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PNPLA3 is the dominant SNP linked to liver disease severity at time of first referral to a tertiary center

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Abstract: BACKGROUND Single nucleotide polymorphisms (SNPs) in genes including PNPLA3, TM6SF2, HSD17B13 and SERPINA1 have been identified as risk modifiers of progression in chronic liver disease (CLD). However, it is unclear whether genotyping for these risk variants is useful in clinical routine. METHODS Liver disease severity was assessed by liver stiffness measurement (LSM) and by presence of clinical manifestations of advanced-chronic liver disease (ACLD) in 779 consecutive CLD patients at the time of referral to a tertiary center. The associations of risk variants with CLD severity were calculated individually and in a combined model using a polygenic risk-score. RESULTS Non-alcoholic fatty liver disease (NAFLD) was the most common etiology (n = 511, 65.6%), and ACLD was present in 217 (27.9%) patients. The PNPLA3-G-allele remained independently associated with higher LSM (adjusted-B: 2.508 [95%CI: 0.887-4.130], P = 0.002) or the presence of ACLD (aOR: 1.562 [95%CI: 1.097-2.226], P = 0.013). SERPINA1-Z-allele was also independently associated with LSM (adjusted-B: 4.558 [95%CI: 1.182-7.934], P = 0.008), while the other risk alleles did not attain statistical significance. Combining these risk alleles into a polygenic risk-score was significantly associated with LSM (adjusted-B: 0.948 [95%CI: 0.153-1.743], P = 0.020). CONCLUSION PNPLA3 risk-variants are linked to liver disease severity at the time of first referral to an outpatient hepatology clinic.

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Liver, Pancreas and Biliary Tract

PNPLA3 is the dominant SNP linked to liver disease severity at time of first referral to a tertiary center

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ABSTRACT

Background: Single nucleotide polymorphisms (SNPs) in genes including *PNPLA3*, *TM6SF2*, *HSD17B13* and *SERPINA1* have been identified as risk modifiers of progression in chronic liver disease (CLD). However, it is unclear whether genotyping for these risk variants is useful in clinical routine.

Methods: Liver disease severity was assessed by liver stiffness measurement (LSM) and by presence of clinical manifestations of advanced-chronic liver disease (ACLD) in 779 consecutive CLD patients at the time of referral to a tertiary center. The associations of risk variants with CLD severity were calculated individually and in a combined model using a polygenic risk-score.

Results: Non-alcoholic fatty liver disease (NAFLD) was the most common etiology ($n = 511$, 65.6%), and ACLD was present in 217 (27.9%) patients. The *PNPLA3*-G-allele remained independently associated with higher LSM (adjusted-B: 2.508 [95%CI: 0.887–4.130], $P = 0.002$) or the presence of ACLD (aOR: 1.562 [95%CI: 1.097–2.226], $P = 0.013$). *SERPINA1*-Z-allele was also independently associated with LSM (adjusted-B: 4.558 [95%CI: 1.182–7.934], $P = 0.008$), while the other risk alleles did not attain statistical significance. Combining these risk alleles into a polygenic risk-score was significantly associated with LSM (adjusted-B: 0.948 [95%CI: 0.153–1.743], $P = 0.020$).

Conclusion: *PNPLA3* risk-variants are linked to liver disease severity at the time of first referral to an outpatient hepatology clinic.

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1. Introduction

Several single nucleotide polymorphisms (SNPs) have been identified and confirmed to modify the risk of progression in chronic liver disease (CLD) of different etiologies. In the first place, the rs738409 G-allele encoding the I148M variant of *patatin-like phospholipase domain-containing protein 3* (*PNPLA3*) was linked with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH) in the general population [1,2]. Moreover, it has been shown to increase the progression to cirrhosis in

alcoholic liver disease (ALD)- [3–6] and alcoholic steatohepatitis (ASH) [7], but also in patients with chronic hepatitis C [8], and to augment the risk for developing hepatocellular carcinoma (HCC) [9,10]. Further genome-wide association studies have linked the *transmembrane 6 superfamily 2* (*TM6SF2*) rs58542926 variant to NAFLD severity [11], and the rs72613567 TA-allele of the *17 β -hydroxysteroid dehydrogenase type 13* (*HSD17B13*) gene to a reduced risks of ALD, chronic hepatitis C, NAFLD and NAFLD-associated cirrhosis [12,13]. Finally, the *SERPINA1* p.E342K allele has recently been associated with disease severity and an increased risk of developing cirrhosis in patients with NAFLD and ALD [14]. Although predominantly studied in NAFLD/NASH, evidence from ALD and viral hepatitis highlight the relevance of these SNPs for disease progression across CLD etiologies, with the variant allele being more

