





Liver disease in adults with α 1-antitrypsin deficiency

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Abstract

Background: The natural history of adult liver disease due to α 1-antitrypsin deficiency (A1AD) remains poorly understood. **Objective:** We investigated whether heterozygosity for the Z-allele predisposes for the development of clinically significant portal hypertension (CSPH). Moreover, we aimed to non-invasively assess the prevalence of liver fibrosis and hepatic steatosis in adults with A1AD treated by pulmonologists.

Methods: SERPINA1 rs28929474 (Z-allele) was genotyped in 315 patients with CSPH (hepatic venous pressure gradient ≥ 10 mmHg; cases) and 248 liver donors (controls). In addition, 31 adults with A1AD (Pi*ZZ/Pi*SZ) and 11 first-degree relatives (Pi*MZ/Pi*MS) underwent liver stiffness and controlled attenuation parameter (CAP) measurement.

Results: Heterozygosity for the Z-allele was observed in 6.7% of patients with CSPH and 2.8% of liver donors. Thus, harboring the Z-allele was associated with increased odds of CSPH (odds ratio: 2.47; 95% confidence interval: 1.03–5.9; $P=0.042$). Among Pi*ZZ/Pi*SZ patients, 23%/3% had liver stiffness values indicative of liver fibrosis ($\geq F2/\geq F3$). Interestingly, 65%/52% of Pi*ZZ/Pi*SZ patients had CAP values indicative of hepatic steatosis ($\geq S1/\geq S2$).

Conclusions: Heterozygosity for the Z-allele predisposes for the development of CSPH, confirming its role as a genetic (co)factor in liver disease. Pi*ZZ/SZ patients rarely develop liver fibrosis $\geq F3$ during adulthood; however, liver fibrosis $\geq F2$ is common. Elevated CAP values hint at underlying hepatic steatosis, which might promote liver fibrosis progression.

Keywords

Alpha 1-antitrypsin deficiency, portal hypertension, liver fibrosis, hepatic steatosis, transient elastography, controlled attenuation parameter

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Key points

1. Summarize the established knowledge on this subject

- α 1-antitrypsin deficiency (A1AD) is one of the most common genetic disorders, since about 1:25 European Caucasians harbor at least one Z-allele and about 1:2000 are homozygous for the Z-allele (Pi*ZZ).
- While the pulmonary manifestation of A1AD has been intensively studied, the natural history of adult liver disease due to A1AD remains poorly understood.

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2. What are the significant and/or new findings of this study?

- In a genetic sub-study (case-control design), heterozygosity for the Z-allele predisposed for the development of clinically significant portal hypertension, confirming its role as a genetic (co)factor.
- Moreover, we evaluated thoroughly characterized α 1-antitrypsin-deficient patients treated by pulmonologists (Pi*ZZ/Pi*SZ), as well as their first-degree relatives (Pi*MZ/Pi*MS), by transient elastography with controlled attenuation parameter (CAP) measurement.
- Pi*ZZ/SZ patients rarely develop fibrosis \geq F3; however, about one-quarter had fibrosis \geq F2, which warrants the use of non-invasive methods in this population.
- Elevated CAP values were observed in about two-thirds of Pi*ZZ/SZ patients and hint at underlying hepatic steatosis, which might promote liver fibrosis progression.

Introduction

α 1-antitrypsin deficiency (A1AD) is a consequence of misfolding and intracellular polymerization of mutant α 1-antitrypsin within the endoplasmic reticulum (ER) of hepatocytes (toxic gain-of-function), which induces a proinflammatory and fibrogenic response by nuclear factor- κ B activation. Moreover, A1AD increases the sensitivity to ER stress in the presence of concomitant liver diseases.¹

In contrast, the lack of α 1-antitrypsin in the lung (loss-of-function) leads to early-onset panlobular basal emphysema, the typical manifestation of A1AD.

A1AD is one of the most common genetic disorders, since about 1:25 European Caucasians harbor at least one Z-allele (*SERPINA1* rs28929474[T]). About 1:2000 is homozygous for the Z-allele (Pi*ZZ). While the pulmonary manifestation of A1AD has been intensively studied, the natural history of adult liver disease due to A1AD remains poorly understood.

