Hepatobiliary phenotypes of adults with alpha-1 antitrypsin deficiency

European Alpha-1 Liver Study Group

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Abbreviations:

AAT	Alpha-1 antitrypsin
AATD	Alpha-1 antitrypsin deficiency
CAP	Controlled attenuation parameter
LSM	Liver stiffness measurements
NAFLD	Non-alcoholic fatty liver disease
NASH	Non-alcoholic steatohepatitis
Pi	Protease inhibitor
Pi*M	Normal AAT allele
Pi*S	Mutant *SERPINA1* allele variant termed 'S'
Pi*Z	Mutant *SERPINA1* allele variant termed 'Z'
Pi*MZ	AAT genotype with heterozygosity for the Pi*Z variant
Pi*SZ	AAT genotype with compound heterozygosity for Pi*Z and Pi*S variant
Pi*ZZ	AAT genotype with homozygosity for the Pi*Z variant
SERPINA1	AAT gene
TE	Transient elastography (FibroScan®)
TM6SF2	Transmembrane 6 superfamily member 2
PNPLA3	Patatin-like phospholipase domain-containing protein 3
HSD17B13	17β-Hydroxysteroid dehydrogenase type 13 gene

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Significance of this study

What is already known about this subject?

- Pi*Z and Pi*S are the most important genetic variants causing alpha1-antitrypsin deficiency (AATD).
- No reliable data on hepatobiliary phenotype exist for individuals with Pi*SS and Pi*SZ genotype, despite the fact that both genotypes are seen in ~1:500 Caucasians.
- The vast majority of AATD subjects remain undiagnosed during their lifetime and this fact complicates AATD phenotyping.

What are the new findings?

- In a large community-based Biobank as well as in a multinational cohort, subjects with Pi*SZ genotype were markedly predisposed to liver fibrosis and seemed to be predisposed to primary liver cancer.
- Compared to the characteristic severe AATD genotype Pi*ZZ, Pi*SZ genotype causes intermediate hepatobiliary phenotype, while Pi*SS does not seem to have major hepatobiliary consequences.

How might it impact on clinical practice in the foreseeable future?

- Our study defines the hepatic risks associated with the major AATD genotypes. These data, together with the individual situation/susceptibility factors, should guide the counseling and management of AATD individuals.
- The observed association with primary liver cancer should promote hepatologic surveillance of AATD individuals and spur longitudinal studies characterizing the development of liver fibrosis and malignancy.

ABSTRACT

Objective: Alpha-1 antitrypsin deficiency (AATD) is a common, potentially lethal inborn disorder caused by mutations in alpha-1 antitrypsin (AAT). Homozygosity for the 'Pi*Z' variant of AAT (Pi*ZZ genotype) causes lung and liver disease, whereas heterozygous 'Pi*Z' carriage (Pi*MZ genotype) predisposes to gallstones and liver fibrosis. The clinical significance of the more common 'Pi*S' variant remains largely undefined and no robust data exist on the prevalence of liver tumors in AATD.

Design: Baseline phenotypes of AATD individuals and non-carriers were analyzed in 482,380 participants in the UK Biobank. 1104 participants of a multinational cohort (586 Pi*ZZ, 239 Pi*SZ, 279 non-carriers) underwent a comprehensive clinical assessment. Associations were adjusted for age, sex, BMI, diabetes, and alcohol consumption.

Results: Among UK Biobank participants, Pi*ZZ individuals displayed the highest liver enzyme values, the highest occurrence of liver fibrosis/cirrhosis (adjusted odds ratio (aOR)=21.7[8.8-53.7]) and primary liver cancer (aOR=44.5[10.8-183.6]). Subjects with Pi*MZ genotype had slightly elevated liver enzymes and moderately increased odds for liver fibrosis/cirrhosis (aOR=1.7[1.2-2.2]) and cholelithiasis (aOR=1.3[1.2-1.4]). Individuals with homozygous Pi*S mutation (Pi*SS genotype) harbored minimally elevated alanine aminotransferase values, but no other hepatobiliary abnormalities. Pi*SZ participants displayed higher liver enzymes, more frequent liver fibrosis/cirrhosis (aOR=3.1[1.1-8.2]) and primary liver cancer (aOR=6.6[1.6-26.9]). The higher fibrosis burden was confirmed in a multinational cohort. Male sex, age≥50years, obesity, and the presence of diabetes were associated with significant liver fibrosis.

Conclusion: Our study defines the hepatobiliary phenotype of individuals with the most relevant AATD genotypes including their predisposition to liver tumors, thereby allowing evidence-based advice and individualized hepatological surveillance.

Keywords: SERPINA1, Fibroscan, Pi*S, liver fibrosis, liver cirrhosis.

INTRODUCTION

AAT deficiency (AATD) is one of the most common, potentially lethal inborn disorders, with AATD-related lung and liver disease being the major drivers of morbidity and mortality.[1] Mutations in the *SERPINA1* gene coding for alpha-1 antitrypsin (AAT) lead to a 'gain of function' proteotoxic liver injury, whereas the lack of AAT in the bloodstream facilitates the development of chronic obstructive pulmonary disease (COPD) and emphysema.[1] The most common severe *SERPINA1* variant is termed 'Pi*Z' (rs28929474).[2, 3] The 'Pi*S' variant (rs17580) is even more prevalent, but less detrimental.[1] The homozygous occurrence of 'Pi*Z' is found in 1:2000 Caucasians[2] and is termed 'Pi*ZZ', whilst heterozygous 'Pi*Z' carriage (termed 'Pi*MZ' genotype) is seen in 1:30 individuals of Northern European descent. The strong predisposition of 'Pi*ZZ' individuals for lung disease is supported by a large body of evidence and reflected in clinical management guidelines.[1] The susceptibility for liver disease is less well documented.[3, 4] 'Pi*ZZ'-related liver disease displays a biphasic pattern with the first peak in early childhood as neonatal cholestasis and the second peak after 50 years of age.[1, 5] Two cross-sectional studies indicate that advanced liver fibrosis is ten to 20 times more common in 'Pi*ZZ' subjects compared to individuals without a 'Pi*Z' mutation (non-carriers) and revealed that the non-invasive liver stiffness measurement (LSM) via transient elastography (TE) constitute a useful surrogate of liver fibrosis.[4, 6] 'Pi*MZ' individuals seem to carry moderately elevated odds for both lung and liver disease, and to be susceptible to gallstone disease.[1, 7] Humans carrying both the 'Pi*Z' and the 'Pi*S' variant (termed 'Pi*SZ') are as frequent as 1:500 in certain geographic regions,[8] while the occurrence of homozygous carriage of the 'Pi*S' variant (termed 'Pi*SS') might be even higher.[9] Two studies demonstrated that 'Pi*SZ' individuals display a less severe lung phenotype than Pi*ZZ subjects,[10, 11] whereas the extent of their liver disease was not systematically studied. Children with 'Pi*SZ' genotype develop a clinically relevant liver disease markedly less often than Pi*ZZ individuals.[12, 13] Similar findings have been reported in adults,[14] but multiple case reports have described "idiopathic" liver cirrhosis in 'Pi*SZ' subjects.[15] Finally, while the 'Pi*SS' genotype is considered to confer minimal if any risks, little clinical data are available to support this directly.[16]

Probably the greatest limitation when studying the AATD phenotype is the fact that the majority of AATD cases remain undiagnosed and the proportion is even higher in individuals with less severe genotypes.[1] A Swedish birth cohort-based study partially addressed this issue, but this study examined the individuals only up to 45 years of age, i.e. before the peak of AATD-related adult liver disease and focused on Pi*ZZ individuals.[17] To provide unbiased information about the hepatobiliary phenotype of individuals with major AATD genotypes, we used the UK Biobank, a community sample from the United Kingdom totaling nearly 500,000 individuals with available 'Pi*Z' and 'Pi*S' genotyping. To corroborate our findings, we prospectively recruited the largest, multi-national cohort of Pi*SZ subjects without previously known chronic liver disease and compared their lung- and liver-related parameters

to those of Pi*ZZ participants, and non-carriers. The goal of our study was to provide data for evidence-based management and counseling of these individuals.

METHODS

Population-based UK Biobank participants (Cohort 1)

The 'UK Biobank' (UKB) is a population-based cohort study conducted in the United Kingdom, which recruited 502,511 volunteers aged 37 to 73 years at baseline. All participants underwent an initial examination, which was the basis for our study and gave informed consent for genotyping and data linkage to medical reports. Ongoing inpatient hospital records beginning in 1996 were used to identify diagnoses according to ICD10 codes (international classification of diseases, 10th revision). Genotyping for both the Pi*Z (rs28929474) and Pi*S (rs17580) mutation of *SERPINA1* was available in 487,503 subjects. Follow-up measurement of liver enzymes was conducted in 16,010 participants.

We excluded participants with viral hepatitis (ICD10: B16-B19: 713 MM, 20 MZ, 2 SZ) or risky alcohol consumption (>60g alcohol/d for men, >40g alcohol/d for women: 3775 MM, 153 MZ, 9 SZ, 3 ZZ, 4 SS). We compared *SERPINA1* variants to well-known genes, that modulate the risk of liver disease, i.e. *PNPLA3* p.I148M (rs738409), *HSD17B13*:T (rs72613567), and *TM6SF2* p.E167K (rs5854926); homozygous carriers were compared with non-carriers.

The presence of the following primary ICD10 codes was evaluated: Fibrosis and cirrhosis (K74.0-2+K74.6), primary liver cancer (C22.0), non-alcoholic fatty liver disease (NAFLD, K76.0), non-alcoholic steatohepatitis (NASH, K75.81), cholelithiasis (K80), emphysema (J43.1+J43.2+J43.8+J43.9), and chronic bronchitis (J44). The study has been approved by the UKB Access Committee (Project #47527). The presence of metabolic syndrome was based on the IDF (International diabetes federation) definition, which consists of central obesity (defined as waist circumference with gender and ethnicity specific values) plus any two of the following four factors: (i) raised serum triglycerides ≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality; (ii) reduced serum HDL cholesterol < 40 mg/dL (1.03 mmol/L) in males or < 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality; (iii) raised systolic blood pressure (BP) ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension; (iv) raised fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes.

Real life cohort without previously known liver disease (Cohort 2)

Study population

1104 individuals were recruited as part of our global Alpha-1 Liver initiative, a multicenter registry effort for AATD-related liver disease carrying out baseline assessment, that are shown herein, as well as a prospective follow-up. The majority of non-carriers and Pi*ZZ participants was previously published.[6, 7] The inclusion criteria were (i) age ≥18 years, (ii) the ability to provide written informed consent, and (iii) no known pregnancy. To prevent an

enrichment with individuals with liver involvement, the following exclusion criteria were used: (i) the presence of known liver disease or a liver co-morbidity identified in clinical and laboratory work-up, (ii) at least two independent visits with elevated liver enzymes in medical records prior to baseline.

All participants underwent clinical and laboratory work-up including standardized questionnaires with demographic parameters, previously known chronic diseases and relevant comorbidities, as well as the evaluation of alcohol and cigarette consumption. The need for augmentation therapy and long-term oxygen therapy and the COPD assessment test (CAT) values constituted determinants of lung phenotype. AAT serum level was measured and genotyping was conducted by the responsible national AAT reference laboratories, that performed PCR analysis and/or isoelectric focusing as described.[6]

The Pi*SZ population consisted of 239 subjects from ten European countries (Germany, UK, Portugal, Spain, Italy, Austria, Belgium, Ireland, Denmark, Poland) and the US (Supplementary table 1). Among the Pi*ZZ participants, 413 (70.5%) were from Germany. Non-carriers (n=279) were defined as individuals with normal serum AAT levels (i.e. >110 mg/dL), in whom the presence of the 'Pi*Z' and 'Pi*S' variant was excluded as described.[6] 219 (78.5%) of them were recruited in Germany, 60 (21.5%) in Austria.

Statistical analysis

All continuous variables were analyzed by unpaired, two-tailed t-tests or Mann-Whitney U test, and by a multivariable model to account for confounders (age, sex, BMI, diabetes mellitus, and alcohol consumption) and shown as mean (standard deviation) (normal distribution) or median [IQR] (non-normal distribution). Categorical variables were displayed as relative (%) frequencies and analyzed using the Chi-square test. ORs were presented with their corresponding 95% confidence intervals (CI). Multivariable logistic regression tested for independent associations. Correlations were assessed by Spearman correlation coefficients, where appropriate. Differences were indicated as statistically significant when $P<0.05$. The data were analyzed using SPSS Statistics version 26 (IBM; Armonk, NY, USA) and Prism version 8 (GraphPad, LaJolla, CA, USA).

RESULTS

Lung- and liver-related parameters of AATD subjects in UK Biobank (Cohort 1)

The 482 380 eligible UK Biobank participants comprised 138 Pi*ZZ (frequency 1:3496), 864 Pi*SZ (1:558), 1014 Pi*SS (1:476), and 17006 Pi*MZ individuals (1:28; Figure 1A). All subgroups displayed a similar age and sex distribution as well as a comparable - mostly low or moderate - alcohol consumption. While diabetes mellitus was infrequent, it was less common in Pi*ZZ subjects compared to non-carriers (5% vs. 2%, p<0.0001; Table 1). As expected, Pi*ZZ individuals showed a significantly lower FEV1/FVC ratio compared to all other genotypes despite the lowest cigarette consumption (Table 1). The percentage of individuals with FEV1/FVC < 70% was the highest among Pi*ZZ participants but was also significantly higher in Pi*SZ individuals compared to non-carriers. Regarding liver-related blood parameters, mean ALT values were significantly higher in all analyzed AATD genotypes compared to non-carriers (Table 1, Figure 2A). Pi*MZ and Pi*SZ subjects presented with higher AST values than non-carriers, however, Pi*ZZ individuals had significantly higher AST values than any other assessed AATD subgroup (Table 1, Figure 2B). Gamma-glutamyl transferase (GGT) values were comparable in non-carriers, Pi*MZ, Pi*SS, and Pi*SZ individuals, while Pi*ZZ subjects significantly more often displayed elevated GGT levels. Alkaline phosphatase (ALP) was significantly elevated in Pi*MZ and Pi*SZ participants when compared to non-carriers, however, comparable to subjects without AATD mutation in Pi*ZZ, Pi*SS individuals (Table 1, Figure 2C). The odds ratio of having elevated AST was the highest in Pi*ZZ individuals (adjusted OR=4.5[2.8-7.3], p<0.0001; Figure 3) and surpassed the odds seen in established genetic liver disease modifiers such as homozygous *PNPLA3* or *TM6SF2* mutation (Figure 3; Supplementary tables 2-4). Pi*ZZ participants also had a moderately increased risk for elevated ALT values (adjusted OR=2.1[1.2-3.6], p<0.0001; Figure 3) with odds comparable to the ones seen in subjects with a homozygous *PNPLA3* or *TM6SF2* mutation (Figure 3; Supplementary tables 2,3). Individuals with Pi*MZ, Pi*SS, and Pi*SZ genotype had all significantly increased risk of presenting with elevated ALT values (OR=1.2-1.5; Figure 3). To determine, whether AATD predisposes to elevated liver enzymes even in metabolically inconspicuous individuals, we reperformed the liver enzyme analysis after exclusion of individuals with the diagnosis NAFLD (Supplementary figure 1) and after exclusion of individuals with metabolic syndrome (Supplementary figure 2**).** Both analyses yielded largely identical results, thereby establishing the effect of AATD mutations even in metabolically inconspicuous individuals. In line, an additional adjustment for the *PNPLA3* allele as the second strongest – and due to the high frequency of the risk allele – most relevant genetic risk factor for metabolic liver disease, did not affect the results (data not shown).

