Alpha-1 antitrypsin Pi*Z allele is an independent risk factor for liver transplantation and death in patients with advanced chronic liver disease

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Graphical abstract

Previous knowledge:
Pi*ZZ (the rare homozygous form of severe AATD) may cause ACLD

Previous knowledge:
The common Pi*MZ genotype increases the risk of ACLD development

Impact of Pi*MZ in patients who have already progressed to ACLD?

Highlights
- Pi*Z allele is significantly associated with liver-related events in patients with ACLD.
- This finding extends to patients harbouring the Pi*MZ genotype.
- Genotyping for the Pi*Z allele might improve prognostication in patients with ACLD.
- Therapies targeting accumulation of abnormal AAT should be assessed in Pi*Z carriers with ACLD.

Lay summary
Alpha-1 antitrypsin deficiency is a genetic disease that affects the lung and the liver. Carrying two dysfunctional copies of the gene causes advanced liver disease. Harbouring one dysfunctional copy increases disease severity in patients with other liver illness. However, the significance of this genetic defect in patients who already suffer from advanced liver disease is unclear. Our study found that harbouring at least one dysfunctional copy of the alpha-1 antitrypsin gene increases the risk of requiring a liver transplantation or dying from a liver disease. This indicates the need for medical therapies aimed at treating the hepatic consequences of this genetic defect.

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Alpha-1 antitrypsin Pi*Z allele is an independent risk factor for liver transplantation and death in patients with advanced chronic liver disease

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**Background & Aims:** Alpha-1 antitrypsin (AAT) deficiency causes/predisposes individuals to advanced chronic liver disease (ACLD). However, the role of the SERPINA1 Pi*Z allele in patients who have already progressed to ACLD is unclear. Thus, we aimed to evaluate the impact of the Pi*Z allele on the risk of liver transplantation/liver-related death in patients with ACLD, while adjusting for the severity of liver disease at inclusion.

**Methods:** A total of 1,118 patients with ACLD who underwent hepatic venous pressure gradient (HVPG) measurement and genotyping for the Pi*Z/Pi*S allele at the Vienna Hepatic Hemodynamic Lab were included in this retrospective analysis. The outcome of interest was liver transplantation/liver-related death, while non-liver-related death and removal/suppression of the primary etiological factor were considered as competing risks.

**Results:** Viral hepatitis was the most common etiology (44%), followed by alcohol-related (31%) and non-alcoholic fatty liver disease (11%). Forty-two (4%) and forty-six (4%) patients harboured the Pi*Z and Pi*S variants, respectively. Pi*Z carriers had more severe portal hypertension (HVPG: 19±6 vs. 15±7 mmHg; p < 0.001) and hepatic dysfunction (Child-Turcotte-Pugh: 7.1±1.9 vs. 6.5±1.9 points; p = 0.050) at inclusion, compared to non-carriers. Contrarily, the Pi*S allele was unrelated to liver disease severity. In competing risk regression analysis, harbouring the Pi*Z allele was significantly associated with an increased probability of liver transplantation/liver-related death, even after adjusting for liver disease severity at inclusion. The detrimental impact of the common Pi*MZ genotype (adjusted subdistribution hazard ratio: 1.56 vs. Pi*MM) was confirmed in a fully adjusted subgroup analysis. In contrast, Pi*S carriers had no increased risk of events.

**Conclusion:** Genotyping for the Pi*Z allele identifies patients with ACLD at increased risk of adverse liver-related outcomes, thereby improving prognostication. Therapies targeting the accumulation of abnormal AAT should be evaluated as disease-modifying treatments in Pi*Z allele carriers with ACLD.

**Lay summary:** Alpha-1 antitrypsin deficiency is a genetic disease that affects the lung and the liver. Carrying two dysfunctional copies of the gene causes advanced liver disease. Harbouring one dysfunctional copy increases disease severity in patients with other liver illness. However, the significance of this genetic defect in patients who already suffer from advanced liver disease is unclear. Our study found that harbouring at least one dysfunctional copy of the alpha-1 antitrypsin gene increases the risk of requiring a liver transplantation or dying from a liver disease. This indicates the need for medical therapies aimed at treating the hepatic consequences of this genetic defect.