In 1986, Eriksson and co-workers observed cirrhosis in about one-third of autopsied patients with the Z phenotype (i.e. mostly Pi*ZZ genotype).² Based on these and other observations, A1AD is considered a separate aetiological entity.¹ However, until recently, liver biopsy was the only method for assessing the extent of hepatic involvement. Since liver biopsy is limited by its invasiveness, it is mostly performed in patients with findings suggestive of advanced chronic liver disease (ACLD), and thus, biopsy series in these referral populations might substantially overestimate the risk of liver fibrosis/cirrhosis. Population-based surveys, on the other hand, only capture the tip of the iceberg (e.g. patients who have already progressed to ACLD), rather than providing information on the complete spectrum of sub-clinical liver involvement.^{3,4} Promising treatment approaches are currently moving from preclinical to clinical development.⁵ Thus, it is of high relevance that we improve our understanding of the natural history of this condition and identify patients who have already developed significant liver fibrosis, in order to facilitate the design of such clinical trials.

Besides Pi*ZZ patients, heterozygosity for the Z-allele might be a genetic (co)factor for the development of ACLD.^{6–9} However, this association has not been observed throughout all aetiologies/cohorts, and thus, more data is required to facilitate genetic counselling.^{10,11}

In a genetic sub-study (case-control design), we investigated whether heterozygosity for the Z-allele predisposes for the development of ACLD, as indicated by the presence of clinically significant portal hypertension (CSPH).

Moreover, we assessed thoroughly characterized A1AD patients treated by pulmonologists (Pi*ZZ/Pi*SZ) as well as their first-degree relatives (Pi*MZ/Pi*MS) by transient elastography (TE) with controlled attenuation parameter (CAP) measurement, a well-validated non-invasive method for the assessment of liver fibrosis and hepatic steatosis.

Patients and methods

Sub-studies

In sub-study I, the *SERPINA1* rs28929474(T) (Z-allele) was genotyped in 315 patients who were diagnosed with CSPH at the Medical University of Vienna between 2004 and 2014 (cases) and 248 donors from liver transplantations performed at the Medical University of Innsbruck (controls).¹²

In sub-study II, 31 adults with A1AD (Pi*ZZ/Pi*SZ) and 11 first-degree relatives (Pi*MZ/Pi*MS) underwent liver stiffness and CAP measurement within a prospective study in 2015/2016. Patients were identified based on a registry of subjects with A1AD treated by pulmonologists and directly contacted by the investigators.¹³

Please see the Supplementary material for information on hepatic venous pressure gradient (HVPG) measurement and *SERPINA1* genotyping (sub-study I), as well as liver stiffness and CAP measurement, laboratory tests, the short form (36) health survey (SF-36), and the alcohol use disorders identification test (AUDIT) (sub-study II).

Results

Sub-study I

Characteristics of patients with CSPH. Most patients were male (73.9% (232/314)) with a median age of 52.7 (16) years. Hepatitis C (47.8% (150/314)), alcoholic liver disease (31.2% (98/314)), and non-alcoholic fatty liver disease (NAFLD) (7.3% (23/314)) were the most common aetiologies. The prevalence of diabetes was 19.4% (61/314). The patients had a median HVPG of 17 (8) mmHg and a median model for end-stage liver disease score of 10 (6.8) points.

After excluding one Pi*ZZ patient who underwent liver transplantation, 6.7% (21/314) of patients with CSPH were heterozygous for the Z-allele.

Except for aetiology of liver disease, a comparison of patients with and without the Z-allele revealed no statistically significant differences (Table 1). Interestingly, the Z-allele was overrepresented in patients with

cryptogenic cirrhosis when compared to patients with known aetiology (33.3% (3/9) vs. 5.9% (18/305); $P=0.017$).

Comparison of the proportions of patients heterozygous for the Z-allele. Among liver donors, 2.8% (7/248) were heterozygous for the Z-allele ($P=0.049$ vs. CSPH), which is similar to the expected frequency in the Austrian population (Figure 1). Harboring the Z-allele increased the odds of CSPH (odds ratio: 2.47, 95% confidence interval: 1.03–5.9; $P=0.042$).