Next, we assessed whether the analyzed liver enzymes remain stable or fluctuate over time. Here, we took advantage of follow-up measurements that were available in a subset of UK Biobank individuals and correlated them with baseline values. Serum levels of GGT and ALP showed a strong correlation (rho=0.7-0.85; Supplementary table 5),

whereas the correlation between baseline and follow-up transaminases/bilirubin levels were somewhat weaker (Supplementary table 5), which is in line with previous reports.[18]

In the least studied genotypes Pi*SS and Pi*SZ, male sex, age ≥50 years, and smoking were associated with higher rates of decreased %FEV1/FVC (Figure 4). With regard to transaminases, the presence of BMI ≥30kg/m^2 or diabetes mellitus conferred an increased chance of displaying elevated values. Age ≥50 years was associated with increased AST, but not ALT values (Figure 4).

Table 1: Comparison of lung and liver phenotype in individuals with Pi*SS and Pi*SZ genotype compared to Pi*ZZ, Pi*MZ, and non-carriers (Cohort 1).

	Non-carriers (n=422 506)	**MZ (n=17 006)**	**SS (n=1014)**	**SZ (n=864)**	**ZZ (n=138)**
Characteristics					
Age, mean (SD), y	56.5 (8.1)	56.9 (8.1)	56.4 (8.2)	56.6 (7.8)	56.1 (8.0)
Women, No. (%)	229 360 (54)	9 289 (55)	545(54)	474 (55)	65 (47)
BMI, mean (SD), kg/m^2	27.4 (4.8)	27.3 (4.7)	27.2 (4.6)	27.0 (4.6)	26.8 (4.7)
Alcohol, mean (SD), g/d	8.8 (10.1)	8.6 (9.9)	8.3 (9.8)	8.6 (9.8)	8.3 (8.3)
Risk factors					
BMI>30 kg/m^2, No. (%)	130 070 (31)	5 135 (30)	300 (30)	249 (29)	37 (27)
Diabetes mellitus, No. (%)	22 399 (5)	753 (4)	57 (6)	31 (4)	3 (2)
Lung status					
FEV1/VC, mean (SD), %	75.9 (7.3)[1]	75.9 (7.5)[2]	76.0 (7.4)[3]	75.7 (7.7)[4]	71.5 (12.6)[1,2,3,4]
FEV1/VC<70%, No. (%)	59 476 (14)[5,6]	2 486 (15)[7]	148 (15)[8]	147 (17)[5,9]	44 (32)[6,7,8,9]
Cigarette consumption, mean (SD), py	23.2±18.7[10,11]	23.1±18.9[12,13]	25.7±19.9[10,12,14]	22.5±18.6[15]	14.5±8.2[11,13,14,15]
Liver status					
ALT, mean (SD), % of ULN	56.2 (32.7)[16,17,18,19]	58.8 (31.6)[16]	59.3 (32.4)[17]	59.9 (30.3)[18]	62.5 (25.3)[19]
ALT ≥ULN, No. (%)	26 914 (6.4)[20,21,22,23]	1 235 (7.2)[20,24]	90 (8.9)[21]	76 (8.8)[22]	15 (10.9)[23,24]
AST, mean (SD), % of ULN	63.6 (26.0)[25,26,27]	65.2 (23.9)[25,28]	64.8 (24.5)[29]	66.0 (23.1)[26,30]	75.7 (21.8)[27,28,29,30]
AST ≥ULN, No. (%)	18 490 (4.4)[31.32]	847 (5.0)[31,33]	53 (5.2)[34]	46 (5.3)[35]	20 (14.5)[32,33,34,35]
GGT, mean (SD), % of ULN	73.3 (81.3)	75.6 (76.6)	77.1 (73.4)	76.4 (62.5)	83.1 (75.2)
GGT ≥ULN, No. (%)	68 510 (16.2)[36,37]	2 849 (16.8)[36,38]	185 (18.2)	157 (18.2)	30 (21.7)[37,38]
ALP, mean (SD), % of ULN	72.7 (24.7)[39,40]	75.3 (25.7)[39,41]	73.3 (24.4)[41,42]	76.3 (24.5)[40,42]	72.2 (21.4)
ALP ≥ULN, No. (%)	46 534 (11.0)[43,44]	2 305 (13.6)[43]	121 (11.9)[45]	134 (15.5)[44,45]	16 (11.6)
Bilirubin, mean (SD), mg/dl	0.53 (0.26)	0.54 (0.26)	0.54 (0.27)	0.54 (0.28)	0.56 (0.26)
Bilirubin ≥ULN, No. (%)	11 692 (2.8)	528 (3.1)	31 (3.1)	28 (3.2)	7 (5.1)

Quantitative measures are expressed as mean with standard deviation or relative frequency (%). All analyses were adjusted for age, sex, BMI, presence of diabetes mellitus, and mean alcohol consumption.

Abbreviations: AATD, alpha-1 antitrypsin deficiency; ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; ULN, upper limit of normal (sex-specific).

[1]$p=6.0*10^{-12}$; [2]$p=2.5*10^{-11}$; [3]$p=9.5*10^{-9}$; [4]$p=1.5*10^{-7}$; [5]$p=0.032$; [6]$p=3.0*10^{-9}$;[7]$p=1.2*10^{-8}$; [8]$p=4.9*10^{-8}$; [9]$p=0.000006$; [10]$p=0.018$; [11]$p=0.017$; [12]$p=0.021$; [13]$p=0.022$; [14]$p=0.009$; [15]$p=0.044$; [16]$p=7.9*10^{-30}$; [17]$p=0.001$; [18]$p=0.00007$; [19]$p=0.006$; [20]$p=6.5*10^{-9}$; [21]$p=0.0004$; [22]$p=0.001$; [23]$p=0.009$; [24]$p=0.046$;[25]$p=4.1*10^{-14}$; [26]$p=0.004$; [27]$p=1.3*10^{-9}$; [28]$p=5.1*10^{-9}$;

[29]p=7.4*10^{-8}; [30]p=2.5*10^{-7}; [31]p=0.00004; [32]p=1.5*10^{-9}; [33]p=7.4*10^{-8}; [34]p=0.00001; [35]p=0.00001; [36]p=0.004; [37]p=0.013; [38]p=0.028;[39]p=4.4*10^{-44}; [40]p=0.000003; [41]p=0.019; [42]p=0.003; [43]p=1.0*10^{-24}; [44]p=0.00001; [45]p=0.015.
SI conversion factors: To convert ALT, AST, GGT, and ALP to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Lung- and liver-related diagnoses of AATD subjects in UK Biobank (Cohort 1)

With regard to lung-related ICD codes, the diagnosis of COPD and emphysema was >20x enriched in Pi*ZZ participants compared to non-carriers. It was not more common in Pi*SS and Pi*SZ subjects, while the much more frequent Pi*MZ individuals displayed a moderately increased odds ratio for emphysema (adjusted OR=1.6[1.3-1.9], p<0.0001; Supplementary table 6; Figure 5). Consistently with a previous publication,[19] gallstone disease was enriched in Pi*MZ carriers vs. non-carriers (adjusted OR=1.3[1.2-1.4], p<0.0001), but in none of the other AATD genotypes. The diagnosis liver fibrosis/cirrhosis was nearly 20x more common in Pi*ZZ individuals compared to non-carriers (adjusted OR=21.7[8.8-53.7], p<0.0001), but also markedly enriched in Pi*SZ subjects (adjusted OR=3.1[1.1-8.2], p=0.027) and moderately in Pi*MZ participants (1.7[1.2-2.2]; p=0.001; Supplementary table 6; Figure 5). Both Pi*SZ and Pi*ZZ individuals harbored a numerically higher risk of liver fibrosis/cirrhosis than any of the previously described genetic liver disease modifiers (Figure 6A; Supplementary tables 2-4, 6). Similarly, both Pi*SZ and Pi*ZZ subjects, but none of the other AATD genotypes, possessed a markedly increased risk for the diagnosis of primary liver cancer. Again, the risk of Pi*ZZ individuals for primary liver cancer surpassed the odds seen in individuals with other genetic modifiers, while Pi*SZ was comparable to known risk factors *TM6SF2* and *PNPLA3* (Figure 6, Supplementary tables 2-4, 6).

A sensitivity analysis revealed that in the Pi*SZ and Pi*MZ populations, the 'fibrosis/cirrhosis' phenotype is markedly enriched in males, obese individuals, and subjects ≥50 years old (Supplementary figure 3).

Lung and liver phenotype in a multinational AATD cohort (Cohort 2)

Our multinational cohort consisted of 586 Pi*ZZ subjects, 239 Pi*SZ individuals, and 279 non-carriers, all without previously known or co-existing liver disease (Table 2; Figure 1B). Pi*SZ subjects were underrepresented when compared to the community-based UK Biobank cohort. All three subgroups showed similar rates of diabetes mellitus and alcohol consumption, while differences in other demographic factors were seen (Table 2). When only participants without augmentation therapy were considered, Pi*SZ individuals showed intermediate AAT serum levels (63.8±19.8 mg/dl vs. 139.5±25.1 mg/dl in non-carriers vs. 28.3±16.0 mg/dl in Pi*ZZ, all p<0.0001, Table 2; Supplementary figure 4A). The cut-off AAT level of 99.5 mg/dL differentiated well between Pi*SZ individuals and non-carriers (sensitivity 97.9%, specificity 98.4%, Table 2; Supplementary figure 4A). Pi*SZ individuals had an intermediate lung phenotype as reflected by their CAT scores and need for long-term oxygen treatment, i.e.the

levels/frequencies were higher than in non-carriers, but significantly lower/less frequent compared to Pi*ZZ individuals (Table 2). With regard to liver enzymes, Pi*SZ individuals had lower AST and ALT than Pi*ZZ subjects, while GGT was higher in Pi*SZ subjects than non-carriers. Mean ALP levels were the highest in Pi*SZ individuals (Supplementary figure 4C-F, Supplementary table 7), whereas GLDH and bilirubin levels did not show obvious differences among the subgroups (Supplementary table 7).

In TE, Pi*SZ individuals had intermediate LSM values, i.e. LSMs were higher than in non-carriers (5.2±2.5 kPa vs. 4.6±1.6 kPa, p=0.002), but lower than in Pi*ZZ subjects (5.2±2.5 kPa vs. 6.6±5.2 kPa; p=0.022, Table 2; Supplementary figure 4B) and similar results were seen when only non-obese individuals were assessed (Supplementary table 8). In the entire cohort, thirteen percent of Pi*SZ individuals showed LSM values ≥7.1kPa suggesting liver fibrosis stage of at least 2 on a 0-4 scale[20] compared to 5% of non-carriers (adjusted OR=2.6 [1.1-6.1], p=0.024; Table 2) and 24% of Pi*ZZ subjects (adjusted OR=0.5 [0.2-0.8], p=0.013; Table 2). Pi*SZ individuals with LSM ≥7.1 kPa had significantly higher BMI values and were more frequently diabetic (Supplementary table 9). *Vice versa*, diabetic individuals more frequently displayed elevated AST and ALT values than subjects without diabetes (Supplementary figure 5).

The simultaneously assessed CAP as a surrogate of hepatic steatosis did not show major differences between Pi*SZ and non-carriers, nor between Pi*SZ individuals and Pi*ZZ subjects (Table 2). However, increased liver enzyme levels were seen primarily in Pi*SZ individuals with liver steatosis (as revealed by an analysis of individuals with CAP ≥248dB/m) who displayed higher AST, GGT, and ALP levels than “steatotic” non-carriers (Supplementary figure 6).

Table 2: Characteristics of Pi*SZ individuals in comparison to Pi*ZZ subjects and non-carriers in a multi-center registry cohort (Cohort 2).

