 502 Touch (Echosens, Paris, France). Hepatic steatosis was graded according to cut-offs derived from a recent meta-analysis based on individual patient data: ≥ 248 dB × m⁻¹ for ≥ S1, ≥ 268 dB × m⁻¹ for ≥ S2 and ≥ 280 dB × m⁻¹ for S3.

2.6 | Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 24 (IBM, Armonk, NY, USA), GraphPad Prism 7 (GraphPad Software, La Jolla, CA, USA) and R 3.4.1 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Continuous variables were reported as mean ± standard error of the mean or median (interquartile range), while categorical variables were reported as number of patients with (proportion of patients with) the certain characteristic.

Student’s t test and one-way analysis of variance were used for group comparisons of continuous variables when applicable. Otherwise, Mann-Whitney U test and Kruskal-Wallis one-way analysis of variance were applied. Group comparisons of categorical variables were performed using Chi-squared or Fisher’s Exact test.

The effect of PNPLA3 genotype on transplant-free mortality was investigated using Kaplan-Meier analysis, log-rank test and Cox
regression. Patients entered the survival analyses at the time of HVPG measurement. Patients who received a liver transplantation were censored at the day of surgery. Transplant-free survival time was defined as the time to liver transplantation, death, or end of follow-up.

To further investigate the impact of PNPLA3 G/G genotype on hepatic decompensation, liver-related mortality as well as mortality, considering liver transplantation and, if applicable (nonliver-related) death as competing risks, we used Fine and Gray competing risk regression models (cmprsk: Subdistribution Analysis of Competing Risks; https://CRAN.R-project.org/package=cmprsk).22 Variables that showed differences between PNPLA3 genotypes or those which we considered highly relevant for the endpoint of interest were included as covariates in multivariate analyses.

A \( P \leq 0.05 \) was considered statistically significant.

2.7 | Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the ethics committee of the Medical University of Vienna (No. 1526/2017). All subjects were consented for genetic testing.

3 | RESULTS

3.1 | Characteristics of cohort I and comparison of PNPLA3 genotypes

A total of 372 patients had portal hypertension due to viral hepatitis \( n = 231 \) [62%] or fatty liver disease \( n = 141 \) [38%]; ALD: 104 [28%]/NAFLD: 37 [10%]; Table S1). The median HVPG and model for end-stage liver disease (MELD) score were 16 (10) mm Hg and 10 (4.7) points, respectively. Fifty-six (15%) patients harboured the PNPLA3 G/G genotype, while 163 (44%) and 153 (41%) had the C/C and the C/G genotype, respectively.

PNPLA3 G allele carriers were more commonly male, when compared to C/C patients (Table S1). Moreover, ALD/NAFLD was over-represented among patients harbouring a PNPLA3 G allele. PNPLA3 G allele carriers also showed more severe liver disease, as indicated by higher HVPG and MELD scores as well as lower serum albumin levels. A comparison between PNPLA3 G/G and non-G/G patients yielded similar differences (Table 1). Stratifying patients by aetiology (viral hepatitis and fatty liver disease), we observed trends towards a higher proportion of males and higher HVPG values among PNPLA3 G/G patients of both aetiologies (Table 1).

3.2 | Impact of PNPLA3 genotype on transplant-free mortality in cohort I

Patients were followed for a median of 27.4 (37.6) months. Twenty-five (7%) patients underwent liver transplantation, while 104 patients (28%) died.

While transplant-free survival was similar between PNPLA3 C/C and C/G patients (at 5 years: 72% vs 66% \( P = 0.635 \); Figure S1), we observed substantially impaired transplant-free survival in G/G patients (at 5 years: 47%; \( P = 0.026 \) vs C/C and \( P = 0.003 \) vs C/G). Thus, for further analyses, patients were grouped into PNPLA3 G/G and non-G/G.

Overall, PNPLA3 G/G genotype (adjusted hazard ratio [aHR]: 1.42, 95% confidence interval [95% CI]: 0.879-2.3; \( P = 0.151 \)) tended to increase transplant-free mortality in an analysis adjusted for age, sex, aetiology, HVPG, MELD and serum albumin levels (Table 2 and Figure 1).