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a European multicentre study\textsuperscript{6} indicated that liver stiffness and the probability of cirrhosis increase with the number of Pi*Z alleles, with the highest values in patients with Pi*ZZ followed by Pi*SZ, and Pi*MZ. Pi*ZZ AATD may lead to advanced chronic liver disease (ACLD) even in the absence of additional etiological factors or cofactors, while additional forms of hepatic injury are usually required for carriers of Pi*SZ and Pi*MZ to progress to ACLD. Of note, a considerably higher AATD-related liver disease burden was observed in the presence of metabolic (co)facors.\textsuperscript{7} Analyses of the UK biobank suggested an odds ratio higher than 40 for the development of liver tumours, however, further studies are needed to clarify the role Pi*ZZ in liver tumour development.\textsuperscript{8} Harbouring the Pi*SZ variant was associated with a 3-fold and 7-fold increase in the risk of liver fibrosis/cirrhosis and liver cancer, respectively.\textsuperscript{8} In Pi*MZ individuals an odds ratio of 1.7 for liver fibrosis/liver-related mortality was reported, whereas no significant risks of liver cancers could be demonstrated.\textsuperscript{8–10}

However, despite the overrepresentation of Pi*S allele carriers among patients with ACLD, in particular those with higher disease severity,\textsuperscript{11} data on the impact of AATD-associated mutations on the clinical course of patients who have already progressed to ACLD are scarce. In a study by Schaefer et al.,\textsuperscript{11} in individuals with cirrhosis, including 488 individuals with Pi*MM and 52 with Pi*MZ, the Pi*MZ genotype was associated with more advanced hepatic dysfunction and decompensation. In the subgroup of patients with hypotransferrinaemia or increased transferrin saturation, the PiMZ genotype was accompanied by an increased risk of liver transplantation/death during follow-up, however, there was no statistically significant association in the overall study population. Finally, Pi*MS showed no association with liver disease severity.\textsuperscript{11}

The aim of our study was to evaluate the impact of the SERPINA1 Pi*Z allele on liver-related death or the requirement for liver transplantation, while adjusting for the severity of liver disease at baseline in a large, thoroughly characterized cohort of patients with ACLD who underwent hepatic venous pressure gradient (HVPG) measurement. Moreover, we aimed to investigate the implications of SERPINA1 Pi*S allele on the course of liver disease.

**Patients and methods**

**Study design and patients**

We performed a retrospective, single-centre cohort study in patients with ACLD who underwent HVPG measurement at the Vienna Hepatic Hemodynamic Lab. Inclusion criteria were (i) liver stiffness measurement $\geq 10$ kPa and/or HVPG $\geq 66$ mmHg, (ii) valid HVPG measurement, and (iii) availability of information on SERPINA1 genotype. Furthermore, patients were excluded if any of the following criteria were present: Patients with a history of orthotopic liver transplantation, any active extrahepatic malignancy, patients with non-parenchymal liver diseases, or missing information on important laboratory parameters and/or clinical follow-up. Patients were included between Q2/04 and Q4/20 and recruitment and follow-up over the study period was depicted in Fig. S1. Study-relevant clinical and laboratory information was collected from patients’ medical records.

**HVPG measurement**

Under local anaesthesia and ultrasound guidance, a catheter introducer sheath was inserted into the right internal jugular vein. Subsequently, a hepatic vein was cannulated and the free and wedged hepatic venous pressures were obtained at least as triplicate measurements\textsuperscript{12} by a balloon catheter.\textsuperscript{13}

**Genotyping**

After blood collection of EDTA blood (4 ml), the samples were frozen to $-20$ °C. Prior to DNA isolation, which took place within days to weeks after blood collection, blood was thawed to room temperature. Thereafter, genomic DNA was isolated from 200 μl of blood using the QiAmp Blood Mini Kit (QIAGEN N.V., Hilden, Germany). The optical density of DNA was then measured (DNA content and purity) using a NanoDrop 1000 Spectrophotometer from Thermo Fisher (Thermo Fisher Scientific Inc., Waltham, MA, USA) and 1 μl (equivalent to approximately 30 nanograms) of this DNA was analysed by real-time PCR (7500 Fast Real-Time PCR System, TaqMan SNP Genotyping Assay [Applied Biosystems, Foster City, CA, US]).

**Reporting of ethnicity**

Ethnicity was determined based on geographic origin and data is presented in accordance with recently published guidelines on reporting ethnicity in research articles.\textsuperscript{14}

**Statistical analysis**

All statistical analyses were performed using IBM SPSS Statistics 27 (IBM, New York, NY, USA), R 4.1.2 (R Core Team, R Foundation for Statistical Computing, Vienna, Austria), or GraphPad Prism 8 (GraphPad Software, CA, USA). Categorical variables were reported as absolute (n) and relative frequencies (%), whereas continuous variables as mean ± SD or median (IQR), as appropriate. Student’s t test was used for group comparisons of normally distributed variables and Mann-Whitney U test for non-normally distributed variables. Group comparisons of categorical variables were performed using either Chi-squared or Fisher’s exact test, as appropriate.

Follow-up time was calculated as the time from HVPG measurement to the date of liver transplantation, death, or last follow-up at one of the hospitals of the Vienna hospital association by the reverse Kaplan-Meier method.