Sub-study II

Enrollment. Forty-two A1AD index patients treated by pulmonologists (Pi*ZZ/SZ) were informed about the study. Among these, 31 patients (73.8%) agreed to participate in sub-study II. In addition, 11 first-degree relatives (Pi*MZ/MS) were enrolled.

Table 1. Comparison of patients with clinically significant portal hypertension with and without the Z-allele.

Parameter	All, <i>n</i> = 314	No Z-allele <i>n</i> = 293	Z-allele <i>n</i> = 21	<i>P</i> -value
Age, years	52.7 (16)	52.8 (16)	51.8 (18.2)	0.652
Sex				
Male	232 (73.9%)	216 (73.7%)	16 (76.2%)	0.803
Females	82 (26.1%)	77 (26.3%)	5 (23.8%)	
Aetiology				
Hepatitis C	150 (47.8%)	143 (48.8%)	7 (33.3%)	
Alcoholic liver disease	98 (31.2%)	90 (30.7%)	8 (38.1%)	
NAFLD	23 (7.3%)	22 (7.5%)	1 (4.8%)	
Other	34 (10.8%)	32 (11%)	2 (9.5%)	
Unknown	9 (2.9%)	6 (2%)	3 (14%)	
Diabetes	61 (19.4%)	59 (20.1%)	2 (9.5%)	0.39
HVPG, mmHg	17 (8)	17 (8)	19 (9)	0.632
MELD score, points	10 (6.8)	10 (6.78)	10 (6)	0.393
Platelet count, $G \times L^{-1}$	100 (73)	98.5 (68)	122 (100)	0.118
AST, $U \times L^{-1}$	65 (56)	65 (56)	53 (78)	0.565
ALT, $U \times L^{-1}$	46 (50)	46 (53)	42 (37)	0.772
GGT, $U \times L^{-1}$	129 (150)	125 (152)	149 (90)	0.131
Albumin, $g \times L^{-1}$	35.5 (8.3)	35.7 (8.3)	34.1 (10.2)	0.612
Triglycerides, $mg \times dL^{-1}$	87 (53)	86 (52)	86 (74)	0.482
Cholesterol, $mg \times dL^{-1}$	144 (52)	144 (52)	165 (41)	0.055
LDL, $mg \times dL^{-1}$	81 (39.2)	80.8 (38.8)	81.8 (30.6)	0.162
HDL, $mg \times dL^{-1}$	41 (21)	41 (20)	51 (23)	0.063
Glucose, $mg \times dL^{-1}$	108 (38)	108 (37)	110 (45)	0.996
CRP, $mg \times L^{-1}$	0.34 (0.7)	0.34 (0.7)	0.345 (0.79)	0.955
vWF antigen, %	324 (151)	323 (153)	338 (120)	0.142

NAFLD: non-alcoholic fatty liver disease; HVPG: hepatic venous pressure gradient; MELD: model for end-stage liver disease; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyltransferase; LDL: low-density lipoprotein; HDL: high-density lipoprotein; CRP: C-reactive protein; vWF: von Willebrand factor.

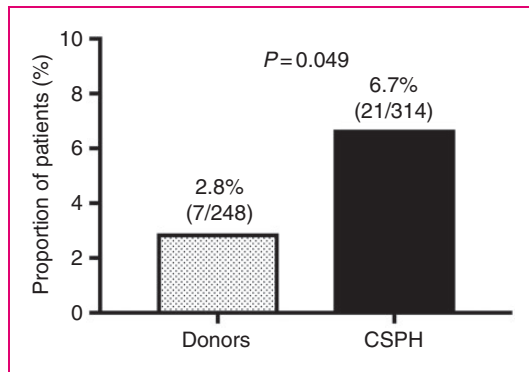


Figure 1. Comparison of the proportion of patients heterozygous for the Z-allele between patients with clinically significant portal hypertension (CSPH; cases) and liver donors (controls).

Patient characteristics. Please see Table 2 and the Supplementary material for patient characteristics.

Liver stiffness. Liver stiffness was comparable between Pi*ZZ/Pi*SZ patients (5.77 ± 0.3 kPa) and their first-degree relatives (Pi*MZ/MS; 5.36 ± 0.6 kPa; $P=0.518$; Figure 2).