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prevalent (*PNPLA3*, *SERPINA1*) and less prevalent (*HSD17B13*) in advanced stages [14–18]. Therefore, we aimed at investigating the usefulness of genotyping for four important SNPs (*PNPLA3*, *TM6SF2*, *HSD17B13*, *SERPINA1*) in clinical routine for the assessment of the severity of CLD.

2. Methods

2.1. Patients and definitions

All patients who attended the hepatic outpatient clinic of the Paracelsus Medical University Salzburg for the first time, between 10/2018 and 07/2020, were evaluated for inclusion in this retrospective cross-sectional analysis. Analysis of the genetic risk variants was commenced as part of the clinical routine in 10/2018 as part of the laboratory evaluation of all patients at first time assessment and data from these 875 subjects were collected for analysis. Patients were only included if information on all SNPs (*PNPLA3*, *TM6SF2*, *HSD17B13*, *SERPINA1*) was available. Patients were excluded, if any of the following criteria were present: Patients with a history of orthotopic liver transplantation ($n = 4$), liver metastasis of other origin than HCC ($n = 4$), cholestatic or autoimmune liver disease (i.e., autoimmune hepatitis [AIH, $n = 18$], primary biliary or sclerosing cholangitis [PBC, $n = 18$ /PSC, $n = 1$]), conditions causing temporarily elevated transaminases in the absence of CLD ($n = 9$), drug-induced liver disease (DILI, $n = 3$) or Wilson disease ($n = 2$), both due to the lack of data of risk variants in these diseases and/or the low number of patients with these etiologies. Finally, all patients with invalid liver stiffness measurement (LSM) were also excluded ($n = 37$). Data were obtained by retrospective evaluation of medical records.

The amount of alcohol consumed was estimated as number of drinks per day/week and significant alcohol abuse was defined as ≥ 3 drinks per day for men and ≥ 2 drinks for women [19]. This threshold was used for allocation of a fatty liver to alcoholic origin in this analysis, also if components of metabolic syndrome as indicators of NAFLD were present. Hepatic decompensation was defined as current or previous presence of ascites, hepatic encephalopathy (HE), or variceal bleeding. ACLD was defined as LSM ≥ 10 kPa, unequivocal clinical signs of cirrhosis or history of hepatic decompensation [20]. Also, LSM was stratified according to the BAVENO-VI consensus recommendations for “suspected ACLD” (≥ 10 kPa), “highly suggestive ACLD” (≥ 15 kPa), and ruling-in clinically significant portal hypertension (CSPH; ≥ 20 –25 kPa) [21]. This study was approved by the ethics committee of Salzburg and performed in accordance with the standards of the Declaration of Helsinki. Since it was a retrospective analysis of available clinical data, the requirement of a written informed consent was waived.

2.2. LSM and controlled attenuation parameter measurement

LSM and controlled attenuation parameter (CAP) measurements were performed by transient elastography using a FibroScan® (Echosens, Paris, France) applying previously published reliability criteria [22]. Measurements were carried out after a fasting period of at least 3 h. The M and XL-probe were chosen based on the expertise of the manufacturer. Patients were instructed to lie in a dorsal position with the right arm in abduction and measurements were performed on the right lobe of the liver, through intercostal spaces [23].

2.3. Genotyping for SNPs

Genomic DNA was collected from peripheral blood samples according to standard procedures for genotyping the following genotype variants: *PNPLA3* I148M, *TM6SF2* E167K, *SERPINA1* and

HSD17B13 [24]. Therefore, the 5-nuclease allelic discrimination Taq-Man genotyping method was performed by using pre-designed assays from Applied Biosystems (Foster City, CA) according to the manufacturer's instructions on the ViiA7 instrument (Applied Biosystems, Foster City, CA). To verify the received results, 10% of samples were genotyped in duplicates.