	Non-carriers (n= 279)	**Pi*SZ (n= 239)**	**Pi*ZZ (n= 586)**	***P value Pi*SZ vs. non-carriers (uni-variable)***	***P value Pi*SZ vs. non-carriers (multi-variable)***	***P value Pi*SZ vs. Pi*ZZ (uni-variable)***	***P value Pi*SZ vs. Pi*ZZ (multi-variable)***
Characteristics							
Age, mean (SD), y	52.4 (14.6)	50.4 (16.1)	54.2 (13.2)	0.142		**0.002**	
Women, No. (%)	137 (49.1)	135 (56.5)	271 (46.2)	0.094		**0.008**	
BMI, mean (SD), kg/m^2	25.6 (4.5)	26.6 (5.5)	25.0 (4.4)	**0.022**		**<0.0001**	
Alcohol, mean (SD), g/d	7.4 (9.7)	7.3 (11.9)	5.6 (9.6)	0.958		0.086	
AAT serum level[#], mean (SD), mg/dL	139.5 (25.1)	63.8 (19.8)	28.3 (16.0)	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**
Risk factors							
BMI ≥30 kg/m², No. (%)	35 (12.9)	51 (23.1)	61 (10.5)	**0.003**		**<0.0001**	
Diabetes mellitus, No. (%)	13 (5.0)	7 (3.5)	20 (4.2)	0.426		0.654	
Relevant alcohol intake[+], No. (%)	32 (11.5)	30 (17.5)	48 (8.2)	0.070		**<0.0001**	
Lung status							
CAT score, mean (SD), points	7.0 (6.0)	14.1 (9.2)	16.5 (8.1)	**<0.0001**	**<0.0001**	**0.022**	**0.004**
Cigarette consumption, mean (SD), py	8.3 (16.5)	11.0 (17.1)	9.9 (13.4)	0.147	0.232	0.501	0.434
Long-term oxygen treatment, No. (%)	1 (0.4)	12 (6.5)	109 (22.6)	**<0.0001**	**0.003**	**<0.0001**	**<0.0001**
Liver status							
Liver stiffness°, mean (SD), kPa	4.6 (1.6)	5.2 (2.5)	6.6 (5.2)	**0.001**	**0.002**	**<0.0001**	**0.022**
Liver stiffness ≥7.1 kPa°, No. (%)	15 (5.4)	24 (12.6)	140 (23.9)	**0.006**	**0.024**	**0.001**	**0.013**
Liver stiffness ≥10.0 kPa (%)°, No. (%)	3 (1.1)	7 (3.7)	76 (13.0)	0.057	0.199	**<0.0001**	**0.006**
CAP°, mean (SD), dB/m	249.5 (58.1)	259.6 (60.7)	264.6 (57.0)	0.122	0.056	0.401	0.132
CAP ≥248 dB/m (%)°, No. (%)	136 (51.9)	67 (57.3)	288 (60.8)	0.334	0.234	0.489	0.296
CAP ≥280 dB/m (%)°, No. (%)	79 (30.2)	46 (39.3)	173 (36.5)	0.080	**0.009**	0.572	0.422

Quantitative measures are expressed as mean with standard deviation or relative frequency (%). All multivariable analyses were adjusted for age, sex, BMI, presence of diabetes mellitus, and mean alcohol consumption. The cut-offs for non-invasive liver parameters were chosen according to etiology-unspecific recommendations: Liver stiffness ≥7.1 kPa indicating significant liver fibrosis (fibrosis stage ≥2 on a 0-4 scale) and ≥10 kPa showing advanced fibrosis (fibrosis stage ≥3). Controlled-attenuation parameter (CAP) ≥248 dB/m suggesting the presence of steatosis grade ≥1, and CAP ≥280 dB/m indicating the presence of steatosis grade 3.

[+] Alcohol intake >12 g/d for women, >24 g/d for men (individuals with alcohol consumption >40 g/d females or >60 g/d males had been excluded *a priori*).

[#] AAT serum levels of individuals, who did not receive AAT augmentation therapy, are shown.

° Liver stiffness and CAP only available in 190 Pi*SZ individuals.

Abbreviations: BMI, body mass index; AAT, alpha-1 antitrypsin; CAT, chronic obstructive pulmonary disease assessment test; CAP, controlled attenuation parameter.
SI conversion factors: To convert AAT to µmol/L, multiply values by 0.184.

DISCUSSION

We analysed the hepatobiliary phenotype of individuals with the most common AATD genotypes using the UK Biobank as a unique, openly available resource with deep genetic, physical, and health data.[21] It does not constitute an entirely representative population sample since 94% of subjects are classed as white British and 6% within ethnic minority groups, compared with 80.5% and 19.5% respectively in UK census data, and it is skewed towards higher income classes.[21] Nevertheless, it represents the best available approximation of such a cohort in that it recruited and systematically genotyped participants independently on their known *SERPINA1* genotype. This approach is crucial since the vast majority of AATD individuals remain undetected and were therefore not considered in previous studies. The AAT genotyping used in our study was extracted from the UK Biobank Axiom™ array and the results remained unknown to the study subjects. The frequencies of analysed genotypes agreed well with their published occurrence in Caucasian population[8] – an observation that further validates our approach.
An important limitation of our study is the difficulty to reliably identify all individuals with NAFLD and NASH since these disorders were not systematically assessed in the UK Biobank baseline visits and may remain underdiagnosed in the clinical routine. To offset this limitation, we repeated the analyses after excluding individuals with the ICD code for NAFLD as well as with presence of metabolic syndrome and demonstrated that the differences persisted in this subgroup. Moreover, liver transaminase levels significantly fluctuated over time and therefore a single measurement is not sufficient to comprehensively evaluate the liver phenotype of AATD individuals. Notably, the limited usefulness of single ALT measurements for evaluation of AATD individuals was reported previously.[22] However, our manuscript aimed to provide “typical liver enzyme levels” seen in subjects with different AATD genotypes.
In the UK Biobank cohort, Pi*ZZ participants suffered a >20 times higher risk of liver fibrosis and cirrhosis as well as ~45 times higher risk of primary liver cancer. The former finding is in line with previous reports demonstrating that signs of advanced fibrosis are nine to 20-fold more common in Pi*ZZ individuals compared to people without AAT mutations as well as the observation that Pi*ZZ individuals are 20 times more likely to require liver transplantation than the general population.[6, 23] The odds of Pi*ZZ subjects to develop advanced liver fibrosis/cirrhosis are substantially higher than the ones reported for other established genetic conditions such as mutations in *PNPLA3, TM6SF2,* or *HSD17B13* gene.[23, 24] Whilst the predisposition to liver fibrosis is now supported by a solid body of evidence, reports on liver cancer in Pi*ZZ individuals are very limited[1] and further

analyses are needed. Pi*ZZ participants had the highest AST/ALT values, but their ALP levels were similar to the ones seen in non-carriers and they did not present with an increased risk of cholelithiasis. Since gallstones consist mainly of lipids such as cholesterol, the alterations in lipid metabolism that were observed in Pi*ZZ individuals (i.e. lower serum levels of triglycerides, very low-density lipoproteins and low-density lipoproteins compared to controls) and indicate an impaired hepatic secretion of lipids might play a role.[6] Collectively, our data revealing a marked susceptibility of Pi*ZZ individuals to end-stage liver disease should prompt their thorough hepatological monitoring. The availability of genetic information allowed us to systematically study AATD genotypes, that are not assessed in clinical routine such as Pi*MZ and Pi*SS. With regard to Pi*MZ, our data confirmed previous findings of a mild increase in transaminases as well as a moderately increased risk of liver fibrosis/cirrhosis and cholelithiasis.[7, 19, 24, 25] On the other hand, the increased occurrence of emphysema was seen in some, but not all population-based studies.[1] With regard to the Pi*SS genotype, our data are novel and support the current opinion that these individuals display no or only minimal predisposition to both lung and liver disease. It provides an important guidance for physicians and a relief for the carriers of this genotype.

A focus of our work was on the Pi*SZ phenotype, that is underrepresented in clinical routine compared to Pi*ZZ subjects. This might be due to their less conspicuous AAT serum levels as well as their less pronounced disease phenotype.[10, 11, 26] Consistently with published data, the Pi*SZ individuals available in the UK Biobank displayed no or only minimal lung phenotype,[10, 11] while our multi-center cohort was skewed towards more lung-diseased individuals, likely due to the fact that it often prompted the diagnosis of AATD. Although Pi*SZ individuals display normal or only minimally elevated transaminases, both analyzed cohorts revealed a marked predisposition to liver fibrosis. The UK Biobank cohort also suggested an increased susceptibility to primary liver cancer that was not assessed in the second cohort. The more pronounced association with liver fibrosis compared to lung emphysema might be attributable to the fact that the liver phenotype constitutes a “gain-of-function” toxicity while lung injury seems to arise due to a loss-of-function situation. Accordingly, the intermediate AAT serum levels seen in Pi*SZ individuals might be sufficient to protect the lung from proteolytic damage, while misfolding and polymerization of AAT may generate biologically relevant proteotoxic stress in the liver. The identified hetero-polymerization between Pi*S and Pi*Z [27] might be responsible for the greater liver fibrosis burden than that of the Pi*MZ state despite the absence of any Pi*SS signal (suggesting no clinically significant challenge with Pi*S misfolding alone). While Pi*SZ subjects display clear predisposition to liver fibrosis and primary liver cancer, their susceptibility is markedly lower than the one seen in Pi*ZZ individuals, which is consistent with the observed lower levels of intracellular polymers and a less pronounced lung phenotype.[10, 11]

In addition to the characterization of the hepatobiliary phenotype of AATD individuals, we demonstrated that male sex, obesity, diabetes, and higher age are associated with increased risk of liver fibrosis/cirrhosis as well as primary liver cancer. Notably, the same factors were previously implicated in liver fibrosis development in Pi*MZ and Pi*ZZ individuals.[4, 6, 7, 25, 28] Among them, obesity and diabetes are potentially modifiable and their effects as drivers of non-alcoholic fatty liver disease extends beyond AATD.[29, 30] They are associated with increased oxidative stress and lipolysis and may aggravate the endoplasmic reticulum stress occurring in AATD.[31, 32] Male sex is another parameter linked to AATD since the production of AAT is stimulated by testosterone and males therefore produce higher amounts of the potentially toxic protein.[33]

In conclusion, our data characterize the hepatobiliary phenotype of adults with major AATD genotypes with a focus on Pi*SZ and should help in patient management and counselling. While Pi*ZZ individuals need a closer monitoring, the surveillance of Pi*MZ and Pi*SZ subjects needs to be adjusted to the overall clinical context that includes the presence of hepatic co-morbidities/metabolic risk factors, other genetic factors as well as the presence/absence of baseline liver fibrosis as evaluated by non-invasive methods. The association with primary liver cancer should spur hepatological surveillance of both Pi*ZZ and Pi*SZ individuals. However, further studies are warranted to determine whether screening is needed for all Pi*SZ/Pi*ZZ individuals or only those with advanced liver fibrosis/cirrhosis. Longitudinal assessment is needed to define the rate of disease development and tumor occurrence in the individuals with different AATD genotypes.

Declarations:

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Study concept and design: M.F., C.V.S., P.S.

Acquisition of data: M.F., C.V.S., V.P., K.H., M.P., M.C.R., F.B., P.E., K.T., M.M., B.B., V.W., J.C.W., J.V., F.N., J.G., M.M., A.N., B.S., H.Z., S.J., N.A., L.J., R.G., C.G., K.M.S., M.T., A.K., B.G., D.T., A.M., J.R.H., D.A.L., F.L., N.T.G., V.C., W.J.G., C.T., A.M.T., N.G.M, P.S.

Analysis and interpretation of data: M.F., C.V.S., P.S.

Drafting of the manuscript: M.F., C.V.S., P.S.

Critical revision of the manuscript for important intellectual content: all authors

Figures and tables: M.F., C.V.S., P.S.

Statistical analysis: M.F., C.V.S.

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Study supervision: M.F., C.V.S., P.S.

All authors had full access to all of the data and approved the final version of this manuscript. All authors take responsibility for the integrity of the data and the accuracy of the data analysis.

REFERENCES

1 Strnad P, McElvaney NG, Lomas DA. Alpha1-Antitrypsin Deficiency. *The New England journal of medicine* 2020;382:1443-55.

2 Greene CM, Marciniak SJ, Teckman J, Ferrarotti I, Brantly ML, Lomas DA, et al. alpha1-Antitrypsin deficiency. *Nature reviews Disease primers* 2016;2:16051.

3 Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. *The New England journal of medicine* 2009;360:2749-57.

4 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;69:1357-64.

5 Patel D, Teckman JH. Alpha-1-Antitrypsin Deficiency Liver Disease. *Clinics in liver disease* 2018;22:643-55.

6 Hamesch K, Mandorfer M, Pereira VM, Moeller LS, Pons M, Dolman GE, et al. Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation. *Gastroenterology* 2019;157:705-19.e18.

7 Schneider CV, Hamesch K, Gross A, Mandorfer M, Moeller LS, Pereira V, et al. Liver Phenotypes of European Adults Heterozygous or Homozygous for Pi*Z Variant of AAT (Pi*MZ vs Pi*ZZ genotype) and Non-carriers. *Gastroenterology* 2020.

8 Blanco I, Bueno P, Diego I, Perez-Holanda S, Lara B, Casas-Maldonado F, et al. Alpha-1 antitrypsin Pi*SZ genotype: estimated prevalence and number of SZ subjects worldwide. *Int J Chron Obstruct Pulmon Dis* 2017;12:1683-94.

9 de Serres FJ, Blanco I. Prevalence of alpha1-antitrypsin deficiency alleles PI*S and PI*Z worldwide and effective screening for each of the five phenotypic classes PI*MS, PI*MZ, PI*SS, PI*SZ, and PI*ZZ: a comprehensive review. *Therapeutic advances in respiratory disease* 2012;6:277-95.

10 Lara B, Miravitlles M. Spanish Registry of Patients With Alpha-1 Antitrypsin Deficiency; Comparison of the Characteristics of PISZ and PIZZ Individuals. *Copd* 2015;12 Suppl 1:27-31.

11 Franciosi AN, Hobbs BD, McElvaney OJ, Molloy K, Hersh C, Clarke L, et al. Clarifying the Risk of Lung Disease in SZ alpha1-antitrypsin Deficiency. *Am J Respir Crit Care Med* 2020.

12 Ruiz M, Lacaille F, Berthiller J, Joly P, Dumortier J, Aumar M, et al. Liver disease related to alpha1-antitrypsin deficiency in French children: The DEFI-ALPHA cohort. *Liver international : official journal of the International Association for the Study of the Liver* 2019;39:1136-46.

13 Teckman JH, Rosenthal P, Abel R, Bass LM, Michail S, Murray KF, et al. Baseline Analysis of a Young alpha-1-Antitrypsin Deficiency Liver Disease Cohort Reveals Frequent Portal Hypertension. *J Pediatr Gastroenterol Nutr* 2015;61:94-101.

14 Townsend SA, Edgar RG, Ellis PR, Kantas D, Newsome PN, Turner AM. Systematic review: the natural history of alpha-1 antitrypsin deficiency, and associated liver disease. *Alimentary pharmacology & therapeutics* 2018;47:877-85.