The impact of SERPINA1 single nucleotide variants on liver-related death or the requirement for liver transplantation was assessed using competing risk analysis considering non-liver-related death, or date of removal/suppression of the primary etiological factor (initiation of antiviral therapy/reported alcohol abstinence), as defined by Baveno VII,\textsuperscript{15} as competing risks. Therefore, Fine and Gray competing risks regression models (cmprsk: subdistribution analysis of competing risks, https://CRAN.R-project.org/package=cmprsk)\textsuperscript{16} were calculated. Baseline characteristics that were significantly different between patients with or without the respective SERPINA1 single nucleotide variants or which we considered of particular importance for the endpoint of interest (i.e., age, indicators of hepatic dysfunction, and HVPG; patatin-like phospholipase domain containing 3 [PNPLA3] GG genotype in an additional analysis) were included into the multivariable competing risk model as covariables. The Child-Turcotte-Pugh (CTP) and United Network for Organ Sharing (UNOS) model for end-stage liver disease (MELD) (2016) scores have significant overlap in terms of included variables. Therefore, we generated separate models with either CTP or UNOS MELD (2016) scores. To confirm our findings without the consideration of competing risks, we have
additionally calculated Cox regression models and included them into the supplementary information.

The level of significance was set at a 2-sided p value <0.05.

Ethics
The study has been conducted in accordance with the principles of the Declaration of Helsinki and its amendments and has been approved by the local ethics committee, which waived the requirement for written informed consent. However, all patients signed a written informed consent for genetic testing.

Results

Study population
Overall, 2,550 patients underwent HVPG measurement within the study period (Fig. 1). After applying inclusion and exclusion criteria, 1,118 patients were finally included in our study. Mean age was 55±12 years and most patients were male (n = 776, 69%; Table 1). Viral hepatitis was the leading etiology of liver disease (n = 495, 44%), followed by alcohol-related liver disease (n = 351, 31%), non-alcoholic fatty liver disease (NAFLD; n = 121, 11%), and other liver disease etiologies (n = 151, 14%). Regarding portal hypertension severity, mean HVPG was 15±7 mmHg and 62% of patients (n = 577) had varices, of whom 135 (12%) had a history of variceal bleeding. Mean UNOS MELD (2016) was 12±5 points and mean CTP score was 7±2 points. Almost half of patients had already experienced decompensation at study inclusion (n = 509, 45%), whereas 40 patients had one (Pi*MZ n = 39; 4%; Pi*SZ n = 1; 0.1%) and two Pi*S alleles (Pi*SS; 0.1%). Due to the low frequency of liver related death or the requirement for liver transplantation in univariable analysis (SHR 1.88; 95% CI 1.20-2.41; p = 0.036), the common heterozygous form of AAT-related liver disease genotypes, the Pi*MZ genotype was associated with an increased risk of liver transplantation/liver-related death (HR 1.44; 95% CI 1.11-1.88; p = 0.007) in univariable analysis (Table 1).

Impact of SERPINA1 rs28929474 genotype/the Pi’Z allele on risk of liver transplantation/liver-related death
Median follow-up time was 64.6 (95% CI 59.4-70.9) months. Overall, 319 patients (28.5%) achieved etiological cure and 100 patients (8.9%) underwent liver transplantation. During the study period, 377 patients (33.7%) died and 297 of these deaths were considered liver related.

Fig. 2 shows the cumulative incidences of liver transplantation/liver-related death stratified according to the presence of the Pi’Z allele. In competing risk regression analysis, harbouring the Pi’Z allele was significantly associated with liver-related death or the requirement for liver transplantation in univariable analysis (subdistribution hazard ratio [SHR] 2.09; 95% CI 1.37-3.19; p <0.001). Importantly, this result was confirmed (model 1: adjusted SHR [aSHR] 1.75; 95% CI 1.15-2.65; p = 0.030) after adjustment for age, HVPG, and CTP stage, as well as for age, HVPG, UNOS MELD (2016), and decompensation status (model 2: aSHR 1.80; 95% CI 1.18-2.74; p = 0.007; Table 2).

When only considering patients with the Pi’M1M1 (n = 1,030) and the Pi’MZ (n = 39; i.e., the common heterozygous form of AAT-related liver disease) genotypes, the Pi’MZ genotype was associated with liver-related death or the requirement for liver transplantation in univariable analysis (SHR 2.17; 95% CI 1.42-3.33; p = 0.001; Table S2). Results were confirmed in Cox regression analyses as depicted in Tables S2 and S3.

Impact of SERPINA1 rs28929474 genotype/the Pi’Z allele on risk of liver transplantation/liver-related death in different etiologies of liver disease
Detailed information is provided in the Fig. S3.

Impact of the Pi’Z allele when accounting for PNPLA3 GG genotype
The distribution of PNPLA3 genotypes (CC: n = 489 [44%], GC: n = 464 [41%], GG: n = 165 [15%]) was similar compared to previously published data from a partly overlapping cohort.