2.4. Polygenic risk score

A polygenic risk score was calculated assigning one point for each risk allele. Specifically, *PNPLA3* C/C as wild type was counted as 0 points, while *PNPLA3* G/C was counted as 1 point and *PNPLA3* G/G as 2 points. Patients with *TM6SF2* E/E were assigned 0 points, patients with *TM6SF2* E/K 1 point and *TM6SF2* K/K 2 points. For *HSD17B13*, the T/T variant was counted as 0 points, while patients with *HSD17B13* T/TA were deducted –1 point and patients with *HSD17B13* TA/TA were deducted –2 points. Finally, patients with *SERPINA1* M/M or M/S were assigned 0 points, while patients with *SERPINA1* M/Z or S/Z were assigned 1 point and patients with *SERPINA1* Z/Z 2 points. This resulted in a polygenic risk score between –2 and 4 points.

2.5. Statistical analyses

Statistical analyses were performed using IBM SPSS Statistics 26 (SPSS Inc., Armonk, New York, USA) and GraphPad Prism 8 (GraphPad Software, La Jolla, California, USA). Depending on their distribution, continuous variables were reported as mean $\pm$ standard deviation (SD) or median (IQR). Categorical variables were presented as numbers and proportions of patients. Comparisons of continuous variables were carried out by using Student's *t*-test or Mann-Whitney U test, as applicable. For comparisons of more than two groups, Kruskal-Wallis one-way analysis of variance was used. Proportions of patients were compared using χ^2 -squared test. Multivariable linear regression analyses were used to assess the associations between SNPs and LSM. Additionally, multivariable logistic regression analyses were used to determine their association with the presence of ACLD. The following covariables were used for regression analyses: Age, body mass index (BMI) and significant alcohol consumption. To assess the influence of risk variants, genotypes were compared binomially as presence of *PNPLA3* G-allele (C/C vs. G/C or G/G), *TM6SF2* K-allele (E/E vs. E/K or K/K), *HSD17B13* TA-allele (T/T vs. T/TA or TA/TA), and *SERPINA1* Z-allele (M/M or M/S vs. M/Z or S/Z or Z/Z). To test whether the allele frequency observed in our study conformed to an ideal population in Hardy-Weinberg equilibrium, we calculated χ^2 -squared tests as previously described with values of ≥ 3.84 indicating a significant difference [25]. A two-sided *P* value ≤ 0.05 was considered as statistically significant.

3. Results

3.1. Study population and patient characteristics (Fig. 1, Table 1)

In total, 875 patients were evaluated for inclusion in this study (Fig. 1). Following other exclusion criteria, 779 patients could finally be analyzed. The majority of patients were male ($n = 476$, 61.1%) with a mean age of 51.7 ± 15.8 years. NAFLD was the most common etiology of liver disease ($n = 511$, 65.6%), followed by ALD ($n = 118$, 15.1%), chronic hepatitis C virus ($n = 79$, 10.1%) and chronic hepatitis B virus disease ($n = 44$, 5.6%). Mean BMI was 26.7 ± 5.0 kg/m², median LSM was 6.0 kPa (IQR: 4.6–9.2) and median CAP was 273 dB/m (IQR: 223–321). 171 (22.0%), 102 (13.1%) and 77 (9.9%) patients had LSM ≥ 10.0 , ≥ 15.0 and ≥ 20.0 kPa. Overall, ACLD was present in 217 (27.9%) patients.

Fig. 1. Study flowchart.

Abbreviations: ACLD advanced chronic liver disease AIH autoimmune hepatitis DILI drug-induced liver disease HCC hepatocellular carcinoma OLT orthotopic liver transplantation PBC primary biliary cholangitis PSC primary sclerosing cholangitis SNP single nucleotide polymorphism TIPS transjugular intrahepatic portosystemic shunt.