Since previous studies on the impact of PNPLA3 on liver disease severity observed more consistent effects in fatty liver disease, as compared to viral hepatitis, we analysed these groups separately (Table 2 and Figure 1). While PNPLA3 showed no impact in patients with viral hepatitis \( n = 231 \); aHR: 0.845, 95% CI: 0.358-1.99; \( P = 0.7 \), harbouring the G/G genotype doubled the transplant-free mortality risk among patients with fatty liver disease \( n = 141 \); aHR: 2.16, 95% CI: 1.15-4.06; \( P = 0.017 \), independently of potential confounding factors (age, sex, HVPG, MELD and serum albumin levels). Interestingly, even in the subgroup of patients who had already progressed to clinically significant portal hypertension, PNPLA3 G/G genotype substantially increased transplant-free mortality \( n = 129 \); aHR: 2.11, 95% CI: 1.09-4.09; \( P = 0.027 \).

3.3 | Influence of PNPLA3 G/G genotype on hepatocellular carcinoma development, hepatic decompensation and liver-related mortality in patients with fatty liver disease

Among patients without a history of HCC, 5 (16%) and 9 (9%) patients were diagnosed with HCC during follow-up in the subgroup of patients with and without the PNPLA3 G/G genotype, respectively. HCC incidence rates were numerically higher among patients harbouring the PNPLA3 G/G genotype (8% per person-year; 95% CI: 3-17) when compared to non-G/G patients (4% per person-year; 95% CI: 2-7).

To further investigate the impact of PNPLA3 G/G genotype on mortality, we conducted a competing risk regression analysis adjusted for age, sex, aetiology, HVPG, MELD, and serum albumin levels, considering liver transplantation as a competing risk (Table 3). The mortality risk among patients harbouring the PNPLA3 G/G genotype was about twice as high when compared to non-G/G patients (adjusted subdistribution hazard ratio [aSHR]: 2.2, 95% CI: 1.22-3.98; \( P = 0.009 \)).

Among the 50 deaths in patients with fatty liver disease, 10 were nonliver-related (G/G: 18% \( [3/17] \); non-G/G: 21% \( [6/33] \)). Harbouring the PNPLA3 G/G genotype doubled the risk of liver-related mortality among patients with fatty liver disease (aSHR: 2.2, 95% CI: 1.08-4.46; \( P = 0.029 \)), considering liver transplantation and nonliver-related death as competing risks and adjusting for potential confounding factors (Table 3 and Figure 2).

In an analysis considering liver transplantation and nonliver-related death as competing risks, PNPLA3 G/G genotype was associated with a twofold increase in risk of hepatic decompensation...
TABLE 1  Comparison of patient characteristics between patatin-like phospholipase domain-containing protein 3 (PNPLA3) G/G and non-G/G in A the overall cohort, B among patients with viral hepatitis and C among patients with alcoholic (ALD)/non-alcoholic fatty liver disease (NAFLD) (cohort I)

| Patient characteristics | A | B | C |
|-------------------------|---|---|---|
|                         | All patients, n = 372 | Viral hepatitis, n = 231 | ALD/NAFLD, n = 141 |
| Age, years              | 52.5 (14.7) | 51.8 ± 0.7 | 58.6 (17.5) |
| Sex                     | 240 (76%) | 155 (75%) | 85 (77%) |
| Female                  | 76 (24%) | 51 (25%) | 25 (23%) |
| Aetiology               | 13 (4%) | 8 (4%) | 5 (5%) |
| ALD/NAFLD               | 110 (35%) | 5 (4%) | — |
| Viral                   | 206 (65%) | 9 (4%) | — |
| HCC                     | 15 (10) | 13 (9) | 18.4 ± 0.6 |
| HVPG, mm Hg             | 1.01 | 0.944-1.11 | 0.975 |
| MELD, points            | 10 (4) | 9 (4) | 11 (6.6) |
| Albumin, g x L⁻¹        | 37.2 (7.9) | 37.7 (7.4) | 35.8 (8.3) |

BMI, body mass index; HCC, hepatocellular carcinoma; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease.