Harbouring the PNPLA3 GG genotype was associated with an increased risk of liver transplantation/liver-related death (HR 1.44; 95% CI 1.11-1.88; p = 0.007) in univariable analysis. Interestingly, the impact of the Pi’Z allele was independent of the PNPLA3 GG genotype (Table S4).

Impact of SERPINA1 rs17580 genotype/the Pi’S allele on risk of liver transplantation/liver-related death
Next, we evaluated the impact of the Pi’S allele on the clinical course of patients who had already progressed to ACLD. When

Patient characteristics according to SERPINA1 rs28929474 genotype/the Pi’Z allele
At baseline, Pi’Z carriers were slightly older (57.8 ± 11.9 vs. 54.9 ± 12.0 years; p = 0.124) and more commonly had NAFLD than patients without Pi’Z (24% vs. 10%; p = 0.012), while viral hepatitis was comparatively uncommon (26% vs. 45%; p = 0.012; Table 1). HVPG was significantly higher in Pi’Z carriers (19 ± 6 vs. 15 ± 7 mmHg; p <0.001), with an increased (though not statistically significant) proportion of Pi’Z carriers having a history of variceal bleeding (21% vs. 12%; p = 0.058). Moreover, a higher number of patients had already experienced decompensation (62 vs. 45%; p = 0.030) and CTP score tended to be higher in Pi’Z carriers (7.1 ± 1.9 vs. 6.5 ± 1.9 points; p = 0.050).

| Study cohort | n = 1,118 |
|--------------|-----------|
| All patients | n = 2,550 |
| History of OLT | n = 64 |
| PSVD/INCPH or no ACLD | n = 559 |
| Any active extrahepatic malignancy | n = 108 |
| HVPG-measurement unsuccessful/unreliable | n = 126 |
| Information on clinical FU | n = 54 |
| No information on AAT genotype available | n = 523 |

Fig. 1. Study flowchart. AAT, alpha-1 antitrypsin; ACLD, advanced chronic liver disease; FU, follow-up; HVPG, hepatic venous pressure gradient; INCPH, idiopathic non-cirrhotic portal hypertension; PSVD, porto-sinusoidal vascular disease.
comparing patients with Pi*S allele(s) to non-carriers, no significant differences were observed at study inclusion (Table S5).

Table 1. Comparison of patient characteristics according to the SERPINA1 rs28929474 genotype/Pi*Z allele. Values in bold designate p values < 0.05. ALD, alcohol-related liver disease; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CRP, C-reactive protein; CTP, Child-Turcotte-Pugh; GGT, gamma-glutamyl transferase; HCC, hepatocellular carcinoma; HVPG, hepatic venous pressure gradient; INR, international normalized ratio; NAFLD, non-alcoholic fatty liver disease; UNOS MELD (2016), United Network for Organ Sharing model for end-stage liver disease (2016) score.

| Patient characteristics | All patients n = 1,118 | Pi*Z non-carriers | Pi*Z carriers | p value |
|-------------------------|------------------------|------------------|----------------|--------|
| Age, years, mean ± SD   | 55.0 ± 12.0            | 54.9 ± 12.0      | 57.8 ± 11.9    | 0.124  |
| Sex, n (%)              |                        |                  |                |        |
| Male                    | 776 (69%)              | 754 (69%)        | 31 (74%)       | 0.611  |
| Female                  | 342 (31%)              | 331 (31%)        | 11 (26%)       |        |
| Etiology, n (%)         |                        |                  |                |        |
| ALD                     | 351 (31%)              | 338 (31%)        | 13 (31%)       | 0.012  |
| NAFLD                   | 121 (11%)              | 111 (10%)        | 10 (24%)       |        |
| Viral                   | 495 (44%)              | 484 (45%)        | 11 (26%)       |        |
| Other                   | 151 (14%)              | 143 (13%)        | 8 (19%)        |        |
| HVPG, mmHg, mean ± SD   | 15 ± 7                 | 15 ± 7           | 19 ± 6         | <0.001 |
| UNOS MELD (2016) score, point, mean ± SD | 12 ± 5 | 12 ± 5 | 13 ± 5 | 0.161 |
| CTP score, mean ± SD    | 6.5 ± 1.9              | 6.5 ± 1.9        | 7.1 ± 1.9      | 0.160  |
| A, n (%)                | 694 (62%)              | 674 (63%)        | 20 (48%)       | 0.143  |
| B, n (%)                | 324 (29%)              | 307 (29%)        | 17 (41%)       |        |
| C, n (%)                | 100 (9%)               | 95 (9%)          | 5 (12%)        |        |
| Varices, n (%)          | 577 (62%)              | 548 (62%)        | 29 (76%)       | 0.194  |
| History of variceal bleeding, n (%) | 135 (12%) | 126 (12%) | 9 (21%) | 0.058 |
| Decompensated, n (%)    | 509 (46%)              | 483 (45%)        | 26 (62%)       | 0.030  |
| HCC, n (%)              | 149 (13%)              | 146 (14%)        | 3 (7%)         | 0.229  |
| Sodium, mmol x L⁻¹, mean ± SD | 138.0 ± 3.5 | 138.0 ± 3.5 | 137.6 ± 3.4 | 0.450 |
| Creatinine, mg x dl⁻¹, median (IQR) | 0.8 (0.7–0.9) | 0.8 (0.7–0.9) | 0.7 (0.7–0.8) | 0.128 |
| Bilirubin, mg x dl⁻¹, median (IQR) | 1.1 (0.7–1.8) | 1.0 (0.7–1.8) | 1.3 (1.0–2.1) | 0.010 |
| Albumin, g x L⁻¹, mean ± SD | 36.4 ± 5.8 | 36.5 ± 5.8 | 34.5 ± 5.7 | 0.032 |
| CRP, mg x L⁻¹, median (IQR) | 0.3 (0.1–0.7) | 0.3 (0.1–0.7) | 0.3 (0.1–0.7) | 0.280 |
| INR, mean ± SD          | 1.3 ± 0.3              | 1.3 ± 0.3        | 1.4 ± 0.3      | 0.075  |
| AST, U x L⁻¹, median (IQR) | 52 (35–79) | 52 (35–80) | 55 (38–72) | 0.411 |
| ALT, U x L⁻¹, median (IQR) | 38 (24–68) | 38 (24–60) | 36 (27–51) | 0.526 |
| GGT, U x L⁻¹, median (IQR) | 105 (57–185) | 104 (57–185) | 140 (55–190) | 0.502 |