3.2. Prevalence of risk alleles (Table 1)

Overall, the *PNPLA3* wild-type (C/C) was present in 394 patients (50.6%), whereas the heterozygous (G/C) and the homozygous mutant-type (G/G) were present in 303 (38.9%) and 82 (10.5%) patients. Thus, *PNPLA3* rs738409 G-allele frequency was 0.2997, and χ^2 was 4.2027 when compared to an ideal population in Hardy-Weinberg equilibrium. The *TM6SF2* E/E genotype was present in 641 patients (82.3%), while 133 patients (17.1%) harbored the heterozygous allele (*TM6SF2* E/K) and 5 patients (0.6%) the homozygous K-allele (*TM6SF2* K/K). *TM6SF2* rs58542926 K-allele frequency was 0.0918 and χ^2 was 0.4511. Furthermore, *HSD17B13* wild-type was found in 59.2% ($n = 461$), the heterozygous allele *HSD17B13* T/TA in 33.9% ($n = 264$) and the homozygous TA-allele in 6.9% ($n = 54$) patients ($\chi^2 = 3.5731$). For *SERPINA1*, the allele distribution was as follows: 707 with M/M (90.8%), 26 with M/S (3.3%), 44 with M/Z (5.6%) and 2 with Z/Z (0.3%).

3.3. Association with liver disease (Table 2, Supplementary Tables 1–4)

First, we compared LSM and CAP values as well as transaminase levels across patients with different genotypes of the respective single SNPs (univariable analyses). Differences were only observed across *PNPLA3* genotypes for LSM, AST and ALT (Supplementary Table 1). For all other genotypes, no differences in these parameters were observed following univariable analyses. Similarly, the prevalence of HCC, ACLD and type-2 diabetes mellitus (T2DM) only significantly differed among *PNPLA3* genotypes while no differences existed for the others (Supplementary Table 2).

Next, logistic and linear regressions were calculated to assess the impact of the individual risk alleles on disease severity in the presence of age, BMI and alcohol consumption (Table 2A, multivariable analyses). *PNPLA3* G-allele was independently associated with higher LSM (adjusted B: 2.508 [95%CI: 0.887–3.140], $P = 0.002$) and higher odds of ACLD (aOR: 1.562 [95%CI: 1.097–2.226], $P = 0.013$). *TM6SF2* K-allele did not increase LSM ($P = 0.208$) or the risk of ACLD ($P = 0.503$). *HSD17B13* risk allele expression showed a numerical trend in the overall cohort but was neither associated with LSM ($P = 0.067$) nor with presence of ACLD ($P = 0.462$). As for *SERPINA1*, LSM was significantly associated with presence of Z-allele (adjusted B: 4.558 [95%CI: 1.182–7.934], $P = 0.008$), whereas it was not associated with ACLD (aOR: 1.858 [95%CI: 0.939–3.678], $P = 0.075$).

Next, we aimed to assess the influence of each risk allele in the presence/absence of other risk alleles (Table 2B). *PNPLA3* G-allele remained independently associated with LSM (adjusted B: 2.409 [95%CI: 0.793–4.026], $P = 0.004$) or ACLD (aOR: 1.568 [95%CI: 1.098–2.238], $P = 0.013$). However, only *SERPINA1* Z-allele was independently associated with LSM (adjusted B: 4.620 [95%CI: 1.260–7.979], $P = 0.007$) while the other risk alleles were neither signif-

icantly associated with LSM nor ACLD after correcting for *PNPLA3*. These associations were similar when including T2DM and low-density lipoprotein (Supplementary Table 3) or additionally adding gender (Supplementary Table 4) as covariables.

3.4. Polygenic risk score (Table 3)

Finally, we created a polygenic risk score to investigate whether a simple coding system for risk alleles might increase significance of risk stratification. Overall, -2 points, were allocated to 17 patients (2.3%) and -1 points were allocated 131 patients (16.8%). The majority of patients had 0 points ($n = 301$, 38.6%). Moreover, 1 point was observed in 28.6% ($n = 223$), 2 points in 12.1% ($n = 94$) and 3 points in 1.5% ($n = 12$) patients. The maximum score of 4 points was seen in one patient (0.1%). Notably, this score was significantly associated with LSM (adjusted B: 0.948 [95%CI: 0.153–1.743], $P = 0.020$). However, it was not predictive for ACLD ($P = 0.177$).