15 Strnad P, Brantly ML, Bals R. Alpha-1-Antitrypsin Deficiency (ERS Monograph), 2019.

16 McGee D, Schwarz L, McClure R, Peterka L, Rouhani F, Brantly M, et al. Is PiSS Alpha-1 Antitrypsin Deficiency Associated with Disease? *Pulmonary medicine* 2010;2010:570679.

17 Mostafavi B, Piitulainen E, Tanash HA. Survival in the Swedish cohort with alpha-1-antitrypsin deficiency, up to the age of 43-45 years. *Int J Chron Obstruct Pulmon Dis* 2019;14:525-30.

18 Lazo M, Selvin E, Clark JM. Brief communication: clinical implications of short-term variability in liver function test results. *Annals of internal medicine* 2008;148:348-52.

19 Ferkingstad E, Oddsson A, Gretarsdottir S, Benonisdottir S, Thorleifsson G, Deaton AM, et al. Genome-wide association meta-analysis yields 20 loci associated with gallstone disease. *Nature Communications* 2018;9:5101.

20 Friedrich-Rust M, Poynard T, Castera L. Critical comparison of elastography methods to assess chronic liver disease. *Nature reviews Gastroenterology & hepatology* 2016;13:402-11.

21 Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, et al. The UK Biobank resource with deep phenotyping and genomic data. *Nature* 2018;562:203-9.

22 Clark VC, Dhanasekaran R, Brantly M, Rouhani F, Schreck P, Nelson DR. Liver test results do not identify liver disease in adults with α(1)-antitrypsin deficiency. *Clin Gastroenterol Hepatol* 2012;10:1278-83.

23 Adam R, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, et al. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). *J Hepatol* 2012;57:675-88.

24 Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. *The New England journal of medicine* 2018;378:1096-106.

25 Strnad P, Buch S, Hamesch K, Fischer J, Rosendahl J, Schmelz R, et al. Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. *Gut* 2019;68:1099-107.

26 Green CE, Vayalapra S, Hampson JA, Mukherjee D, Stockley RA, Turner AM. PiSZ alpha-1 antitrypsin deficiency (AATD): pulmonary phenotype and prognosis relative to PiZZ AATD and PiMM COPD. *Thorax* 2015;70:939-45.

27 Laffranchi M, Berardelli R, Ronzoni R, Lomas DA, Fra A. Heteropolymerization of α-1-antitrypsin mutants in cell models mimicking heterozygosity. *Human molecular genetics* 2018;27:1785-93.
28 Bowlus CL, Willner I, Zern MA, Reuben A, Chen P, Holladay B, et al. Factors associated with advanced liver disease in adults with alpha1-antitrypsin deficiency. *Clin Gastroenterol Hepatol* 2005;3:390-6.
29 Williams R, Aspinall R, Bellis M, Camps-Walsh G, Cramp M, Dhawan A, et al. Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. *Lancet* 2014;384:1953-97.
30 Stender S, Kozlitina J, Nordestgaard BG, Tybjærg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. *Nat Genet* 2017;49:842-7.
31 Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. *Physiological reviews* 2018;98:2133-223.
32 Lebeaupin C, Vallée D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. *J Hepatol* 2018;69:927-47.
33 Rudnick DA, Liao Y, An JK, Muglia LJ, Perlmutter DH, Teckman JH. Analyses of hepatocellular proliferation in a mouse model of alpha-1-antitrypsin deficiency. *Hepatology* 2004;39:1048-55.

FIGURE LEGENDS

Figure 1: Overview of analyzed cohorts.

A) Cohort 1: Population-based study analyzing UK Biobank participants aged 37 to 73 years at baseline. B) Cohort 2: Prospectively recruited individuals available in a multinational, cross-sectional Alpha-1 Liver initiative.

Figure 2: Liver related parameters in individuals heterozygous for the Pi*Z variant (Pi*MZ), homozygous for the Pi*S variant (Pi*SS), heterozygous for both Pi*S and Pi*Z (Pi*SZ), and homozygous for the Pi*Z variant (Pi*ZZ) compared to non-carriers (Cohort 1).

422 506 non-carriers, 17 006 Pi*MZ subjects, 1014 Pi*SS individuals, 864 Pi*SZ subjects, and 138 Pi*ZZ individuals underwent laboratory analysis. P values were adjusted for age, sex, BMI, alcohol consumption, and presence of diabetes mellitus. Scatter plots of serum level of alanine aminotransferase (ALT; A), aspartate aminotransferase (AST; B), and alkaline phosphatase (ALP; C), all normalized to the sex-specific upper limit of normal (ULN).

Figure 3: Risk of Pi*SS and Pi*SZ subjects to show elevated AST or ALT compared to heterozygous (Pi*MZ) and homozygous (Pi*ZZ) Pi*Z carriers as well as homozygous carriers of PNPLA3 p.I148M (rs738409), HSD17B13:T (rs72613567), and TM6SF2 p.E167K (rs5854926) (Cohort 1).

Adjusted odds ratios (aOR) with their corresponding 95% confidence intervals (CI) are shown for aspartate aminotransferase (AST; A) and alanine aminotransferase (ALT; B). The risk to display levels higher than the corresponding sex-dependent upper limit of normal (ULN) was compared to the respective non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus.

Figure 4: Rate of Pi*SS and Pi*SZ subjects with decreased Tiffenau Index, elevated AST, or elevated ALT in different subpopulations (Cohort 1).

Relative frequencies (%) are shown and visualized by a color coding (right panel). Decreased Tiffenau-index is defined as FEV1/VC <70%. Smokers are defined as “ever-smokers” and non-smokers are defined as “never-smokers”. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index (kg/m^2); DM, diabetes mellitus; FEV1, forced expiratory volume in 1 second; VC, vital capacity.

Figure 5: Odds ratios of ICD10 diagnoses in individuals heterozygous or homozygous for the Pi*Z variant (Pi*MZ/Pi*ZZ), homozygous for Pi*S variant (Pi*SS) and heterozygous for Pi*S and Pi*Z (Pi*SZ) (Cohort 1).

Adjusted odds ratios (aOR) with their corresponding 95% confidence intervals (CI) are shown for Pi*MZ, Pi*SS, Pi*SZ, and Pi*ZZ subjects compared to non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus. If in one group no cases are available, the corresponding aOR was set as 1[1;1].

Figure 6: Odds ratios of ICD10 diagnoses in individuals heterozygous (or homozygous for Pi*Z (Pi*MZ/Pi*ZZ), heterozygous for Pi*S and Pi*Z (Pi*SZ) or homozygous for displayed genetic liver fibrosis modifiers.

Genetic liver fibrosis modifiers include *PNPLA3* p.I148M (rs738409), *HSD17B13*:T (rs72613567), and *TM6SF2* p.E167K (rs5854926)). Adjusted odds ratios (aOR) with their 95% confidence intervals (CI) compared to the corresponding non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus.

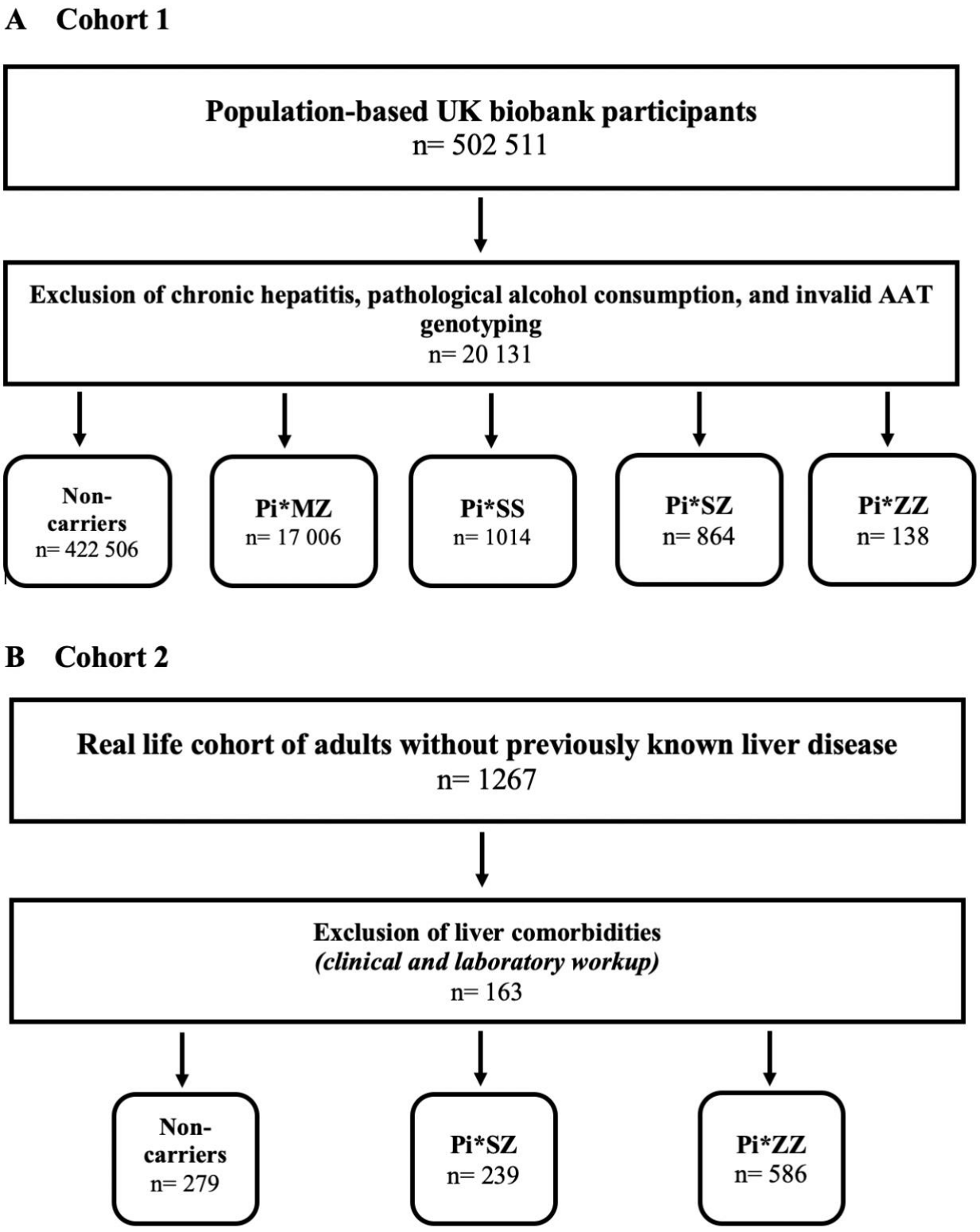

Figure 1: Overview of analyzed cohorts.

A) Cohort 1: Population-based study analyzing UK Biobank participants aged 37 to 73 years at baseline. B) Cohort 2: Prospectively recruited individuals available in a multinational, cross-sectional Alpha-1 Liver initiative.

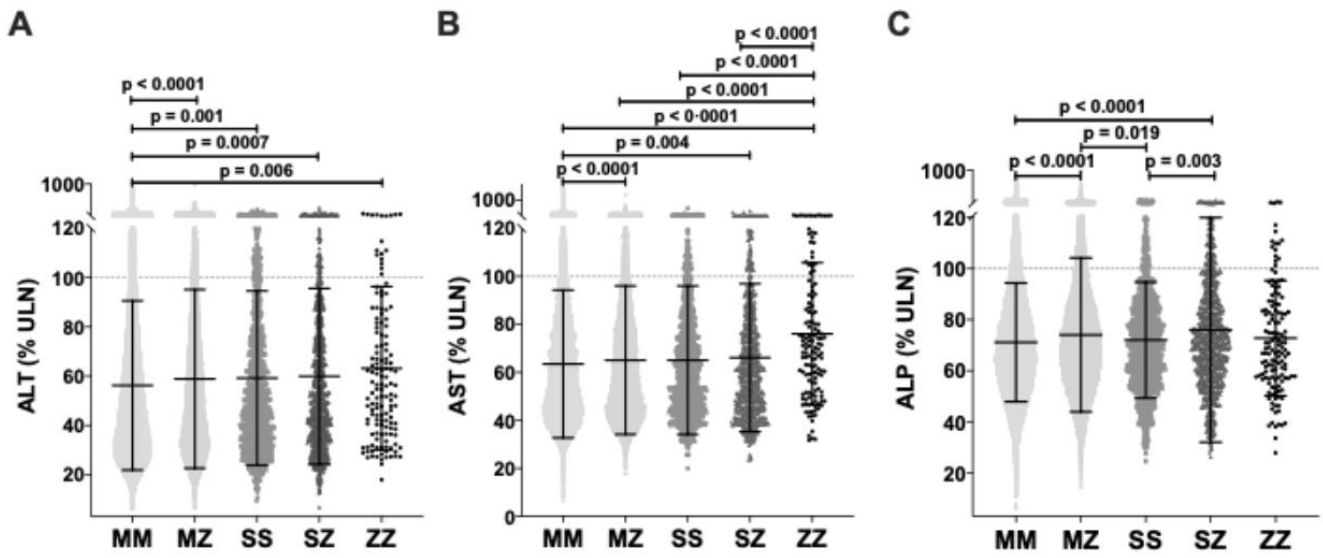

Figure 2: Liver related parameters in individuals heterozygous for the Pi*Z variant (Pi*MZ), homozygous for the Pi*S variant (Pi*SS), heterozygous for both Pi*S and Pi*Z (Pi*SZ), and homozygous for the Pi*Z variant (Pi*ZZ) compared to non-carriers (Cohort 1).

422 506 non-carriers, 17 006 Pi*MZ subjects, 1014 Pi*SS individuals, 864 Pi*SZ subjects, and 138 Pi*ZZ individuals underwent laboratory analysis. P values were adjusted for age, sex, BMI, alcohol consumption, and presence of diabetes mellitus. Scatter plots of serum level of alanine aminotransferase (ALT; A), aspartate aminotransferase (AST; B), and alkaline phosphatase (ALP; C), all normalized to the sex-specific upper limit of normal (ULN).