SHR SERPINA1 Pi*Z carriers vs. non-carriers: 2.09 (95% CI: 1.37–3.19); p < 0.001

Fig. 2. Cumulative incidences of liver transplantation/liver-related death in SERPINA1 Pi*Z carriers vs. non-carriers with etiological cure and non-liver-related death as competing risks. SHR, subdistribution hazard ratio.

was confirmed when adjusting for covariables, as demonstrated in Table S6.

Discussion

Liver transplantation is currently the only established treatment option for severe AATD-related liver disease. Our study provides evidence of the significance of the Pi*Z allele and associated deficiency genotypes Pi*MZ (and Pi*SZ and Pi*ZZ) on disease progression beyond the development of ACLD, suggesting that pharmacological interventions targeting the toxic gain-of-function by decreasing the production or increasing the degradation of Z protein have the potential to ameliorate liver disease progression in patients with ACLD.

In an unselected cohort of patients undergoing HVPG measurement, we demonstrated that Pi*Z carriage was not very common in patients with ACLD (4%). This is less frequent than in comparable studies (Schafer et al. [9.6%], Strnad et al. [13.8%])19,10 which may be explained by the high proportion of patients with viral etiology in our study. In these patients, Pi*Z carriage seemed to be less detrimental, as indicated by a numerically lower SHR for liver transplantation/liver-related death, compared to other etiologies. Besides having more severe portal hypertension compared to non-carriers, Pi*Z carriers also had more advanced hepatic dysfunction at the time of evaluation. Contrarily, the Pi*S allele showed no significant differences among included patients, indicating that – in the
absence of the Z allele – it is unrelated to liver disease and its progression in patients with ACLD. This is in line with a population-based study investigating its potential impact on the progression to cirrhosis. Considering its association with liver transplantation/liver-related death, carriers of the Pi’Z allele were at increased risk, even after multivariable adjustment. This is in accordance with data from the UK Biobank and a Finnish population-based study, indicating an increased risk of liver-related mortality. Of note, this is the first study adjusting for portal hypertension severity (i.e., HVPG) at baseline and thereby accounting for an additional important prognostic indicator. Since the mean UNOS MELD (2016) of our study population was low (i.e., 12 points), and thus, most patients had no indication for liver transplantation at baseline, we included the requirement for liver transplantation (together with death) in a composite endpoint, rather than analysing liver transplantation as a competing event. Regarding the impact of Pi’Z in different etiologies of liver disease, the question arises whether heterozygosity for the Pi’Z allele is considered solely as a disease modifier or whether AATD may even be seen as the primary etiological factor in some of these patients. Interestingly, patients with viral hepatitis (i.e., those with the most evident primary etiological factor) were underrepresented among Pi’Z carriers, while NAFLD was overrepresented. This is in line with previous studies reporting a more consistent association between Pi’MZ genotype and ACLD in patients with fatty liver disease, as compared to other common etiologies. One explanation may be that NAFLD – which was not always biopsy-proven – may have been misdiagnosed. However, the newly proposed MAFLD term also accounts for the concept that genetic predisposition vs. environmental factors vs. metabolic syndrome varies on a case-by-case basis. However, the overrepresentation of NAFLD etiology among patients harbouring the Pi’Z allele may also be explained by the interaction between the metabolic syndrome and the Pi’Z allele, as individuals with Pi’ZZ and metabolic syndrome showed more accumulated abnormal AAT in hepatocytes, indicating that the presence of metabolic syndrome – which overlaps with NAFLD – may amplify the detrimental impact of the Pi’Z variant. However, in univariable analyses, we observed numerically increased risks of liver transplantation/death within all included etiologies. Even so, sample size/statistical power was limited when sub-stratifying patients according to disease etiology and did not allow for adjustment for baseline disease severity. Accordingly, no firm conclusions can be drawn from this etiology-specific data. Moreover, due to the role of the Pi’Z allele as a disease-modifying factor/a risk factor for disease progression, the question arises whether the removal/suppression of the primary etiological factor (i.e., viral eradication/suppression in HCV/HBV or alcohol abstinence) is similarly beneficial in these patients, or whether the genetic background hinders disease progression/promotes progressive disease. Although a recent study found no impact of other genetic variants in this context, the specific role of the Pi’Z allele may be addressed by future studies.