Among Pi*ZZ/Pi*SZ patients, 22.6% (7/31) and 3.2% (1/31) had liver stiffness values indicative of liver fibrosis \geq F2 and \geq F3, respectively (Figure 3).

Risk factors for liver fibrosis \geq F2. Combining all subjects included in sub-study II, subjects with liver fibrosis \geq F2 had higher body mass index (BMI; 27.6 ± 1.4 vs. 24.5 ± 0.7 $\text{kg} \times \text{m}^{-2}$; $P=0.049$) and serum gamma-glutamyltransferase levels (GGT; 45 (62) vs. 27 (19) U/L; $P=0.013$) (Table 2). In contrast, lower serum high-density lipoprotein (HDL) cholesterol levels (50.1 ± 4.1 vs. 66.2 ± 2.9 $\text{mg} \times \text{dL}^{-1}$; $P=0.014$) were observed in the liver fibrosis \geq F2 group. Moreover, we observed trends towards a higher prevalence of hepatic steatosis \geq S1 (87.5% (7/8) vs. 53% (18/34); $P=0.078$) and \geq S2 (75% (6/8) vs. 35.3% (12/34); $P=0.078$), as well as higher HOMA-IR (homeostatic model assessment of insulin resistance) levels (3.31 (2.61) vs. 2.01 (1.99) $\text{mg} \times \mu\text{U} \times \text{dL}^{-1} \times \text{mL}^{-1}$; $P=0.107$) in subjects with liver fibrosis \geq F2. As expected, subjects with liver fibrosis \geq F2 showed lower platelet counts (174 (23) vs. 221(83) $\text{G} \times \text{L}^{-1}$; $P < 0.001$).

If analysed as a continuous variable, liver stiffness tended to correlate with the same parameters (Supplementary Table 2).

CAP values. Pi*ZZ/Pi*SZ patients had numerically higher CAP values (267 ± 9 $\text{dB} \times \text{m}^{-1}$) than their first-degree relatives (Pi*MZ/MS; 237 ± 22 $\text{dB} \times \text{m}^{-1}$; $P=0.148$; Figure 2).

Interestingly, 64.5% (20/31) of Pi*ZZ/Pi*SZ patients had CAP values indicative of hepatic steatosis \geq S1 and 51.6% (16/31) showed values suggestive of \geq S2 (Figure 3).

CAP correlated positively with age and metabolic parameter such as BMI ($\rho=0.449$; $P=0.003$), HbA1c ($\rho=0.394$; $P=0.013$), and HOMA-IR ($\rho=0.474$; $P=0.002$) (Supplementary Table 2). We observed direct correlations with high-sensitive C-reactive protein (hsCRP; $\rho=0.418$; $P=0.008$) as a marker of inflammation, and von Willebrand factor antigen (vWF; $\rho=0.451$; $P=0.003$) as a marker of endothelial dysfunction. Moreover, CAP tended to correlate positively with liver stiffness ($\rho=0.304$; $P=0.053$).

Concomitant liver diseases. Overall, only three subjects (7.1% (3/42)) reported at-risk drinking (AUDIT \geq 8 points); however, serum carbohydrate-deficient transferrin levels were $< 2.6\%$ in all individuals (Table 2).¹⁴ Three subjects (7.1% (3/42)) were hepatitis B surface and core antibody-positive, while we did not observe hepatitis B surface antigen positivity. There was no evidence of other chronic liver diseases.

Please see the Supplementary material for information on hepatitis A/B vaccination and hepatitis E seroprevalence, as well as health-related quality of life.

Discussion

To assess the role of the Z-allele as a genetic (co)factor for the development of ACLD, we investigated whether heterozygosity for the Z-allele predisposes for the development of CSPH (sub-study I). Harboring the Z-allele was associated with approximately 2.5-fold increased odds of CSPH. This might be explained by the impact of A1AD on hepatic inflammation and fibrosis.¹ Interestingly, the Z-allele was overrepresented in patients with cryptogenic cirrhosis (33.3%) when compared to patients with known aetiology (5.9%). This confirms a previous report of Graziadei et al., who observed a prevalence of 26.9% in patients who underwent liver transplantation for cryptogenic cirrhosis.⁷ Thus, heterozygosity for the Z-allele might have implications in patients without a known aetiology. This prompts *SERPINA1* genotyping in patients with cryptogenic cirrhosis, as well as in patients with findings indicative of an otherwise unexplained chronic liver disease. The group of patients with cryptogenic cirrhosis might have comprised a considerable proportion of NAFLD patients (i.e. ‘burnt-out non-alcoholic steatohepatitis’); however, a previous study did not observe a link between A1AD alleles and liver fibrosis in NAFLD.^{15–17} Moreover, we did not observe an overrepresentation of the Z-allele in NAFLD patients.