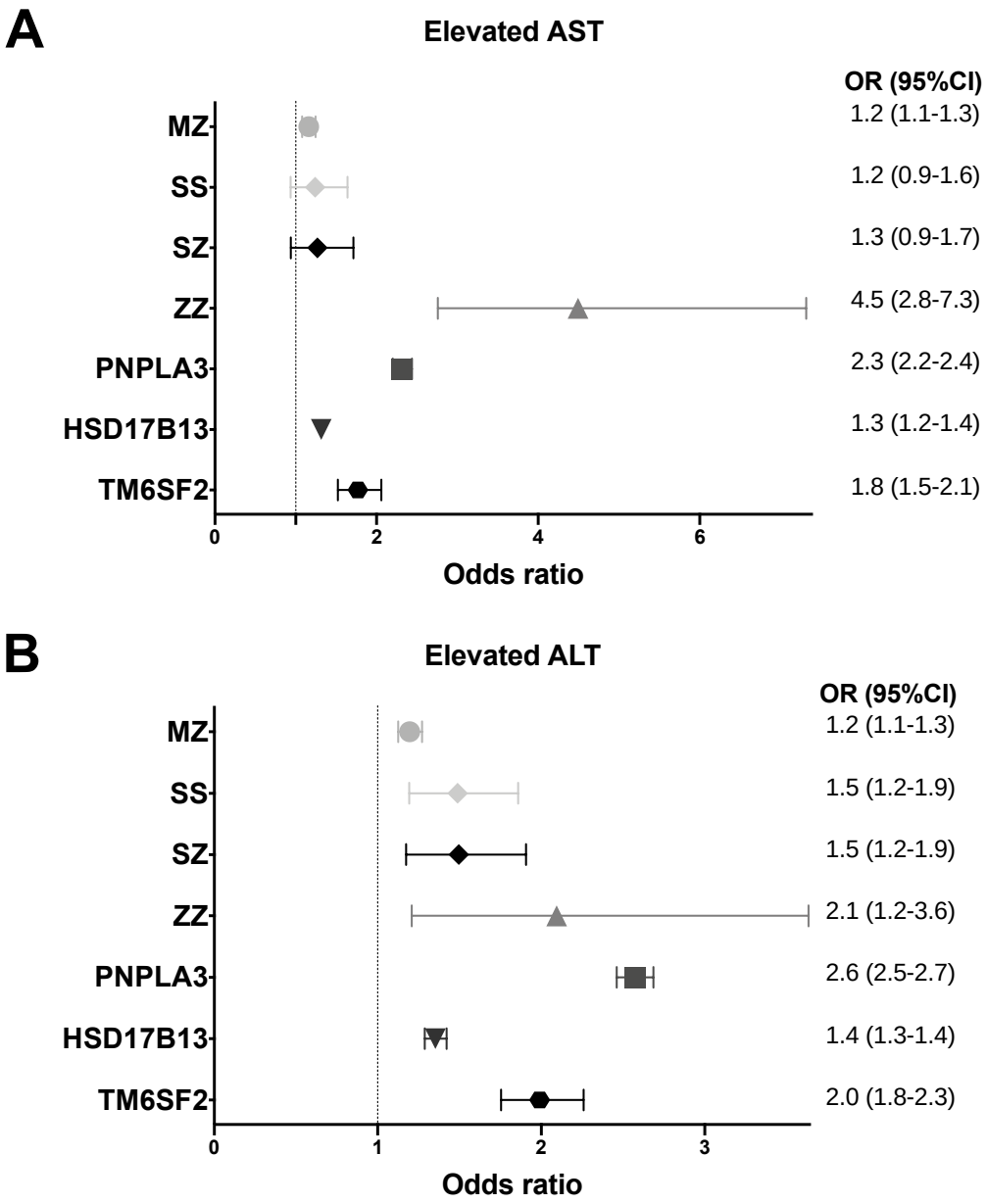

Figure 3: Risk of Pi*SS and Pi*SZ subjects to show elevated AST or ALT compared to heterozygous (Pi*MZ) and homozygous (Pi*ZZ) Pi*Z carriers as well as homozygous carriers of PNPLA3 p.I148M (rs738409), HSD17B13:T (rs72613567), and TM6SF2 p.E167K (rs5854926) (Cohort 1).

Adjusted odds ratios (aOR) with their corresponding 95% confidence intervals (CI) are shown for aspartate aminotransferase (AST; A) and alanine aminotransferase (ALT; B). The risk to display levels higher than the corresponding sex-dependent upper limit of normal (ULN) was compared to the respective non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus.

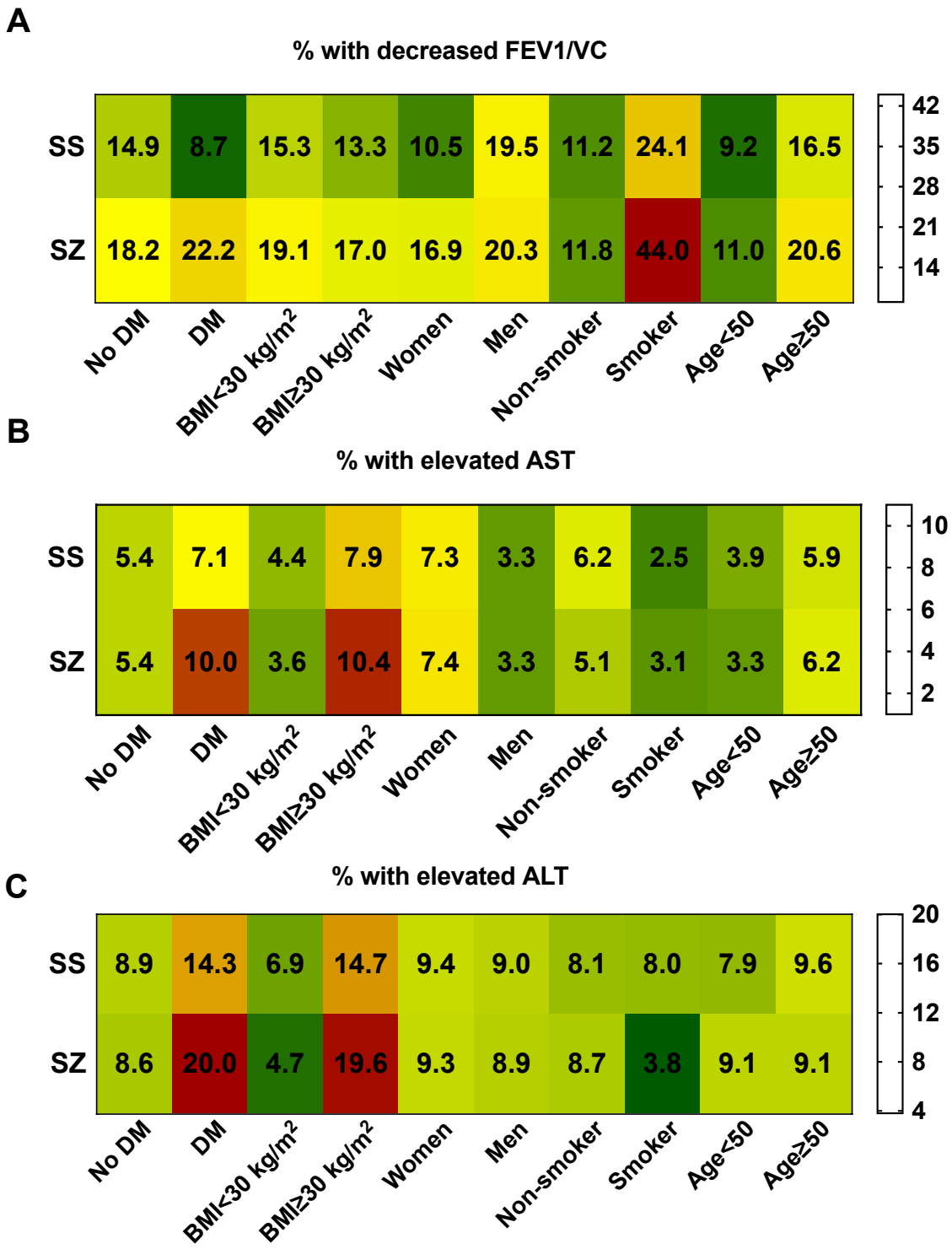

Figure 4: Rate of Pi*SS and Pi*SZ subjects with decreased Tiffenau Index, elevated AST, or elevated ALT in different subpopulations (Cohort 1).

Relative frequencies (%) are shown and visualized by a color coding (right panel). Decreased Tiffenau-index is defined as FEV1/VC <70%. Smokers are defined as "ever-smokers" and non-smokers are defined as "never-smokers". Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index (kg/m²); DM, diabetes mellitus; FEV1, forced expiratory volume in 1 second; VC, vital capacity.

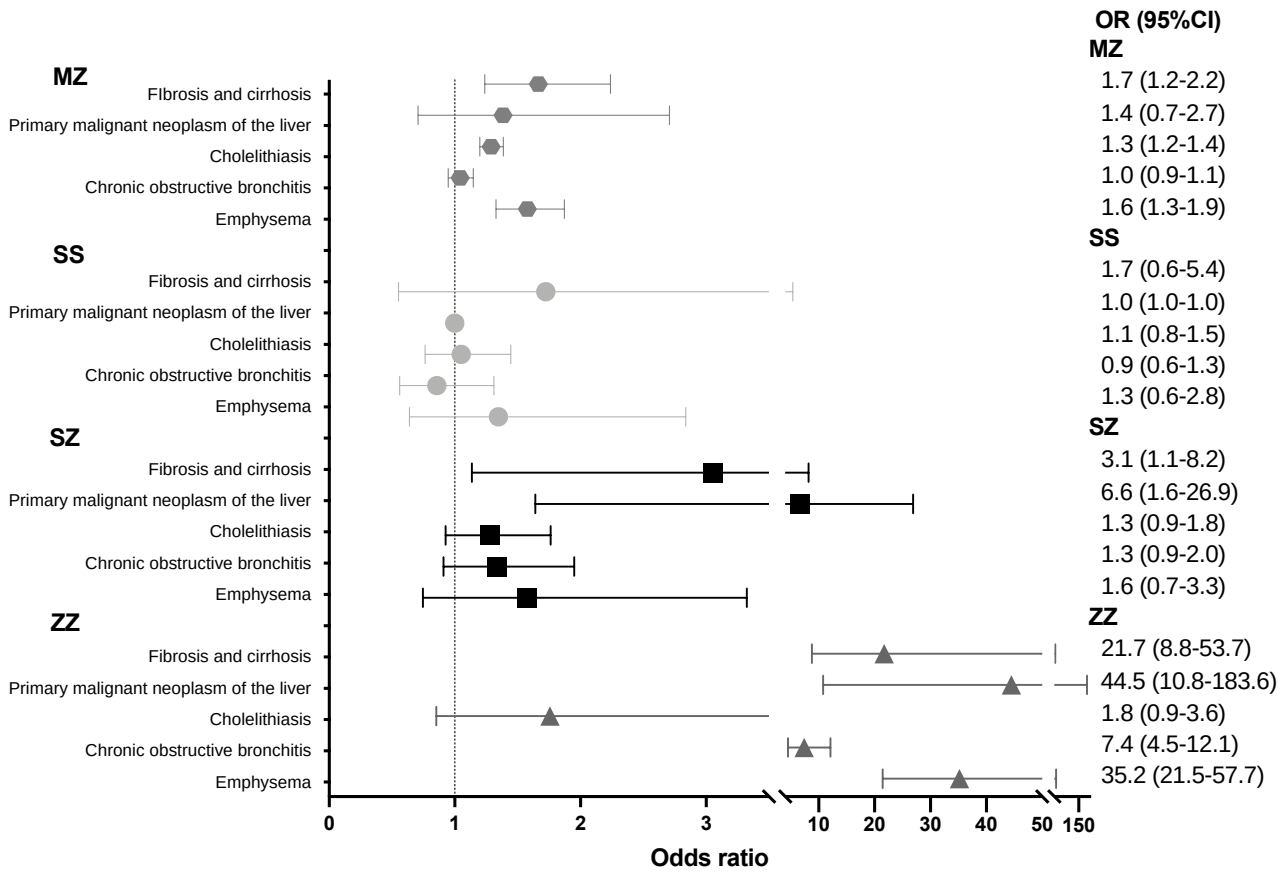

Figure 5: Odds ratios of ICD10 diagnoses in individuals heterozygous or homozygous for the Pi*Z variant (Pi*MZ/Pi*ZZ), homozygous for Pi*S variant (Pi*SS) and heterozygous for Pi*S and Pi*Z (Pi*SZ) (Cohort 1).

Adjusted odds ratios (aOR) with their corresponding 95% confidence intervals (CI) are shown for Pi*MZ, Pi*SS, Pi*SZ, and Pi*ZZ subjects compared to non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus. If in one group no cases are available, the corresponding aOR was set as 1[1;1].