TABLE 2  Cox regression analyses on the influence of patatin-like phospholipase domain-containing protein 3 (PNPLA3) G/G genotype on transplant-free survival in A the overall cohort, B among patients with viral hepatitis and C among patients with alcoholic (ALD)/non-alcoholic fatty liver disease (NAFLD) (cohort I)

| Patient characteristics | A | B | C |
|-------------------------|---|---|---|
|                         | All patients, n = 372 | Viral hepatitis, n = 231 | ALD/NAFLD, n = 141 |
| Age, years              | 1.03 | 1.03 | 1.04 |
| Sex, male vs female     | 0.689 | 0.648 | 0.619 |
| Aetiology, viral vs ALD/NAFLD | 0.651 | 0.648 | 0.575 |
| HVPG, per mm Hg         | 0.986 | 0.974 | 0.989 |
| MELD, per point         | 0.917 | 0.907 | 0.923 |
| Albumin, per g x L⁻¹    | 1.42 | 0.845 | 2.16 |
| PNPLA3 G/G              | 1.01-1.05 | 0.845-1.263 | 1.01-1.08 |
|                        | 0.15 | 0.202 | 0.288 |
|                        | 0.004 | 0.041 | 0.008 |
|                        | 0.079 | 0.079 | 0.356 |
|                        | 0.001 | 0.002 | 0.001 |
|                        | 0.151 | 0.7 | 0.017 |

HR, hazard ratio; 95% CI, 95% confidence interval; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease.

(aSHR: 2.1, 95% CI: 1.1-4; P = 0.024), after adjusting for potential confounding factors (Table 3 and Figure 2).

Finally, another competing risk regression analysis (considering liver transplantation as a competing risk) adjusted for age, sex, aetiology, HVPG, MELD and serum albumin levels restricted to patients who had already progressed to clinically significant portal hypertension, confirmed that harbouring the PNPLA3 G/G genotype substantially increased mortality (aSHR: 2.33, 95% CI: 1.27-4.29; P = 0.006) in this subgroup.

3.4 Impact of PNPLA3 genotype on hepatic steatosis in cohort II

Cohort II comprised 153 patients and was comparable to cohort I in terms of liver disease severity (median HVPG: 18 (10) mm Hg; median MELD: 10 (5) points; Table S2). The mean body mass index was 25.7 ± 0.4 kg m⁻², with 85 (56%) patients being overweight/obese (body mass index [BMI] ≥ 25 kg m⁻²). Twenty-four (16%) patients were obese (BMI ≥ 30 kg m⁻²) and 33 (22%) had diabetes mellitus type 2.

Similar to cohort I, we observed a trend towards an overrepresentation of fatty liver disease among patients harbouring a PNPLA3 G allele. Except for HCC prevalence, all other relevant patient characteristics (eg BMI) were comparable between PNPLA3 genotype groups.

Interestingly, PNPLA3 genotype had no influence on controlled attenuation parameter (C/C: 242 ± 7 vs C/G: 252 ± 7 vs G/G: 223 ± 13 dB m⁻¹; P = 0.639; Table S2 and Figure S2) or the prevalence of values ≥ 248 dB m⁻¹ (C/C: 28 [44%] vs C/G: 33 [54%] vs G/G: 12 [41%]; P = 0.443) in this population of patients with portal hypertension due to fatty liver disease or viral hepatitis.
DISCUSSION

Previous studies established firm associations between the PNPLA3 G allele and NAFLD/NASH and disease severity. More recently, the PNPLA3 G allele has also been linked to ALD-induced cirrhosis. As opposed to this, its effects in viral hepatitis were less consistent.

We observed an overrepresentation of ALD/NAFLD among carriers of the PNPLA3 G allele, supporting its particular relevance in these aetiologies (cohort I; trend in cohort II). This is in line with a study by Fatelli et al which also found a higher frequency of the PNPLA3 G allele in patients with cirrhosis due to fatty liver disease, compared to viral cirrhosis. Similarly, Friedrich and co-workers observed an increased prevalence of the PNPLA3 G/G genotype (vs a reference population) in ALD patients listed for liver transplantation. Of note, the highest frequency of the PNPLA3 G/G genotype was observed in patients with cryptogenic cirrhosis, which might have comprised a considerable proportion of patients with NAFLD.