Table 2. Comparison of subjects with significant liver fibrosis ($\geq F2$), or without (F0/F1), as defined by a liver stiffness cut-off of 7.1 kPa.

Parameter	All, <i>n</i> = 42	Liver fibrosis F0/F1, <i>n</i> = 34	Liver fibrosis $\geq F2$, <i>n</i> = 8	<i>P</i> -value
Age, years	54.2 (27.5)	49.1 (27.7)	63.7 (15.4)	0.21
Sex				
Male	25 (59.5%)	16 (47.1%)	7 (87.5%)	0.114
Females	17 (40.5%)	18 (52.9%)	1 (12.5%)	
BMI, $\text{kg} \times \text{m}^{-2}$	25.1 \pm 0.6	24.5 \pm 0.7	27.6 \pm 1.4	0.049
<25 $\text{kg} \times \text{m}^{-2}$	21 (50%)	18 (52.9%)	3 (37.5%)	0.061
25-29.9 $\text{kg} \times \text{m}^{-2}$	16 (38.1%)	14 (41.2%)	2 (25%)	
$\geq 30 \text{ kg} \times \text{m}^{-2}$	5 (11.9%)	2 (5.9%)	3 (37.5%)	
Group				
Treated by pulmonologists	31 (73.8%)	24 (70.6%)	7 (87.5%)	0.419
First-degree relatives	11 (26.2%)	10 (29.4%)	1 (12.5%)	
SERPINA1 genotype				
ZZ	28 (66.7%)	21 (61.8%)	7 (87.5%)	0.486
MZ	10 (23.8%)	9 (26.5%)	1 (12.5%)	
SZ	3 (7.1%)	3 (8.8%)	0 (0%)	
MS	1 (2.4%)	1 (2.9%)	0 (0%)	
CAP, $\text{dB} \times \text{m}^{-1\text{a}}$	260 \pm 9	253 \pm 10	285 \pm 18	0.148
$\geq 248 \text{ dB} \times \text{m}^{-1}$ ($\geq S1$) ^a	25 (59.5%)	18 (53%)	7 (87.5%)	0.078
$\geq 268 \text{ dB} \times \text{m}^{-1}$ ($\geq S2$) ^a	18 (42.9%)	12 (35.3%)	6 (75%)	0.056
$\geq 280 \text{ dB} \times \text{m}^{-1}$ ($S3$) ^a	15 (35.7%)	10 (29.4%)	5 (62.5%)	0.11
Platelet count, $\text{G} \times \text{L}^{-1}$	209 (82)	221 (83)	174 (23)	< 0.001
Prothrombin time, %	90.8 \pm 2.6	92.3 \pm 3	84.5 \pm 4.2	0.242
AP, $\text{U} \times \text{L}^{-1}$	67.9 \pm 2.2	67.5 \pm 2.4	69.9 \pm 5.4	0.668
AST, $\text{U} \times \text{L}^{-1}$	27.1 \pm 1.5	26.5 \pm 1.4	29.8 \pm 5.0	0.397
ALT, $\text{U} \times \text{L}^{-1}$	24 (24)	22.5 (25)	27 (26)	0.498
GGT, $\text{U} \times \text{L}^{-1}$	31 (19)	27 (19)	45 (62)	0.013
Bilirubin, $\text{mg} \times \text{dL}^{-1}$	0.62 (0.43)	0.63 (0.41)	0.58 (0.52)	0.888
Albumin, $\text{g} \times \text{L}^{-1}$	44.1 (4.4)	44.1 (4.6)	43.9 (4.0)	0.440
Triglycerides, $\text{mg} \times \text{dL}^{-1}$	90 (64)	80 (60)	94.5 (233)	0.352
Cholesterol, $\text{mg} \times \text{dL}^{-1}$	198 \pm 6	199.6 \pm 7	191 \pm 11	0.579
LDL, $\text{mg} \times \text{dL}^{-1}$	113 \pm 5	114 \pm 6	110 \pm 9	0.756
HDL, $\text{mg} \times \text{dL}^{-1}$	63.1 \pm 2.6	66.2 \pm 2.9	50.1 \pm 4.1	0.014
HbA1c, %	5.3 (0.6)	5.3 (0.6)	5.4 (1)	0.63
HOMA-IR, $\text{mg} \times \mu\text{U} \times \text{dL}^{-1} \times \text{mL}^{-1}$	2.16 (2.11)	2.01 (1.99)	3.31 (2.61)	0.107
hsCRP, $\text{mg} \times \text{L}^{-1}$	0.13 (0.243)	0.13 (0.25)	0.09 (0.1)	0.218
vWF antigen, %	136.5 (63)	136.5 (59)	153.5 (120)	0.368
$\alpha 1$ -antitrypsin, $\text{mg} \times \text{dL}^{-1}$	73.7 (68.3)	73.9 (69.3)	66.6 (59.9)	0.519
SF-36 PCS score, points	38.9 \pm 2	38.9 \pm 2.2	39.1 \pm 4.8	0.978
SF-36 MCS score, points	55.5 (8.6)	56.0 (9.4)	55.2 (8.4)	1
AUDIT, points	3 (4)	3 (4)	1.5 (4)	0.32
< 8 points	39 (92.9%)	31 (91.2%)	8 (100%)	0.609
≥ 8 points	3 (7.1%)	3 (8.8%)	0 (0%)	