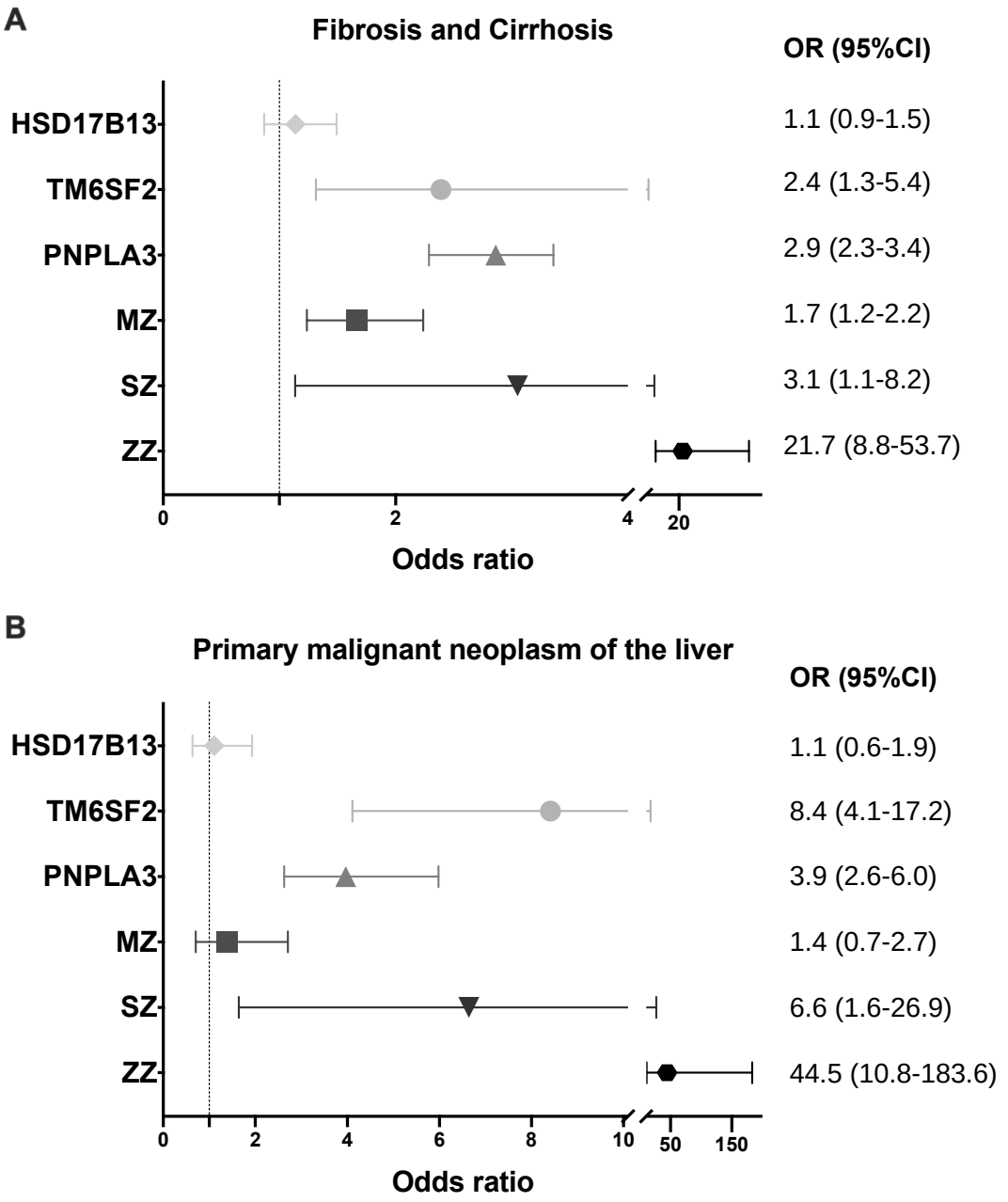

Figure 6: Odds ratios of ICD10 diagnoses in individuals heterozygous (or homozygous for Pi*Z (Pi*MZ/Pi*ZZ), heterozygous for Pi*S and Pi*Z (Pi*SZ) or homozygous for displayed genetic liver fibrosis modifiers.

Genetic liver fibrosis modifiers include *PNPLA3* p.I148M (rs738409), *HSD17B13*:T (rs72613567), and *TM6SF2* p.E167K (rs5854926)). Adjusted odds ratios (aOR) with their 95% confidence intervals (CI) compared to the corresponding non-carriers. Odds ratios were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus.

SUPPLEMENT

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Material and Methods

Ethics

The institutional review board of RWTH Aachen University (EK 173/15) and the institutional ethics committees of the participating centers provided ethical approval. The study was conducted according to the Declaration of Helsinki (Hong Kong Amendment) as well as Good Clinical Practice (European guidelines) and was registered with ClinicalTrials.gov (NCT02929940).

Recruitment of Real Life Alpha-1 Liver Cohort

All individuals included in this study were recruited as part of the global Alpha-1 Liver cohort through various collaborations and campaigns: Firstly, a collaboration with rare liver disease networks, where patients with known or suspected AATD were referred to the study group by other physicians. In the framework of the European Reference Network (ERN) for hepatological diseases (ERN Rare-Liver, www.rare-liver.eu), which was founded in 2016, the University Hospital Aachen became the coordinating center for AATD-related liver disease. A registry grant from the European Association for the Study of the Liver (EASL) enabled us to extend this network beyond the ERN Rare-Liver structure. Secondly, the cooperation with non-hepatologic AATD networks including national and global patient advocacy groups, respiratory specialists, as well as lung-centered AATD registries expanded our ability to recruit AATD individuals. These platforms were used to inform patients about the liver examination days taking place in participating countries. Thirdly, we carried out our own awareness campaign consisting of an AATD liver-related website (www.alpha1-liver.eu), e-mail service, and a telephone hotline. Additionally, we prepared information flyers and handed them out to patients, are present on social media, organize talks at patient meetings, as well as contribute to patient-centered journals in various countries.

Non-carriers were recruited as volunteers on liver examination days or as genetically unrelated companions of subjects with AATD.

Assessment of liver disease

As part of the liver assessment, all participants completed standardized questionnaires in personal interviews and a physical examination including blood sampling and TE was performed. A required fasting period of four hours was communicated in advance. If the fasting time fell below this time, the individuals were excluded (3 Pi*SZ, 3 non-carriers). To obtain serum samples, blood was centrifuged, aliquoted, and stored at -80°C. EDTA blood for genetic examinations was stored at +4°C. Non-invasive determination of liver stiffness was performed as previously

described.[1, 2] The measurement was conducted by experienced investigators using the M or XL probe. A measurement was considered if at least ten valid measurements were available and the interquartile range of the median LSM was ≤30%. Failure to meet these quality criteria led to exclusion (5 Pi*SZ, 2 non-carriers, 2 Pi*ZZ). The chosen cut-offs (7.1 kPa for significant (i.e. fibrosis stage ≥2) and 10 kPa for advanced liver fibrosis (i.e. fibrosis stage ≥3)) were in line with our previous publications on AATD individuals[3, 4] as well as with etiology-unspecific recommendations. [5, 6] For controlled attenuation parameter (CAP) as a surrogate of hepatic steatosis, [6] following, previously published cut-offs were used: [3, 4] 248 dB/m for mild (i.e. steatosis grade ≥1) and 280 dB/m for severe steatosis (i.e. steatosis grade =3). The individual mean, weekly alcohol consumption was evaluated in a face-to-face conversation. Individuals with excessive mean consumption (>40 g/d women, >60 g/d men) were excluded (5 Pi*ZZ, 3 Pi*SZ, 1 non-carrier). The personal interview and physical examination were used to detect signs of preexisting chronic liver disease (e.g. previously elevated liver enzymes, previously known diagnosis of chronic liver disease, liver transplant). The only exception was non-alcoholic fatty liver disease (NAFLD) given its high prevalence in Caucasian population. Because of that, only individuals with a histologically proven non-alcoholic steatohepatitis (NASH) were excluded (4 Pi*SZ). A detailed laboratory work-up was performed to detect additional liver co-morbidities. As part of that, we assessed the presence of chronic hepatitis B and C virus infections (1 Pi*SZ excluded), the presence of autoimmune hepatitis (no exclusions), and hereditary hemochromatosis (2 Pi*SZ subjects with an otherwise unexplained significant increase in both ferritin [>500 ng/mL] and transferrin saturation [>45%]) were excluded. Serum alanine transaminase (ALT) or aspartate transaminase (AST) activities >5x of the sex-specific upper limit of normal (ULN) or alkaline serum phosphatase (ALP) >2x of the sex-specific ULN at the time of recruitment also led to exclusion (2 Pi*ZZ) since they interfere with the determination of liver stiffness by TE.

REFERENCES (Supplement)

1. Clark VC, Marek G, Liu C, et al. Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. *J Hepatol* 2018; 69(6): 1357–64.
2. Kumpers J, Fromme M, Schneider CV, et al. Assessment of liver phenotype in adults with severe alpha-1 antitrypsin deficiency (Pi*ZZ genotype). *J Hepatol* 2019; 71(6): 1272–4.
3. Hamesch K, Mandorfer M, Pereira VM, et al. Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation. *Gastroenterology* 2019; 157(3): 705–19.e18.
4. Schneider CV, Hamesch K, Gross A, et al. Liver Phenotypes of European Adults Heterozygous or Homozygous for Pi*Z Variant of AAT (Pi*MZ vs Pi*ZZ genotype) and Non-carriers. *Gastroenterology* 2020.
5. Friedrich-Rust M, Poynard T, Castera L. Critical comparison of elastography methods to assess chronic liver disease. *Nature reviews Gastroenterology & hepatology* 2016; 13(7): 402–11.
6. Karlas T, Petroff D, Sasso M, et al. Individual patient data meta-analysis of controlled attenuation parameter (CAP) technology for assessing steatosis. *J Hepatol* 2017; 66(5): 1022–30.

Supplementary table 1: Characteristics and liver status of Pi*SZ individuals from contributing countries.

	Ger-many (n= 51)	UK (n= 54)	Portu-gal (n= 51)	Ireland (n= 21)	Spain (n= 18)	Poland (n= 11)	Den-mark (n= 9)	USA (n= 8)	Austria (n= 8)	Italy (n= 4)	Bel-gium (n= 4)
Characteristics											
Age, mean (SD), y	53.7 (16.8)	47.9 (14.5)	48.4 (15.7)	52.9 (16.8)	47.9 (14.9)	43.7 (20.3)	60.7 (11.2)	54.8 (20.1)	56.1 (17.3)	54.8 (8.9)	36.0 (10.2)
Women, No. (%)	31 (60.8)	27 (50.0)	27 (52.9)	15 (71.4)	11 (61.1)	4 (36.4)	4 (44.4)	6 (75.0)	6 (75.0)	2 (50.0)	2 (50.0)
BMI, mean (SD), kg/m^2	26.2 (5.3)	26.7 (6.1)	27.1 (5.8)	28.3 (6.1)	24.2 (3.3)	24.0 (3.3)	28.3 (4.8)	32.1 (5.0)	23.6 (3.1)	28.4 (4.5)	23.3 (2.1)
Liver status											
LSM°, mean (SD), kPa	5.3 (3.8)	5.3 (1.5)	5.1 (1.7)	-	4.5 (1.2)	5.0 (2.5)	4.9 (1.6)	6.0 (1.6)	6.5 (1.2)	5.4 (2.4)	8.3 (2.8)
LSM ≥7.1 kPa°, No. (%)	5 (9.8)	7 (13.2)	4 (10.8)	-	1 (5.6)	1 (12.5)	1 (11.1)	1 (25.0)	1 (33.3)	1 (25.0)	2 (66.7)
LSM ≥10.0 kPa (%)°, No. (%)	3 (5.9)	1 (1.9)	1 (2.7)	-	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.3)
ALT, mean (SD), % of ULN	72.1 (61.0)	71.8 (29.9)	72.0 (53.3)	54.6 (22.3)	66.5 (44.1)	111.3 (81.5)	82.9 (52.8)	73.2 (27.4)	53.8 (18.8)	54.8 (20.1)	95.3 (56.2)
ALT ≥ULN, No. (%)	7 (14.0)	9 (18.0)	9 (17.6)	1 (4.8)	2 (11.1)	4 (36.4)	2 (22.2)	1 (12.5)	0 (0)	0 (0)	1 (25.0)
AST, mean (SD), % of ULN	68.8 (35.8)	65.5 (23.0)	72.1 (54.7)	58.7 (15.2)	64.2 (23.2)	91.7 (60.8)	75.2 (39.4)	71.0 (26.0)	90.4 (77.2)	59.0 (16.1)	61.2 (28.5)
AST ≥ULN, No. (%)	4 (8.0)	4 (11.1)	8 (15.7)	1 (4.8)	2 (11.1)	2 (18.2)	1 (11.1)	2 (25.0)	1 (12.5)	0 (0)	0 (0)
GGT, mean (SD), % of ULN	109.2 (238.8)	87.3 (89.9)	101.3 (133.9)	72.7 (60.1)	53.2 (34.3)	73.2 (69.7)	115.4 (132.2)	-	73.2 (82.1)	53.8 (31.9)	157.9 (207.1)
GGT ≥ULN, No. (%)	8 (16.0)	8 (22.2)	8 (16.0)	3 (14.3)	2 (11.1)	3 (27.3)	2 (22.2)	-	1 (12.5)	1 (25.0)	1 (25.0)

Quantitative measures are expressed as mean with standard deviation or as relative frequency (%).

° LSM only available in 190 Pi*SZ individuals. Abbreviations: BMI, body mass index; LSM, liver stiffness measurement; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ULN, upper limit of normal (sex-specific).

SI conversion factors: To convert ALT, AST, GGT, and ALP to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Supplementary table 2: Comparison of lung, biliary, and liver phenotype in *PNPLA3* p.I148M (rs738409) homozygotes, heterozygotes, and non-carriers (Cohort 1).

	PNPLA3 I148M Non-carrier (n=296 718)	**Heterozygous PNPLA3 I148M carriers (n=162 975)**	**Homozygous PNPLA3 I148M carriers (n=22 986)**	**p-value (multivariate) non-carrier vs. heterozygous**	**p-value (multivariate) non-carrier vs. homozygous**
Characteristics					
Age, mean (SD), y	56.5 (8.1)	56.5 (8.1)	56.6 (8.1)		
Women, No. (%)	161 002 (54)	88 588 (54)	12 428 (54)		
BMI, mean (SD), kg/m^2	27.5 (4.8)	27.4 (4.8)	27.3 (4.7)		
Alcohol, mean (SD), g/d	8.8 (10.1)	8.7 (10.1)	8.7 (10.1)		
Risk factors					
BMI>30 kg/m^2, No. (%)	91 987 (31)	49 527 (30)	6 883 (30)		
Diabetes mellitus, No. (%)	15 383 (5)	8 535 (5)	1 325 (6)		
Liver status					
ALT, mean (SD), % of ULN	54.9 (30.6)	57.8 (34.6)	64.6 (41.7)	**<0.0001**	**<0.0001**
ALT ≥ULN, No. (%)	16 102 (5.4)	12 172 (7.5)	2824 (12.3)	**<0.0001**	**<0.0001**
AST, mean (SD), % of ULN	62.8 (24.0)	64.6 (27.3)	68.7 (31.5)	**<0.0001**	**<0.0001**
AST ≥ULN, No. (%)	11 306 (3.8)	8 159 (5.0)	1 894 (8.2)	**<0.0001**	**<0.0001**
GGT, mean (SD), % of ULN	75.4 (80.7)	75.1 (81.6)	75.9 (84.7)	0.87	0.092
GGT ≥ULN, No. (%)	48 396 (16.3)	26 234 (16.1)	3 810 (16.6)	0.60	0.062
ALP, mean (SD), % of ULN	72.9 (24.8)	72.8 (24.7)	72.0 (24.7)	0.22	**<0.0001**
ALP ≥ULN, No. (%)	33 311 (11.2)	17 930 (11.0)	2 397 (10.4)	0.052	**<0.0001**
Bilirubin, mean (SD), mg/dl	0.53 (0.26)	0.53 (0.26)	0.54 (0.27)	**<0.0001**	**<0.0001**
Bilirubin ≥ULN, No. (%)	8 173 (2.8)	4 589 (2.8)	712 (3.1)	0.41	**0.005**
ICD10 codes					
Cholelithiasis, No. (%)	11 867 (4.0)	6 308 (3.9)	788 (3.4)	0.12	**<0.0001**
Fibrosis and Cirrhosis, No. (%)	436 (0.15)	353 (0.22)	97 (0.42)	**<0.0001**	**<0.0001**
Primary liver cancer, No. (%)	98 (0.03)	69 (0.04)	30 (0.13)	0.10	**<0.0001**
Chronic Bronchitis, No. (%)	7 830 (2.6)	4 410 (2.5)	593 (2.6)	**0.041**	0.97
Emphysema, No. (%)	1 632 (0.6)	841 (0.5)	119 (0.5)	0.15	0.41

Quantitative measures are expressed as mean with standard deviation or relative frequency (%). All analyses were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus. Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; ULN, upper limit of normal (sex-specific).