Recently, the Pi’MZ genotype has been associated with hepatic decompensation, the requirement for liver transplantation and liver-related death in a cohort of patients with compensated ACLD; NAFLD was the main etiology (45%) and the prevalence of obesity (median BMI >30 kg x m$^{-2}$) and metabolic comorbidities (e.g., diabetes prevalence ~50%) was high. In this cohort, n = 49/574 (9%) were Pi’Z allele carriers, compared to 3.8% in our study. This may be explained by the high prevalence of NAFLD in the US cohort, as patients with NAFLD included in our study also more commonly harboured the Pi’Z allele. Importantly, the metabolic syndrome (MetS) has been shown to fuel AATD-related liver disease, as both periodic-acid-Schiff (PAS)-positive diastase-resistant globules in hepatocytes and liver fibrosis were more common in MetS. Accordingly, it cannot be assumed that Pi’Z allele carriage has the same impact on liver phenotype and the development of liver-related clinical events in patients with and without MetS/NAFLD. Furthermore, the analyses were not adjusted for portal hypertension severity (i.e., HVPG), which differed across genotypes in our study and drives the development of first hepatic decompensation. Therefore, our study provides important data that confirms the significance of heterozygosity of this genetic variant on disease progression in a European cohort. The same considerations apply when comparing our work to the Schaefer et al. study, which did not account for portal hypertension at the time of study inclusion and only showed a trend towards an increase in liver-related death, as the presence of the Pi’Z allele, i.e., the hepatic Pi’Z accumulation, is druggable. This is an important difference to PNPLA3 – which is not druggable – as our observation regarding the disease-modifying

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### Table 2. Multivariable competing risk regression analysis of risk of liver transplantation/liver-related death, with etiological cure and non-liver-related death as competing risks.

| Patient characteristics | Model 1 | Model 2 |
|-------------------------|---------|---------|
|                         | aSHR (95%CI) | p value | aSHR (95%CI) | p value |
| Age, year               | 1.03 (1.02–1.04) | <0.001 | 1.03 (1.02–1.04) | <0.001 |
| HVPG, mmHg              | 1.03 (1.01–1.04) | 0.004 | 1.03 (1.01–1.05) | 0.003 |
| CTP stage A             | 1.12 | <0.001 | 1.12 | <0.001 |
| CTP stage B             | 2.00 (1.52–2.62) | <0.001 | 2.00 (1.52–2.62) | <0.001 |
| CTP stage C             | 4.24 (2.91–6.17) | <0.001 | 4.24 (2.91–6.17) | <0.001 |
| UNOS MELD (2016) score  | 1.09 (1.06–1.12) | <0.001 | 1.09 (1.06–1.12) | <0.001 |
| dACLD                   | 1.10 (0.84–1.43) | 0.500 | 1.10 (0.84–1.43) | 0.500 |
| SERPINA1 Z allele       | 1.75 (1.15–2.65) | 0.009 | 1.80 (1.18–2.74) | 0.007 |

aSHR, adjusted subdistribution hazard ratio; CTP, Child-Turcotte-Pugh score; dACLD, decompensated advanced chronic liver disease; HVPG, hepatic venous pressure gradient; UNOS MELD (2016), United Network for Organ Sharing model for end-stage liver disease (2016) score.
effect of Pi*Z allele may even have therapeutic implications in the future. Notably, the association between the Pi*Z allele and the outcome of interest was confirmed in an analysis accounting for PNPLA3 GG genotype.