While liver disease severity at the time of HVPG measurement showed a stepwise increase with each PNPLA3 G allele in cohort I, no such association was observed in cohort II. In our opinion, the cross-sectional findings on liver disease severity at the time of HVPG measurement should not be overinterpreted, since the duration of disease/time to cirrhosis are unknown. Furthermore, the numbers of patients with HCC at the time of HVPG measurement were small in both cohorts, which substantially limits the significance of our findings on HCC prevalence.

Although there is a broad body of evidence derived from cross-sectional analyses, comparatively few longitudinal studies assessed the impact of PNPLA3 genotype on clinical endpoints other than HCC development. Thus, the results of our study provide valuable

**FIGURE 1** A. Transplant-free survival in patients with the patatin-like phospholipase domain-containing protein 3 (PNPLA3) G/G genotype, or without (cohort I). B. Subgroup of patients with fatty liver disease (cohort I). aHR, adjusted hazard ratio; 95%CI, 95% confidence interval.

**TABLE 3** Competing risk regression analyses on the influence of patatin-like phospholipase domain-containing protein 3 (PNPLA3) G/G genotype on A mortality, B liver-related mortality and C hepatic decompensation in patients with alcoholic (ALD)/non-alcoholic fatty liver disease (NAFLD) (cohort I).

| Patient characteristics | A Mortality | B Liver-related mortality | C Hepatic decompensation |
|-------------------------|-------------|--------------------------|--------------------------|
|                         | SHR 95% CI  | P value                  | SHR 95% CI  | P value                  | SHR 95% CI  | P value                  |
| Age, years              | 1.04 1.01-1.08 | 0.011                  | 1.05 1.01-1.1 | 0.008                  | 1.03 0.998-1.05 | 0.068                |
| Sex, male vs female     | 0.713 0.344-1.48 | 0.36                  | 0.76 0.335-1.73 | 0.51                  | 1.83 0.943-3.57 | 0.074                |
| HVPG, per mmHg          | 0.972 0.918-1.03 | 0.34                  | 0.961 0.901-1.03 | 0.23                  | 1.01 0.956-1.07 | 0.66                 |
| MELD, per point         | 0.976 0.897-1.06 | 0.58                  | 1 0.921-1.09 | 0.98                  | 0.978 0.915-1.04 | 0.5                 |
| Albumin, per g x L^{-1} | 0.922 0.876-0.97 | 0.002                 | 0.925 0.876-0.978 | 0.006                | 0.951 0.911-0.944 | 0.025              |
| PNPLA3 G/G              | 2.2 1.22-3.98 | 0.009                  | 2.2 1.08-4.46 | 0.029                 | 2.1 1.1-4.4 | 0.024                 |

^aConsidering liver transplantation as a competing risk.

^bConsidering liver transplantation and nonliver-related death as competing risks.

SHR, subdistribution hazard ratio; 95% CI, 95% confidence interval; HVPG, hepatic venous pressure gradient; MELD, model for end-stage liver disease.
information on the prognostic impact of the PNPLA3 G allele in patients who have already developed portal hypertension.

Friedrich and colleagues investigated the effect of PNPLA3 genotype in patients listed for liver transplantation and reported increased risks of (further) hepatic decompensation (ie ascites and hepatic encephalopathy) and mortality in carriers of the G allele. However, since the proportion of patients who underwent liver transplantation differed substantially throughout the PNPLA3 genotypes, this study does not allow to draw conclusions on the impact of PNPLA3 genotype on mortality. Furthermore, due to the transplant setting, patients with ongoing alcohol consumption were excluded from this study, which might limit the generalisability of its findings. Following alcohol withdrawal, Rausch and colleagues observed a delayed regression of liver stiffness measured by transient elastography in PNPLA3 G allele carriers. Moreover, an analysis based on data of the Steroids or Pentoxifylline for Alcoholic Hepatitis (STOPAH) trial revealed an association between PNPLA3 genotype and severe alcoholic hepatitis development as well as medium-term mortality. This effect was limited to

![Graph A](image)