SI conversion factors: To convert ALT, AST, GGT, and ALP to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Supplementary table 3: Comparison of lung, biliary, and liver phenotype in *TM6SF2* p.E167K (rs5854926) homozygotes, heterozygotes, and non-carriers (Cohort 1).

	TM6SF2 p.E167K Non-carriers (n=412 855)	**Heterozygous TM6SF2 p.E167K carriers (n=66 393)**	**Homozygous TM6SF2 p.E167K carriers (n=2 636)**	**p-value (multivariate) non-carrier vs. heterozygous**	**p-value (multivariate) non-carrier vs. homozygous**
Characteristics					
Age, mean (SD), y	56.5 (8.1)	56.7 (8.1)	56.8 (8.1)		
Women, No. (%)	224 243 (54)	35 925 (54)	1 442 (55)		
BMI, mean (SD), kg/m^2	27.4 (4.8)	27.4 (4.7)	27.2 (4.6)		
Alcohol, mean (SD), g/d	8.8 (10.1)	8.8 (10.1)	8.6 (9.9)		
Risk factors					
BMI>30 kg/m^2, No. (%)	127 210 (31)	20 163 (30)	776 (29)		
Diabetes mellitus, No. (%)	21 312 (5)	3 683 (6)	194 (79		
Liver status					
ALT, mean (SD), % of ULN	55.9 (32.0)	58.9 (32.0)	64.0 (45.1)	**<0.0001**	**<0.0001**
ALT ≥ULN, No. (%)	25 372(6.1)	5 398 (8.1)	293 (11.71)	**<0.0001**	**<0.0001**
AST, mean (SD), % of ULN	63.5 (25.7)	64.7 (25.4)	68.4 (34.3)	**<0.0001**	**<0.0001**
AST ≥ULN, No. (%)	17 655 (4.5)	3 474 (5.2)	192 (7.3)	**<0.0001**	**<0.0001**
GGT, mean (SD), % of ULN	75.1 (80.8)	76.1 (83.3)	80.6 (95.2)	**0.013**	**<0.0001**
GGT ≥ULN, No. (%)	66 891 (16.2)	10 961 (16.5)	464 (17.6)	**0.043**	**0.009**
ALP, mean (SD), % of ULN	73.0 (24.9)	71.4 (24.1)	68.7 (23.8)	**<0.0001**	**<0.0001**
ALP ≥ULN, No. (%)	46 714 (11.3)	6 615 (10.0)	217 (8.2)	**<0.0001**	**<0.0001**
Bilirubin, mean (SD), mg/dl	0.53 (0.26)	0.54 (0.26)	0.56 (0.27)	**<0.0001**	**<0.0001**
Bilirubin ≥ULN, No. (%)	11 463 (2.8)	1 903 (2.9)	90 (3.4)	0.27	0.096
ICD10 codes					
Cholelithiasis, No. (%)	16 187 (3.9)	2 644 (4.0)	105 (4.0)	0.36	0.89
Fibrosis and Cirrhosis, No. (%)	695 (0.17)	169 (0.25)	12 (0.46)	**<0.0001**	**<0.0001**
Primary liver cancer, No. (%)	139 (0.03)	49 (0.07)	8 (0.30)	**<0.0001**	**<0.0001**
Chronic Bronchitis, No. (%)	10 779 (2.6)	1 703 (2.6)	65 (2.5)	0.33	0.90
Emphysema, No. (%)	2 224 (0.5)	348 (0.5)	15 (0.6)	0.51	0.92

Quantitative measures are expressed as mean with standard deviation or relative frequency (%). All analyses were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus. Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; ULN, upper limit of normal (sex-specific). SI conversion factors: To convert ALT, AST, GGT, and ALP to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Supplementary table 4: Comparison of lung, biliary, and liver phenotype in *HSD17B13*:T (rs72613567) homozygotes, heterozygotes, and rs72613567:T non-carriers (Cohort 1).

	HSD17B13 TA/TA (n=35 375)	**HSD17B13 T/TA (n=188 270)**	**HSD17B13 T/T (n=257 543)**	**p-value (multivariate) TA/TA vs. T/TA**	**p-value (multivariate) TA/TA vs. T/T**
Characteristics					
Age, mean (SD), y	56.8 (8.0)	56.7 (8.1)	56.5 (8.1)		
Women, No. (%)	19 054 (54)	102 544 (54)	139 615 (54)		
BMI, mean (SD), kg/m^2	27.4 (4.7)	27.4 (4.8)	27.4 (4.8)		
Alcohol, mean (SD), g/d	9.0 (10.1)	8.9 (10.1)	8.7 (10.0)		
Risk factors					
BMI>30 kg/m^2, No. (%)	10 854 (319	57 407 (31)	79 686 (31)		
Diabetes mellitus, No. (%)	1 798 (5)	9 503 (5)	13 863 (5)		
Liver status					
ALT, mean (SD), % of ULN	54.8 (29.8)	55.4 (31.1)	57.3 (34.1)	**<0.0001**	**<0.0001**
ALT ≥ULN, No. (%)	1 899 (5.4)	10 916 (5.8)	18 187 (7.1)	**<0.0001**	**<0.0001**
AST, mean (SD), % of ULN	62.8 (23.8)	63.1 (24.9)	64.2 (26.7)	**0.045**	**<0.0001**
AST ≥ULN, No. (%)	1 345 (3.8)	7 429 (3.9)	12 530 (4.9)	0.12	**<0.0001**
GGT, mean (SD), % of ULN	73.2 (74.3)	74.7 (79.9)	76.0 (83.1)	**<0.0001**	**<0.0001**
GGT ≥ULN, No. (%)	5 462 (15.4)	30 015 (15.9)	42 738 (16.6)	**0.003**	**<0.0001**
ALP, mean (SD), % of ULN	72.6 (25.0)	73.0 (24.7)	72.7 (24.8)	**0.014**	0.092
ALP ≥ULN, No. (%)	3 872 (10.9)	21 197 (11.3)	28 412 (11.0)	0.11	0.62
Bilirubin, mean (SD), mg/dl	0.53 (0.26)	0.53 (0.26)	0.53 (0.27)	0.20	**0.001**
Bilirubin ≥ULN, No. (%)	1 021 (2.9)	5 055 (2.7)	7 353 (2.9)	**0.048**	**0.017**
ICD10 codes					
Cholelithiasis, No. (%)	1 459 (4.1)	7 508 (4.0)	9 949 (3.9)	0.25	**0.017**
Fibrosis and Cirrhosis, No. (%)	60 (0.17)	316 (0.17)	506 (0.20)	0.98	0.35
Primary liver cancer, No. (%)	14 (0.04)	71 (0.04)	111 (0.04)	0.83	0.72
Chronic Bronchitis, No. (%)	929 (2.6)	5 029 (2.7)	6 563 (2.5)	0.45	0.66
Emphysema, No. (%)	189 (0.5)	1 054 (0.6)	1 336 (0.5)	0.59	0.86

Quantitative measures are expressed as mean with standard deviation or relative frequency (%). All analyses were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus. Abbreviations: ALT, alanine aminotransferase; ALP, alkaline phosphatase; AST, aspartate aminotransferase; BMI, body mass index; GGT, gamma-glutamyl transferase; ULN, upper limit of normal (sex-specific).

SI conversion factors: To convert ALT, AST, GGT, and ALP to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Supplementary table 5: Correlation of serum ALT, AST, GGT, ALP, and bilirubin in the initial and first follow-up examination (Cohort 1).

	Overall	**Non-carriers (n=15 391)**	**MZ (n=548)**	**SS (n=31)**	**SZ (n=40)**
ALT	.604**	.605**	.607**	.408*	.472**
AST	.602**	.598**	.640**	.806**	.461**
GGT	.822**	.820**	.838**	.709**	.727**
ALP	.768**	.767**	.778**	.788**	.790**
Bilirubin	.651**	.649**	.659**	.533**	.623**

Spearman correlation coefficients between the highlighted parameters are shown. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase. P-values are considered significant at $p < 0.05$ (*) and $p < 0.01$ (**).

Supplementary table 6: Comparison of lung, biliary, and liver phenotype by primary ICD10 codes in participants with Pi*SS and Pi*SZ genotype compared to Pi*ZZ, Pi*MZ, and non-carriers (Cohort 1).

	Non-carriers (n=422 506)	MZ (n=17 006)	SS (n=1014)	SZ (n=864)	ZZ (n=138)
ICD10 codes					
Cholelithiasis, No. (%)	16 314 (3.9)[1]	835 (4.9)[1]	41 (4.0)	40 (4.6)	8 (5.8)
Fibrosis and Cirrhosis, No. (%)	748 (0.2)[2,3,4]	50 (0.3)[2,5]	3 (0.3)[6]	4 (0.5)[3,7]	4 (2.9)[4,5,6,7]
Primary liver cancer, No. (%)	168 (0.05)[8,9]	9 (0.1)[10,11]	0 (0)	3 (0.3)[8,10]	2 (1.4)[9,11]
Chronic Bronchitis, No. (%)	10 916 (2.6)[12]	461 (2.7)[13]	24 (2.4)[14]	28 (3.2)[15]	20 (14.5)[12,13,14,15]
Emphysema, No. (%)	2 191 (0.5)[16,17]	144 (0.8)[16,18]	8 (0.8)[19]	7 (0.8)[20]	20 (14.5)[17,18,19,20]

Relative frequencies (%) of the respective diagnoses are shown. All analyses were adjusted for age, sex, BMI, alcohol consumption, and diabetes mellitus.

[1]$p=7.1*10^{-12}$; [2]$p=0.001$; [3]$p=0.027$; [4]$p=3.4*10^{-9}$; [5]$p=0.000003$; [6]$p=0.002$; [7]$p=0.011$; [8]$p=0.008$; [9]$p=1.5*10^{-7}$; [10]$p=0.039$; [11]$p=0.00001$; [12]$p=1.5*10^{-15}$; [13]$p=5.9*10^{-14}$; [14]$p=1.5*10^{-9}$; [15]$p=1.6*10^{-7}$; [16]$p=1.9*10^{-7}$; [17]$p=3.8*10^{-45}$; [18]$p=8.6*10^{-31}$; [19]$p=3.4*10^{-12}$; [20]$p=1.2*10^{-11}$.

Supplementary table 7: Liver-related blood parameters in Pi*SZ subjects compared to Pi*ZZ individuals and non-carriers (Cohort 2).

	Non-carriers (n= 279)	**Pi*SZ (n= 239)**	**Pi*ZZ (n= 586)**	***P value Pi*SZ vs. non-carriers (uni-variable)***	***P value Pi*SZ vs. non-carriers (multi-variable)***	***P value Pi*SZ vs. Pi*ZZ (uni-variable)***	***P value Pi*SZ vs. Pi*ZZ (multi-variable)***
Liver-related blood parameters							
ALT, mean (SD), % of ULN	65.9 (29.8)	71.8 (48.4)	78.8 (47.6)	0.106	0.248	0.062	**0.011**
ALT ≥ULN, No. (%)	32 (11.5)	36 (15.4)	107 (18.9)	0.198	0.174	0.237	0.124
AST, mean (SD), % of ULN	62.5 (22.5)	69.6 (40.4)	74.1 (31.1)	**0.019**	0.117	0.106	**0.015**
AST ≥ULN, No. (%)	14 (5.1)	25 (11.4)	66 (12.7)	**0.009**	**0.015**	0.603	0.701
GGT, mean (SD), % of ULN	57.7 (45.7)	90.1 (131.3)	96.7 (122.4)	**0.001**	**0.001**	0.529	0.702
GGT ≥ULN, No. (%)	36 (13.0)	37 (17.5)	131 (24.1)	0.164	0.069	0.051	**0.045**
ALP, mean (SD), % of ULN	58.5 (18.5)	72.9 (35.4)	66.1 (23.6)	**<0.0001**	**<0.0001**	**0.011**	**0.014**
ALP ≥ULN, No. (%)	6 (2.3)	31 (14.5)	31 (7.0)	**<0.0001**	**<0.0001**	**0.002**	**0.008**
GLDH, mean (SD), % of ULN)	53.7 (69.8)	59.6 (97.7)	77.0 (252.4)	0.619	0.768	0.637	0.626
GLDH ≥ULN, No. (%)	16 (6.3)	5 (10.4)	51 (13.2)	0.311	0.384	0.582	0.650
Bilirubin, mean (SD), mg/dl	0.52 (0.33)	0.59 (0.33)	0.56 (0.31)	**0.023**	0.058	0.188	0.427
Bilirubin ≥ULN, No. (%)	17 (6.1)	14 (6.5)	15 (3.6)	0.868	0.970	0.100	0.566

Quantitative measures are expressed as mean with standard deviation or relative frequency (%), and all multivariable analysis were adjusted for age, sex, BMI, presence of diabetes mellitus, and mean alcohol consumption.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; GLDH, glutamate dehydrogenase; ULN, upper limit of normal (sex-specific).