4. Discussion

In this study, we aimed at investigating the usefulness of genotyping of four important SNPs (*PNPLA3*, *TM6SF2*, *HSD17B13*, *SERPINA1*) in clinical routine in the assessment of the severity of chronic liver disease. Therefore, we analysed a cohort of 779 consecutive patients with symptomatic and/or suspected liver disease at the time of their first contact with a hepatologist at a tertiary care center. We confirmed the established association of *PNPLA3* with disease severity but identified *SERPINA1* as another central disease modifying gene in liver disease patients.

At first, we identified a different distribution of *PNPLA3* variants in our cohort when compared to an ideal population in Hardy-Weinberg-equilibrium while their distribution was not different for *HSD17B13*, *TM6SF2*, and *SERPINA1*. Furthermore, the prevalence of *PNPLA3* risk allele in our cohort was higher than reported in literature while it was similar for *TM6SF2*, *HSD17B13* and *SERPINA1* [14,26].

These data suggest that *PNPLA3* risk variants seem to be enriched in patients getting specialist referral for the assessment of suspected liver disease while no such indication was observed for other genotypes. Thus, the effect of *PNPLA3* risk variant carriage may translate into a higher risk of developing clinically overt liver disease. Although this was not observed for *HSD17B13*, *TM6SF2*, and *SERPINA1*, these variants are significantly less common in the general population which might attenuate their influence on disease severity on a population level despite a strong biological effect and pathophysiological influence in the individual patient. Given the well-established role of *TM6SF2* and *HSD17B13* variants in liver disease, the insignificant associations for these variants in our study is likely due to a lack of statistical power. The association of *PNPLA3* with liver disease at our unselected cohort goes in line with data from the population based National Health and Nutrition Examination Survey (NHANES) study demonstrating increased mortality from liver disease in carriers of *PNPLA3* risk variants [27]. In particular, *PNPLA3* risk variants were associated with LSM and the clinical diagnosis of ACLD in our cohort while no such link was found for the other genotypes. Again, this observation contributes to the increasing knowledge that the *PNPLA3* risk allele might indeed be the central genetic risk factor for developing symptomatic liver disease [28]. Although not from unselected consecutive patients, previous data have demonstrated that *PNPLA3* variants are a strong risk factor for development of liver cirrhosis and HCC [4,29,30]. Thus, genotyping for *PNPLA3* may provide information beyond the assessment of liver disease severity at the time of assessment (i.e., when liver disease starts to be clinically evident). Therefore, it might impact follow-up strategies such as intervals of

Table 1

Patient and disease characteristics.

Abbreviations: CAP controlled attenuation parameter IQR interquartile range SD standard deviation T2DM type 2 diabetes mellitus.

Characteristics	Overall <i>n</i> = 779			
<i>Clinical data</i>				
Age, y, mean ± SD	51.7 ± 15.8			
Male sex, <i>n</i> (%)	<i>n</i> = 476 (61.1%)			
BMI, kg/m ² , mean ± SD	26.7 ± 5.0			
LSM, kPa, median (IQR)	6.0 (4.6–9.2)			
LSM ≥ 10 kPa, <i>n</i> (%)	<i>n</i> = 171 (22.0%)			
LSM ≥ 15 kPa, <i>n</i> (%)	<i>n</i> = 102 (13.1%)			
LSM ≥ 20 kPa, <i>n</i> (%)	<i>n</i> = 77 (9.9%)			
CAP, dB, median (IQR)	273.0 (223.0–321.0)			
<i>Etiology of liver disease, n (%)</i>				
Non-alcoholic fatty liver disease (NAFLD)	<i>n</i> = 511 (65.6%)			
Alcoholic liver disease (ALD)	<i>n</i> = 118 (15.1%)			
Hepatitis C virus infection (HCV)	<i>n</i> = 79 (10.1%)			
Hepatitis B virus infection (HBV)	<i>n</i> = 44 (5.6%)			
Unknown liver disease	<i>n</i> = 27 (3.5%)			
ACLD	<i>n</i> = 217 (27.9%)			
HCC	<i>n</i> = 14 (1.8%)			
T2DM	<i>n</i> = 88 (11.3%)			
<i>Genotypes, n (%)</i>				
PNPLA3 C/C	<i>n</i> = 394 (50.6%)	<i>p</i>	<i>q</i>	χ ²
PNPLA3 G/C	<i>n</i> = 303 (38.9%)	0.700	0.300	4.2027
PNPLA3 G/G	<i>n</i> = 82 (10.5%)			
SERPINA1 M/M	<i>n</i> = 707 (90.8%)	0.969	0.031	2.2879
SERPINA1 M/S	<i>n</i> = 26 (3.3%)			
SERPINA1 M/Z	<i>n</i> = 44 (5.6%)			
SERPINA1 Z/Z	<i>n</i> = 2 (0.3%)			
TM6SF2 E/E	<i>n</i> = 641 (82.3%)	0.908	0.092	0.4511
TM6SF2 E/K	<i>n</i> = 133 (17.1%)			
TM6SF2 K/K	<i>n</i> = 5 (0.6%)			
HSD17B13 T/T	<i>n</i> = 461 (59.2%)	0.761	0.239	3.5731
HSD17B13 T/TA	<i>n</i> = 264 (33.9%)			
HSD17B13 TA/TA	<i>n</i> = 54 (6.9%)			