^aAvailable in 41 patients.

BMI: body mass index; CAP: controlled attenuation parameter; AP: alkaline phosphatase; AST: aspartate transaminase; ALT: alanine transaminase; GGT: gamma-glutamyltransferase; LDL: low-density lipoprotein; HDL: high-density lipoprotein; HbA1c: glycated hemoglobin; HOMA-IR: homeostatic model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; vWF: von Willebrand factor; SF-36: short form (36) health survey; PCS: physical component summary; MCS: mental component summary; AUDIT: alcohol use disorders identification test.

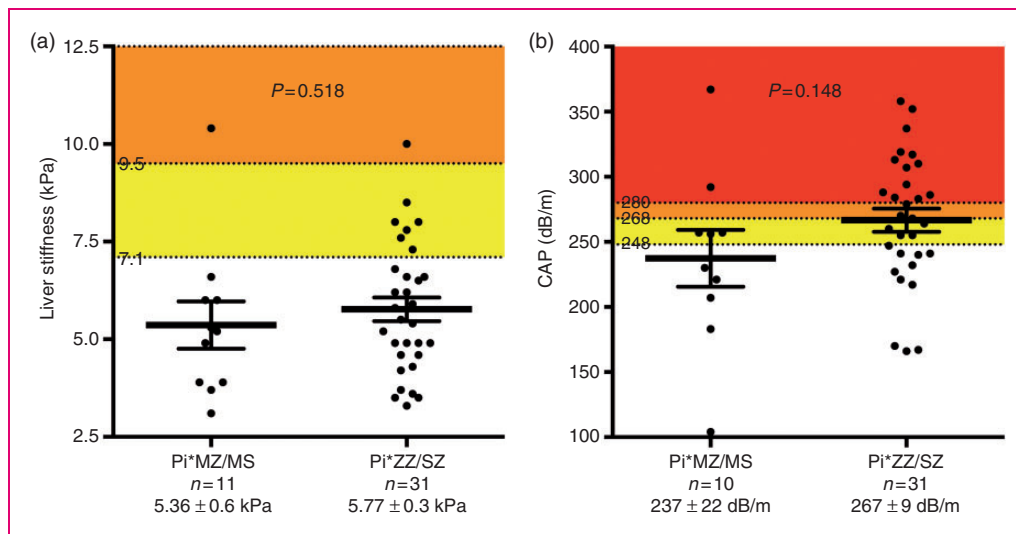


Figure 2. Comparison of (a) liver stiffness and (b) controlled attenuation parameter (CAP) values between Pi*ZZ/SZ patients and their first-degree relatives (Pi*MZ/MS).