The anti-protease AAT, mainly expressed in hepatocytes and secreted into the bloodstream, protects the lungs from proteolytic degradation by neutrophil elastase. In AATD-related liver disease, AAT is misfolded and intracellular polymerized, resulting in enhanced protein degradation and/or aggregation in the ER of hepatocytes, generating proteotoxic stress and hepatocellular injury. The resulting ER stress and/or environmental triggers stimulate AAT production, thereby causing a vicious cycle. Therapeutic approaches for AATD-related liver disease include RNA interference to decrease AAT production and secretion, and autophagy enhancers to reduce protein accumulation. Finally, polymerization inhibitors may facilitate both secretion and degradation. Recently, the small-interfering RNA fazirsiran targeting the accumulation of abnormal AAT should be evaluated as disease-modifying therapies in Pi*Z allele carriers with ACLD. The development pipeline of treatments for AATD-related liver disease shows promise.

Abbreviations

AAT, Alpha-1 antitrypsin; AATD, Alpha-1 antitrypsin deficiency; ACLD, Advanced chronic liver disease; CTP, Child-turcotte-pugh score; ER, Endoplasmic reticulum; GWAS, Genome wide association studies; HCC, Hepatocellular carcinoma; (a[S])HR, [Adjusted [subdistribution]] hazard ratio; HVPG, Hepatic venous pressure gradient; NAFLD, Non-alcoholic fatty liver disease; SERPINA1, Serpin family a member 1; UNOS MELD ratio; HVPG, Hepatic venous pressure gradient; NAFLD, Non-alcoholic fatty liver disease etiologies or genotypes, which cannot be rule-out selection bias. However, a high number of HVPG measurements are performed at our institution for risk stratification and treatment monitoring purposes. Other studies investigating the impact of Pi*Z allele on liver diseases were restricted to specific liver disease etiologies or genotypes, which was not the case for our study, as we aimed to maximize sample size. In this context, we have merged all carriers of the Pi*Z allele (of whom n = 39/42 were Pi*MZ) for the main analysis, however, the impact of the Pi*MZ (n = 39; as compared to the Pi*MM, n = 1,030) genotype was confirmed in a subgroup analysis. Of note, merging all carriers of the Pi*Z allele (i.e., not considering the Pi*S allele) in the main analysis allows for direct comparisons with other studies investigating risk/protective alleles (i.e., PNPLA3 and HSD17B13), while this would have not been possible when analysing only haplotypes (i.e., the Pi*Z and Pi*S allele). Of note, the findings of our study may only apply to individuals of European descent, as only a small number of those of non-European descent were included. Although we cannot formally rule-out overlap between the patient populations of Schaefer et al.’s study11 and the present study, this seems extremely unlikely, given the high geographical distance (liver transplant centres for the East/West of Austria) between the recruiting centres. Finally, our study did not include a validation cohort, as we are not aware of another adequately sized cohort linking information on HVPG and genetic data. In conclusion, we demonstrate the profound detrimental impact of the Pi*Z allele on the outcome of ACLD. Genotyping for the Pi*Z allele identifies patients at increased risk, thereby improving prognostication. Finally, the role of medical therapies targeting the accumulation of abnormal AAT should be evaluated as disease-modifying treatments in Pi*Z allele carriers with ACLD.

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Conflicts of interest

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Please refer to the accompanying ICMJE disclosure forms for further details.

Authors’ contributions
Concept of the study (L.B., B.Sc., and M.M.), data collection (L.B., B.Sc., and M.M.), statistical analysis (L.B., B.Sc., and M.M.), drafting of the manuscript (L.B., B.Sc., and M.M.), and revision for important intellectual content and approval of the final manuscript (all authors).

Data availability statement
The data that support the findings of this study are available from the corresponding author upon reasonable request.