Table 2

(A) Linear regression analyses (liver stiffness measurement, LSM) and logistic regression analyses (advanced chronic liver disease, ACLD) investigating individual associations of risk alleles with LSM and the presence of ACLD (multivariable analysis). (B) All genetic loci in a combined linear and logistic regression analysis.

Covariables for both approaches were: Age (yrs), BMI (kg/m²), significant alcohol abuse (women ≥ 2 drinks per day, men ≥ 3 drinks per day).

Abbreviations: ACLD advanced chronic liver disease CAP controlled attenuation parameter CI confidence interval IQR interquartile range LSM liver stiffness measurement.

A	LSM	ACLD
PNPLA3 (<i>G</i> = 1)	2.508 (0.887–4.130), <i>P</i> = 0.002	1.562 (1.097–2.226), <i>P</i> = 0.013
TM6SF2 (<i>K</i> = 1)	1.361 (−0.758–3.481), <i>P</i> = 0.208	0.853 (0.535–1.359), <i>P</i> = 0.503
HSD17B13 (<i>TA</i> = 1)	1.551 (−0.106–3.208), <i>P</i> = 0.067	1.144 (0.800–1.635), <i>P</i> = 0.462
SERPINA1 (<i>Z</i> = 1)	4.558 (1.182–7.934), <i>P</i> = 0.008	1.858 (0.939–3.678), <i>P</i> = 0.075
B	LSM	ACLD
PNPLA3 (<i>G</i> = 1)	2.409 (0.793–4.026), <i>P</i> = 0.004	1.568 (1.098–2.238), <i>P</i> = 0.013
TM6SF2 (<i>K</i> = 1)	1.547 (−0.554–3.648), <i>P</i> = 0.149	0.851 (0.529–1.368), <i>P</i> = 0.504
HSD17B13 (<i>TA</i> = 1)	1.344 (−0.302–2.990), <i>P</i> = 0.109	1.106 (0.771–1.587), <i>P</i> = 0.584
SERPINA1 (<i>Z</i> = 1)	4.620 (1.260–7.979), <i>P</i> = 0.007	1.841 (0.921–3.680), <i>P</i> = 0.084

regular consultations, ultrasound screening for HCC, and contribute to a personalized approach in clinical hepatology.