**FIGURE 2** A, Liver-related mortality and B, hepatic decompensation in fatty liver disease patients with the patatin-like phospholipase domain-containing protein 3 (PNPLA3) G/G genotype, or without, considering liver transplantation and nonliver-related death as competing risks (cohort I). aSHR, adjusted subdistribution hazard ratio; 95% CI, 95% confidence interval.
patients who were abstinent from drinking. However, the proportion of patients with underlying advanced chronic liver disease included in the STOP AH trial remains unclear. In our study, harbouring the PNPLA3 G/G genotype doubled the mortality risk of patients with portal hypertension due to (predominantly alcoholic) fatty liver disease (cohort I). Interestingly, this effect was also observed in the subgroup of patients with clinically significant portal hypertension, in whom splanchnic vasodilatation/hyperdynamic circulation, rather than "intrahepatic" factors such as PNPLA3, is considered as the main determinant of portal pressure. However, this paradigm has already been challenged by improvements in portal pressure after achieving sustained virologic response to hepatitis C treatments, which were observed throughout all strata of portal hypertension severity.

Although the numerically increased rates of HCC development among PNPLA3 G/G patients might have contributed to the observed association between PNPLA3 G/G and mortality (cohort I), considering the small number of patients who were diagnosed with HCC, differences in (liver-related) mortality cannot solely explained by HCC development.

To further explore the mechanism by which the PNPLA3 G/G genotype might promote hepatic decompensation and increase (liver-related) mortality in patients who have already developed portal hypertension, we analysed controlled attenuation parameter (possibly indicating hepatic steatosis) in a similar patient population (cohort II). Notably, PNPLA3 genotype had no impact on controlled attenuation parameter, which would suggest that its effect on hepatic steatosis is less pronounced in patients who have already progressed to advanced chronic liver disease. Importantly, previous studies have demonstrated the disappearance of hepatic steatosis with liver fibrosis progression in NAFLD patients (ie "burnt-out NASH"), a phenomenon which might also occur in ALD and might have attenuated the effect of PNPLA3 genotype on controlled attenuation parameter in our study, although the proportion of patients with controlled attenuation parameter values indicative of hepatic steatosis was still 48%. Moreover, there is only limited data on the performance of controlled attenuation parameter for diagnosing hepatic steatosis in patients with advanced chronic liver disease; however, it was not impacted by the presence of cirrhosis in a recent meta-analysis of individual patient data. In addition, its diagnostic performance seemed to be similar among ALD patients with advanced liver fibrosis or without. Considering these potential limitations as well as the small sample size of cohort II, our findings should be interpreted with caution and require confirmation.

Until recently, the impact of PNPLA3 genotype on hepatic lipid metabolism, and thus, hepatic steatosis, was considered as the primary explanation for its association with liver disease and its progression. Notably, Pingitore et al as well as Bruschi et al demonstrated that the PNPLA3 G allele enhances proinflammatory and profibrogenic features of hepatic stellate cells. Although the association between the PNPLA3 G/G genotype and increased transplant-free mortality in patients with fatty liver disease was independent of baseline HVPG in our study (cohort I), harbouring the PNPLA3 G/G genotype might have potentiated liver disease progression and aggravated portal hypertension during follow-up.

The main limitation of this study is its retrospective design; however, patients were prospectively characterised at the time of HVPG measurement. Moreover, the number of patients with fatty liver disease included in cohort I was limited and our study did not include a validation cohort. Aetiology was determined based on medical history and the diagnostic work up did not include a liver biopsy in most cases. In addition, alcohol abstinence was not systematically assessed. Finally, the impact of PNPLA3 genotype on clinical events and controlled attenuation parameter was not investigated in the same patient cohort, since controlled attenuation parameter became available in 2014 at our institution. However, inclusion criteria and study setting were exactly the same for both cohorts, which resulted in similar patient characteristics. Analysing clinical events in patients evaluated before 2014 allowed for a longer follow-up duration, a higher number of clinical events, and thus, more robust estimates of the impact of PNPLA3 genotype.

In summary, PNPLA3 G/G genotype seems to double the risks of liver-related morbidity and mortality in patients with portal hypertension due to fatty liver disease, even after the development of clinically significant portal hypertension. Further studies are warranted to investigate potential underlying pathophysiological mechanisms unrelated to hepatic steatosis.

AUTHORSHIP

Guarantor of the article: Arnulf Ferlitsch.

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SUPPORTING INFORMATION

Additional supporting information will be found online in the Supporting Information section at the end of the article.

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