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References
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[1] Sharp HL, Bridges RA, Krivit W, Freier EF. Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognized inherited disorder. J Lab Clin Med 1969;73(6):934–939.
[2] Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha-1-antitrypsin deficiency. N Engl J Med 1986;314(12):736–739.
[3] Lomas DA, Hurst JR, Gooptu B. Update on alpha-1 antitrypsin deficiency: new therapies. J Hepatol 2016;65(2):413–424.
[4] Zoller H, Wagner S, Tilg H. Is heterozygosity for the alpha-1 antitrypsin risk allele PiMZ a disease modifier or genetic risk factor? Gastroenterology 2020;159(2):433–434.
[5] Fromme M, Schneider CV, Trautwein C, Brunetti-Pierri N, Strnad P. Alpha-1-antitrypsin deficiency: a re-surfacing adult liver disorder. J Hepatol 2021.
[6] Pons M, Núñez A, Esquinas C, Torres-Durán M, Rodríguez-Hermosa JL, Calle M, et al. Utility of transient elastography for the screening of liver disease in patients with alpha-1-antitrypsin deficiency. J Clin Med 2021;10(8).
[7] Schneider CV, Hamesch K, Gross A, Mandorfer M, Moeller LS, Pereira V, et al. Liver phenotypes of European adults heterozygous or homozygous for PiZ variant of AAT (PiMZ vs PiZZ genotype) and noncarriers. Gastroenterology 2020;159(2):534–548.e11.
[8] Fromme M, Schneider CV, Pereira V, Hamesch K, Pons M, Reichert MC, et al. Hepatobiliary phenotypes of adults with alpha-1-antitrypsin deficiency. Gut 2022;71(2):415–421.
[9] Luukkonen PK, Salomaa V, Åberg F. The PiMZ allele in alpha-1 antitrypsin increases liver-related outcomes in a population-based study. Gastroenterology 2021;160(5):1874–1875.
[10] Semmler G, Balcara L, Oberkoehler H, Zandanell S, Strasser M, Niederseer D, et al. PNPLA3 and SERPIN1A variants are associated with severity of fatty liver disease at first referral to a tertiary center. J Pers Med 2021;11(3).
[11] Schaefer B, Mandorfer M, Viveiros A, Finkenstedt A, Ferenci P, Schneeberger S, et al. Heterozygosity for the alpha-1-antitrypsin Z allele in cirrhosis is associated with more advanced disease. Liver Transpl 2018;24(6):744–751.
[12] Reiberger T, Schwabl P, Trauner M, Peck-Radosavljevic M, Mandorfer M. Measurement of the hepatic venous pressure gradient and transjugular liver biopsy. JHEP Reports 2020;4(6):e100562.
[13] Fertlitsch A, Bota S, Paternostro R, Reiberger T, Mandorfer M, Heinisch B, et al. Evaluation of a new balloon occlusion catheter specifically designed for measurement of hepatic venous pressure gradient. Liver Int: official J Int Assoc Study Liver 2015;35(9):2115–2120.
[14] Flanagin A, Frey T, Christiansen SL. Updated guidance on the reporting of race and ethnicity in medical and scientific journals. Jama 2021;326(7):621–627.
[15] de Franchis R, Bosch J, Garcia-Tsao G, Reiberger T, Ripoll C. Baveno VII – renewing consensus in portal hypertension. J Hepatol 2022;76(4):959–974.
[16] Fine JP, Gray RJ. A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 1999;94(446):496–509.
[17] Mandorfer M, Scheiner B, Stättermayer AF, Schwabl P, Paternostro R, Bauer D, et al. Impact of patatin-like phospholipase domain containing 3 rs738409 G/G genotype on hepatic decompensation and mortality in patients with portal hypertension. Aliment Pharmacol Ther 2018;48(4):451–459.
[18] Strnad P, McEvoyan NG, Lomas DA, Alpha(1)-Antitrypsin deficiency. N Engl J Med 2020;382(15):1443–1455.
[19] Strnad P, Buch S, Hamesch K, Fischer J, Rosendahl J, Schmelz R, et al. Heterozygous carriage of the alpha1-antitrypsin PiZ variant increases the risk to develop liver cirrhosis. Gut 2019;68(10):1109–1107.
[20] Clark VC, Marek G, Liu C, Collinsworth A, Shuster J, Kurtz T, et al. Clinical and histologic features of adults with alpha-1-antitrypsin deficiency in a non-cirrhotic cohort. J Hepatol 2018;69(6):1357–1364.
[21] Chen VL, Burkholder DA, Moran JJ, DiBattista JV, Miller MJ, Chen Y, et al. Hepatic decompensation is accelerated in patients with cirrhosis and alpha-1-antitrypsin PiMZ genotype. JHEP Rep 2022;4(6):100483.
[22] Mandorfer M, Simbrunner B. Prevention of first decompensation in advanced chronic liver disease. Clin Liver Dis 2021;25(2):291–310.
[23] Strnad P, Mandorfer M, Choudhury G, Griffiths W, Trautwein C, Loomba R, et al. LPI0: Aro-AAT reduces Z-AAT protein in PiZZ patients and leads to improvements in clinically relevant liver biomarkers. Hepatology 2021;74(1).
[24] Strnad P, Mandorfer M, Choudhury G, Griffiths W, Trautwein C, Loomba R, et al. Fazirsiran for liver disease associated with alpha(1)-antitrypsin deficiency. N Engl J Med 2022.
[25] Tang Y, Blomenkamp KS, Fickert P, Trauner M, Teckman JH. NetUDCA promotes degradation of α1-antitrypsin mutant Z protein by inducing autophagy through AMPK/ULK1 pathway. PLoS One 2018;13(8):e0200897.
[26] Scheiner B, Stättermayer AF, Schwabl P, Bucics T, Paternostro R, Bauer D, et al. Impact of HSD17B13 rs72613567 genotype on hepatic decompensation and mortality in patients with portal hypertension. Liver Int 2020;40(2):393–404.