Although other SNPs are continuously being discovered in genome-wide association studies, their impact on disease severity seems to be weaker compared to *PNPLA3* [13]. Since *PNPLA3*/*TM6SF2*/*HSD17B13* interact with different steps in lipid homeostasis [31], a potentiation of their harmful effect leading to disease progression could be expected. While *PNPLA3* promotes intracellular lipid accumulation in the liver by impairing mobilization of various lipid species from lipid droplets and reducing the lipidation of very low-density lipoprotein (VLDL) [32], the *TM6SF2* variant is linked to inhibition of VLDL secretion from hepatocytes [33]. Although the exact function of *HSD17B13* remains unknown,

this protein also increases size and accumulation of lipid droplets in hepatocytes [34] while the loss-of-function variant in *HSD17B13* seems to counteract this mechanism. Finally, *SERPINA1* acts differently by augmenting proteotoxicity (i.e., accumulation of alpha-1-antitrypsin) in the endoplasmic reticulum [35]. To test whether the different biological mechanisms of these variants potentiate each other, we combined them into a polygenic risk score. This polygenic risk score was significantly associated with LSM, but not with the presence of ACLD in our study, indicating that carriage of several SNPs might increase an individual's risk for advanced fibrosis. However, the strength of the observed associations of the combined genetic risk score was weaker than that of *PNPLA3* alone. This indicates that *PNPLA3* alone represents a central SNP to which

Table 3

Overview of genetic landscape using a polygenic risk score, and linear and logistic regression analyses investigating the association of polygenic risk score with liver stiffness measurement (LSM) and presence of advanced chronic liver disease (ACLD). Covariables were: Age (yrs), BMI (kg/m²), significant alcohol abuse (women ≥ 2 drinks per day, men ≥ 3 drinks per day). Abbreviations: ACLD advanced chronic liver disease CI confidence interval LSM liver stiffness measurement.

Polygenic risk score			
Points	n	%	Cumulative%
−2	17	2.3	2.3
−1	131	16.8	19.0
0	301	38.6	57.6
1	223	28.6	86.3
2	94	12.1	98.3
3	12	1.5	99.9
4	1	0.1	100.0
Overall	779	100.0	100.0
	LSM		ACLD
Polygenic risk score	0.948 (0.153–1.743) <i>P</i> = 0.020		1.124 (0.949–1.332) <i>P</i> = 0.177

Polygenic risk score: *PNPLA3* → 0/1/2; *TM6SF2* → 0/1/2; *HSD17B13* → 0/–1/–2; *SERPINA1* → 0/1/2.

the other SNPs only add little information. Although one might argue that these variants should be weighted according to the strength of their association with disease severity, we chose to assign one point for each risk allele to treat them equally. Importantly, a customized weighting would need a meta-analysis of all published studies evaluating the four SNPs used in this study (*PNPLA3*, *TM6SF2*, *HSD17B13*, *SERPINA1*) together, which has not been done so far. Arbitrarily choosing a coefficient for each risk allele would artificially modify the relevance of the respective risk allele which is – when seen from a natural perspective – either present (1) or not (0). Importantly, several studies have already aimed to identify the utility of polygenic risk scores to predict disease severity in NAFLD [36–38]. Just recently, Bianco and colleagues [39] identified a polygenic risk score combining *PNPLA3*–*TM6SF2*–*GCKR*–*MBOAT7* as predictive for HCC. However, none has included *TM6SF2* and *HSD17B13*.

Interestingly, *SERPINA1* was independently associated with LSM in our cohort. This goes in line with recent findings of Strnad et al. [14] who found that the *SERPINA1* Z-allele was associated with disease severity and an increased risk to develop cirrhosis in patients with NAFLD and ALD. This association was independent of *PNPLA3*, *TM6SF2* and *MBOAT7*, and stronger than other risk variants for the development of NAFLD/ALD cirrhosis [13]. In our study, the regression coefficient regarding the association with LSM was nearly twice as high as the coefficient of *PNPLA3*, indicating a stronger influence of LSM. Although a numerically higher aOR was also observed for the presence of ACLD, this did not achieve statistical significance, probably due to a significantly lower prevalence of *SERPINA1* risk allele – even in heterozygous form – provides additional information for risk stratification, which likewise warrants genotyping in patients with liver disease. From a clinical point, our data suggest that at least genotyping for *PNPLA3* variants may add additional value in the routine work-up of liver disease patients, while the usefulness of other variants in this setting is not supported by our findings, except for a potential role of the *SERPINA1* variant in the progression to advanced stages of fibrosis. The routine use of genotyping is also further limited by the respective financial burden. However, as these are highly variable in different regions, the decision will need to be made according to the weight of the individual factors.