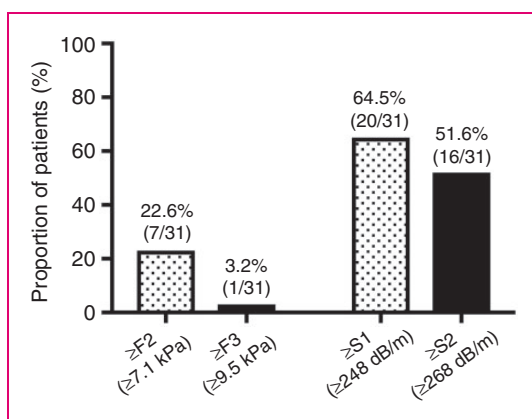


Figure 3. Prevalence of significant and advanced fibrosis (left) as well as controlled attenuation parameter (CAP) values indicative of hepatic steatosis \geq S1 and \geq S2 (right) in patients with α 1-antitrypsin deficiency who were treated by pulmonologists (Pi*ZZ/SZ).

Since sub-study I confirmed the role of the Z-allele as a genetic (co)factor for the development of ACLD, we evaluated thoroughly characterized α 1-antitrypsin-deficient patients treated by pulmonologists (Pi*ZZ/Pi*SZ) as well as their first-degree relatives (Pi*MZ/Pi*MS) by TE with CAP to further investigate the relationship between A1AD genotype and liver phenotype (sub-study II).

In sub-study II, we observed a low prevalence of liver fibrosis \geq F3 (\geq 9.5 kPa; 3.2%), although about one-quarter of patients showed liver stiffness values indicative of liver fibrosis \geq F2 (\geq 7.1 kPa; 22.6%). Interestingly, the prevalence of liver fibrosis \geq F3 in

our study was considerably lower than the proportion of patients with ACLD reported by previous studies.^{2,18}

In the only prospective study performing biopsies to investigate liver involvement in Pi*ZZ patients ($n = 57$), Dawwas et al. observed liver fibrosis \geq F3 in 17.5% of the overall study population.¹⁸ Liver biopsies were only performed in patients with clinical, laboratory or radiological evidence of liver injury, and some patients declined liver biopsy. Thus, the authors might have even underestimated the prevalence of liver fibrosis \geq F3 in their cohort, since the diagnosis might have been missed in patients with sub-clinical liver disease who were not referred for liver biopsy. Mean/median age and BMI were very similar to our study and the authors reported a high participation rate of 89%, which limits the risk of selection bias. Therefore, the different study settings seem to be the only plausible explanation for the discrepancy in the proportion of patients with liver fibrosis \geq F3: Dawwas and co-workers recruited study participants among patients referred to the respiratory clinic of a tertiary care centre.¹⁸ In contrast, the Pi*ZZ/SZ patients included in our study were identified based on a registry of subjects with A1AD treated by pulmonologists and directly contacted by the investigators.¹³ Since our study was not restricted to patients treated at a tertiary care centre, our study population is presumably more representative for the overall population of Pi*ZZ/SZ patients.

Another recently published study used acoustic radiation force impulse (ARFI) elastography, a non-invasive method that is less commonly applied than TE.^{19,20} Importantly, ARFI was performed in only

39.5% of the study population, which puts this study at particularly high risk of selection bias. Moreover, the proportion of study participants with liver stiffness values suggestive of liver fibrosis \geq F2 was comparable between Pi*ZZ (25%) and Pi*MM subjects (22%), which questions the reliability of the measurements. Of note, study participants were fasted for only 3 h and liver stiffness assessed by ARFI elastography has been shown to remain numerically increased, even 3 h after a meal intake.²¹

Kim and colleagues performed liver biopsies in a small series ($n=11$) of Pi*ZZ patients to investigate the value of magnetic resonance elastography for liver fibrosis.²² The proportion of patients with liver fibrosis \geq F2 was 18.2%, which is very similar to our study. However, the small sample size and the strict in- and exclusion criteria substantially limit the conclusions that can be drawn from this study. Nevertheless, its results support the use of non-invasive methods in patients with A1AD, since the diagnostic performance in A1AD was comparable to NAFLD patients who served as a control group.

Interestingly, in our study, about two-thirds of Pi*ZZ/Pi*SZ patients had CAP values indicative of hepatic steatosis \geq S1. This is in line with the histological results of Dawwas et al., which observed hepatic steatosis in 87.5% of patients who underwent liver biopsy (i.e. the sub-group of patients with evidence of liver injury).¹⁸ Importantly, subjects with liver fibrosis \geq F2 had higher BMI and GGT levels, and also tended to show higher CAP values. Of note, a recent meta-analysis based on individual patient data demonstrated that high CAP is not associated with a systematic overestimation of liver fibrosis by TE.²³ CAP itself was linked to a series of metabolic parameters such as BMI, HbA1c and HOMA-IR. Moreover, we observed direct correlations with hsCRP and vWF. To summarize these findings, hepatic steatosis, which can be assessed non-invasively by CAP, seemed to be associated with more advanced liver disease, and also several cardiovascular risk factors. These findings underline that, similar to NAFLD, lifestyle interventions might be of high relevance in patients with A1AD. As clinical studies on specific treatment approaches are still ongoing, it appears reasonable to counsel A1AD patients with hepatic steatosis in analogy to the NAFLD population,²⁴ although some measures might be complicated by the underlying pulmonary manifestation.

Except for three subjects reporting at-risk drinking (however, none of them had liver stiffness values indicative of liver fibrosis \geq F2), there was no evidence of concomitant liver diseases.

Interestingly, less than one-third of Pi*ZZ/Pi*SZ patients were vaccinated against hepatitis A/B

(Supplementary material). Even in the sub-group of patients on α 1-antitrypsin augmentation therapy, only half of the patients were vaccinated, although vaccination is recommended by the label since it is a plasma derivative. Thus, it will require the combined efforts of pulmonologists and hepatologists to increase vaccination rates in this patient population.

Several limitations should be considered when interpreting the findings of our study. First, not all contacted patients (73.8%) participated in our study. However, most patients declined to participate for logistical reasons (e.g. distance to the study center). Moreover, liver stiffness/CAP measured by TE has not been validated against liver biopsy in patients with A1AD. However, its diagnostic value has been confirmed in most aetiologies of chronic liver disease, including NAFLD.^{24–26} All relevant confounding factors for the TE-based assessment of liver fibrosis induce false-high liver stiffness results leading to an overestimation of liver fibrosis stage.²⁷ Thus, they cannot explain the very low prevalence of liver fibrosis \geq F3 observed in our study. Since sample size limits the conclusions of our study, collaborative efforts are required to perform large international studies.^{28,29} However, it can be assumed, that multicenter studies will not be able to characterize subjects in the same detail as our monocenter study.

In conclusion, heterozygosity for the Z-allele predisposes for the development of CSPH, confirming its role as a genetic (co)factor in liver disease. Pi*ZZ/SZ patients rarely develop liver fibrosis \geq F3 during adulthood. However, liver fibrosis \geq F2 is common, which warrants the use of non-invasive methods to identify patients at risk for the development of ACLD. High BMI and GGT values identify patients with an increased pretest probability of having liver fibrosis \geq F2. Elevated CAP values hint at underlying hepatic steatosis, which might promote liver fibrosis progression.

Author contributions

All authors contributed either to research design (MM and TR), and/or the acquisition (genotyping: BS, HZ, and PF; phenotyping: all authors), analysis (MM) or interpretation (all authors) of data. MM drafted the manuscript, which was then critically revised by all other authors. All authors approved the submitted version of the manuscript.

Declaration of conflicting interests

The authors declare that there are no conflicting interests relevant to this work.

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Informed consent

Except for liver donors (requirement of informed consent was waived by the ethics committee of the Medical University of Innsbruck), all subjects were consented for genetic testing.

Ethics approval

All sub-studies were conducted in accordance with the Declaration of Helsinki as reflected by the a priori approval of the ethics committees of the Medical Universities of Vienna (numbers 1964/2014 and 1526/ 2017) and Innsbruck (number UN4218).

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