Our study indicates that neither of the investigated variants are linked to CAP which represents a widely used non-invasive estimate for the extent of hepatic steatosis. However, this is likely due to the nature of CAP measurements being variable, unable to grade hepatic steatosis [40] while being influenced by numerous factors

including alcohol intake, BMI, T2DM or fibrosis, particularly in our cohort of mixed etiologies [22,41,42]. Therefore, our findings do not argue against the fact that these SNPs promote the development of hepatic steatosis, which has been well-documented by numerous studies.

This study has several limitations. To begin with, it is a cross-sectional retrospective study showing only one time point in the course of liver disease. Longitudinal data on liver-related morbidity and mortality are needed to support our findings. However, as genetic markers remain unchanged, the observed association in fact reflects the impact of the variant over the entire observed lifetime. Moreover, since we aimed to assess the utility of genotyping in clinical routine, the cohort being analyzed combines different etiologies of CLD reflecting the heterogeneity of risk factors typical for the general population. Hence, the set environmental risk factors such as diet, physical exercise, amount of alcohol, may be composed differently in any individual included. We aimed to reduce the heterogeneity of the cohort by only including the common fatty liver and viral diseases which are expected to mostly have a relatively continuous chronic course of disease progression in an individual. Although desirable, genotyping for membrane bound O-acyltransferase domain-containing 7 gene (*MBOAT7*) was not available in our cohort as this was not part of the routine clinical program during the study period.

In conclusion, we provide data on the usefulness of genotyping for important risk alleles in a cohort of CLD patients with Caucasian background in clinical routine. Specifically, we confirm the strong association between *PNPLA3* and liver disease severity. Although other SNPs including *SERPINA1* might add information on further risk of developing (severe) liver disease, *PNPLA3* was identified to be of central importance and its use may be justified in the clinical routine. Combining multiple risk alleles into a polygenic score might increase granularity of genetic risk stratification.

Declaration of Competing Interest

None of the authors have potential conflicts of interest with regard to this manuscript to disclose. However, some of the authors have conflicts of interest outside the submitted work: C.D. is part of the scientific advisor board of SPAR Österreich WarenhandelsAG.

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Availability of data

All data generated or analyzed during this study are included in this published article (and its supplementary information files).

Code availability

Not applicable.

Author contributions

Study concept and design (L.B., G.S., E.A.), acquisition of data (L.B., G.S., H.O., S.Z., M.S., L.D., D.N., A.F., F.S., C.D., E.A.), analysis and interpretation of data (L.B., G.S., E.A.), drafting of the manuscript (L.B., G.S., E.A.), critical revision of the manuscript for important intellectual content (L.B., G.S., H.O., S.Z., M.S., L.D., D.N., A.F., F.S., C.D., E.A.). The first draft of the manuscript was written by L.B., G.S. and E.A. and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval

This study was approved by the ethics committee of Salzburg (Ethikkommission für das Bundesland Salzburg; <http://www.salzburg.gv.at/ethikkommission>) and performed in accordance with the standards of the Declaration of Helsinki. Since it was a retrospective analysis of available clinical data, the requirement of a written informed consent was waived.

Consent for publication

Not applicable.

Abbreviations

95%CI 95% confidence interval ACLD advanced chronic liver disease aHR adjusted hazard ratio aOR adjusted odds ratio BMI body mass index cACLD compensated advanced chronic liver disease CAP controlled attenuation parameter CLD chronic liver disease CSPH clinically significant portal hypertension CPS Child-Turcotte-Pugh-Score HCC hepatocellular carcinoma HE hepatic encephalopathy HR hazard ratio *HSD17B13* 17 β -hydroxysteroid dehydrogenase type 13 IQR interquartile range LSM liver stiffness measurement LT liver transplantation MELD model for end-stage liver disease NAFLD non-alcoholic fatty liver disease NASH non-alcoholic steatohepatitis *PNPLA3* patatin-like phospholipase domain-containing protein 3 SD standard deviation *SERPINA1* serpin family A member 1 *TM6SF2* transmembrane 6 superfamily 2 T2DM type-2 diabetes mellitus

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:[10.1016/j.dld.2021.06.015](https://doi.org/10.1016/j.dld.2021.06.015).

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