SI conversion factors: To convert ALT, AST, GGT, ALP, and GLDH to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Supplementary table 8: Characteristics and liver status of non-obese subgroup of Pi*SZ subjects, Pi*ZZ individuals, and non-carriers (Cohort 2).

	Non-carriers, BMI<30 (n= 244)	**Pi*SZ, BMI<30 (n= 188)**	**Pi*ZZ, BMI<30 (n= 525)**	***P value Pi*SZ vs. non-carriers (uni-variable)***	***P value Pi*SZ vs. Pi*ZZ (uni-variable)***
Characteristics					
Age, mean (SD), y	52.4 (15.0)	49.5 (16.7)	54.2 (13.5)	0.061	**0.001**
Women, No. (%)	121 (49.6)	106 (56.4)	241 (45.9)	0.161	**0.014**
BMI, mean (SD), kg/m^2	24.4 (3.1)	(24.3 (3.0)	23.9 (3.0)	0.714	0.187
Alcohol, mean (SD), g/d	7.9 (10.0)	(7.2 (11.8)	5.8 (9.8)	0.494	0.234
Risk factors					
BMI ≥30 kg/m², No. (%)	0 (0)	0 (0)	0 (0)		
Diabetes mellitus, No. (%)	10 (4.4)	6 (3.8)	17 (4.0)	0.785	0.938
Relevant alcohol intake$^+$, No. (%)	30 (12.3)	24 (17.9)	45 (8.6)	0.136	**0.002**
Liver status					
Liver stiffness, mean (SD), kPa	4.5 (1.7)	4.9 (1.6)	6.3 (5.0)	**0.009**	**<0.0001**
ALT, mean (SD), % of ULN	64.2 (27.3)	72.0 (51.7)	76.3 (46.7)	0.063	0.296
ALT ≥ULN, No. (%)	24 (9.9)	28 (15.3)	86 (17.0)	0.090	0.597
AST, mean (SD), % of ULN	62.4 (22.6)	70.3 (43.2)	73.0 (29.9)	**0.028**	0.460
AST ≥ULN, No. (%)	12 (5.0)	20 (11.6)	57 (12.2)	**0.013**	0.817
GGT, mean (SD), % of ULN	57.7 (47.7)	93.1 (143.2)	90.6 (100.8)	**0.004**	0.839
GGT ≥ULN, No. (%)	33 (13.6)	27 (16.0)	110 (22.5)	0.509	0.072
ALP, mean (SD), % of ULN	58.4 (18.8)	71.5 (31.4)	65.4 (23.2)	**<0.0001**	**0.027**
ALP ≥ULN, No. (%)	6 (2.6)	22 (13.3)	24 (6.2)	**<0.0001**	**0.005**
GLDH, mean (SD), % of ULN)	53.9 (73.6)	58.0 (102.7)	76.4 (266.3)	0.764	0.666
GLDH ≥ULN, No. (%)	14 (6.3)	3 (7.5)	44 (12.9)	0.783	0.326
Bilirubin, mean (SD), mg/dl	0.52 (0.31)	0.61 (0.35)	0.56 (0.31)	**0.011**	0.090
Bilirubin ≥ULN, No. (%)	15 (6.2)	13 (7.7)	13 (3.4)	0.547	**0.028**

Quantitative measures are expressed as mean with standard deviation or relative frequency (%).

Abbreviations: BMI, body mass index; AAT, alpha-1 antitrypsin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; GLDH, glutamate dehydrogenase; ULN, upper limit of normal (sex-specific).

SI conversion factors: To convert ALT, AST, GGT, ALP, and GLDH to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Supplementary table 9: Characteristics and liver status of Pi*SZ individuals with and without liver stiffness measurement indicating significant liver fibrosis (Cohort 2).

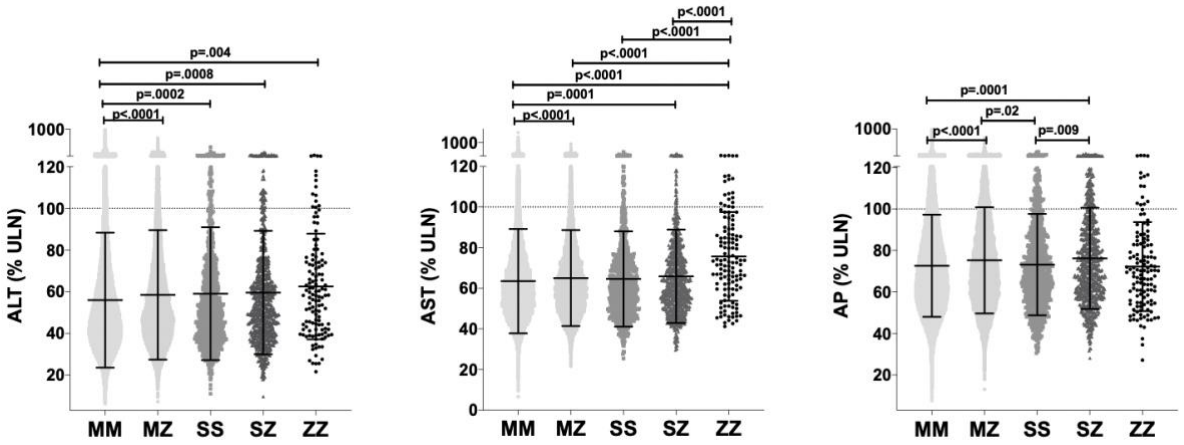
	Pi*SZ, LSM <7.1 kPa *n= 166*	**Pi*SZ, LSM ≥7.1 kPa** *n= 24*	***P value (univariable)***
Characteristics			
Age, median [IQR], y	52.0 [37.2–63.0]	53.5 [44.8–67.8]	0.335
Women, No. (%)	87 (52.4)	13 (54.2)	0.872
BMI, median [IQR], kg/m^2	25.1 [22.5–28.1]	27.8 [24.3–33.7]	**0.017**
Alcohol, median [IQR], g/d	0.0 [0.0–11.0]	0.0 [0.0–20.9]	0.970
AAT serum level[#], median [IQR], mg/dL	59.0 [51.9–68.9]	60.0 [56.0–81.5]	0.230
Risk factors			
BMI ≥30 kg/m², No. (%)	27 (17.0)	8 (33.3)	0.058
Diabetes mellitus, No. (%)	3 (2.2)	3 (14.3)	**0.007**
Relevant alcohol intake[+], No. (%)	23 (18.0)	4 (26.7)	0.415
Liver status			
Liver stiffness, median [IQR], kPa	4.4 [3.9–5.3]	8.5 [7.3–10.1]	**<0.0001**
ALT, median [IQR], % of ULN	54.3 [44.0–78.0]	89.7 [62.8–146.7]	**<0.0001**
ALT ≥ULN, No. (%)	23 (14.3)	9 (37.5)	**0.005**
AST, median [IQR], % of ULN	60.0 [50.0–74.3]	74.3 [54.3–131.4]	**0.015**
AST ≥ULN, No. (%)	13 (8.7)	8 (34.8)	**<0.0001**
GGT, median [IQR], % of ULN	45.0 [35.0–73.8]	105.0 [77.5–219.2]	**<0.0001**
GGT ≥ULN, No. (%)	20 (13.8)	11 (50.0)	**<0.0001**
ALP, median [IQR], % of ULN	60.0 [51.5–74.9]	73.3 [55.2–101.4]	**0.022**
ALP ≥ULN, No. (%)	14 (9.7)	5 (23.8)	0.057
GLDH, median [IQR], % of ULN)	32.0 [22.9–48.0]	75.7 [29.0–367.0]	0.113
GLDH ≥ULN, No. (%)	4 (9.3)	1 (20.0)	0.459
Bilirubin, median [IQR], mg/dl	0.52 [0.40–0.66]	0.58 [0.41–0.75]	0.382
Bilirubin ≥ULN, No. (%)	10 (6.4)	1 (5.0)	0.811

Quantitative measures are expressed as mean with standard deviation (normal distribution), median [interquartile range (IQR)] (non-normal distribution), or relative frequency (%).

Abbreviations: BMI, body mass index; AAT, alpha-1 antitrypsin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase; ALP, alkaline phosphatase; GLDH, glutamate dehydrogenase; ULN, upper limit of normal (sex-specific).

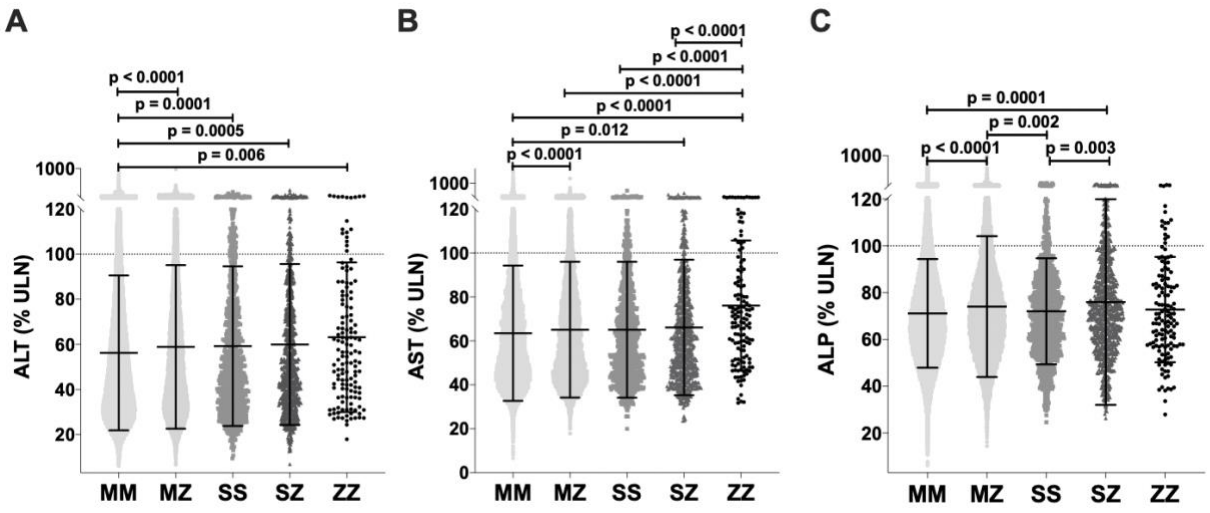
SI conversion factors: To convert ALT, AST, GGT, ALP, and GLDH to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

Supplementary figure 1: Liver enzymes in participants with the highlighted alpha1-antitrypsin genotypes after exclusion of individuals with ICD-10 code NAFLD (Cohort 1).



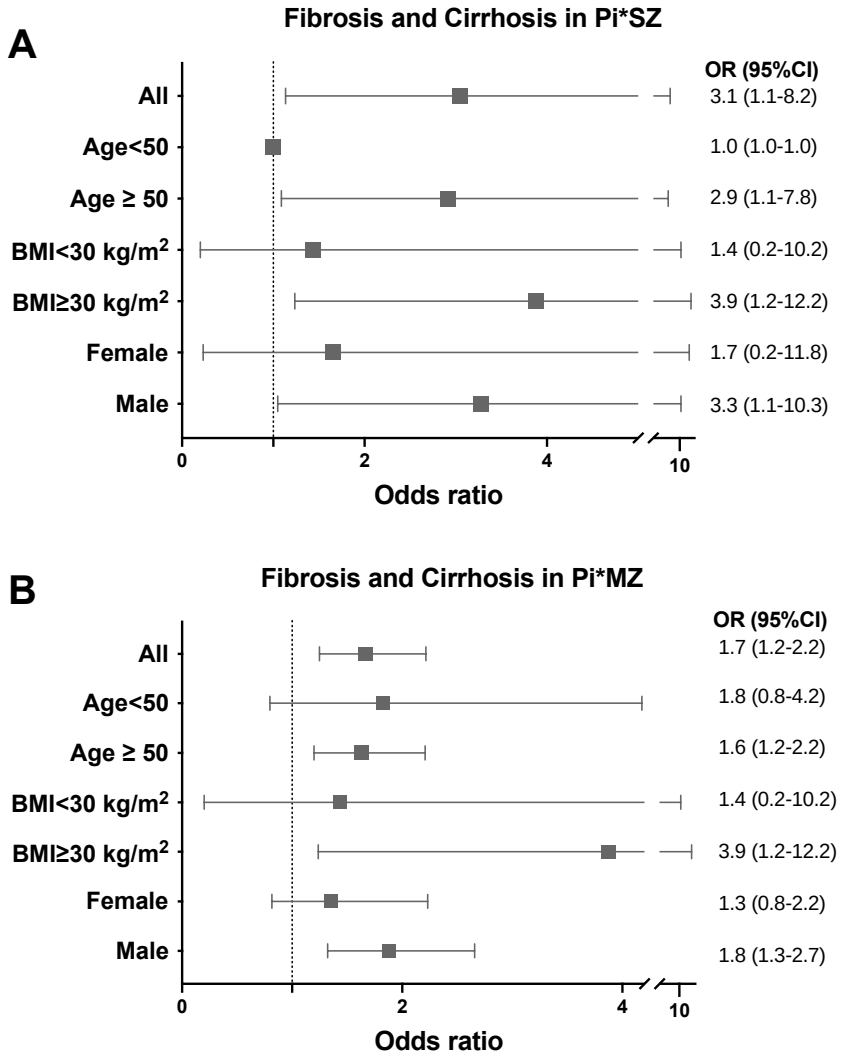
420 196 non-carriers, 16 886 Pi*MZ subjects, 1007 Pi*SS individuals, 856 Pi*SZ subjects, and 137 Pi*ZZ individuals underwent a laboratory analysis. P values were adjusted for age, sex, BMI, mean alcohol consumption, and presence of diabetes mellitus. Scatter plots of serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are shown, all normalized to the sex-specific upper limit of normal (ULN).

Supplementary figure 2: Liver enzymes in participants with the highlighted alpha1-antitrypsin genotypes after exclusion of individuals with metabolic syndrome (Cohort 1).



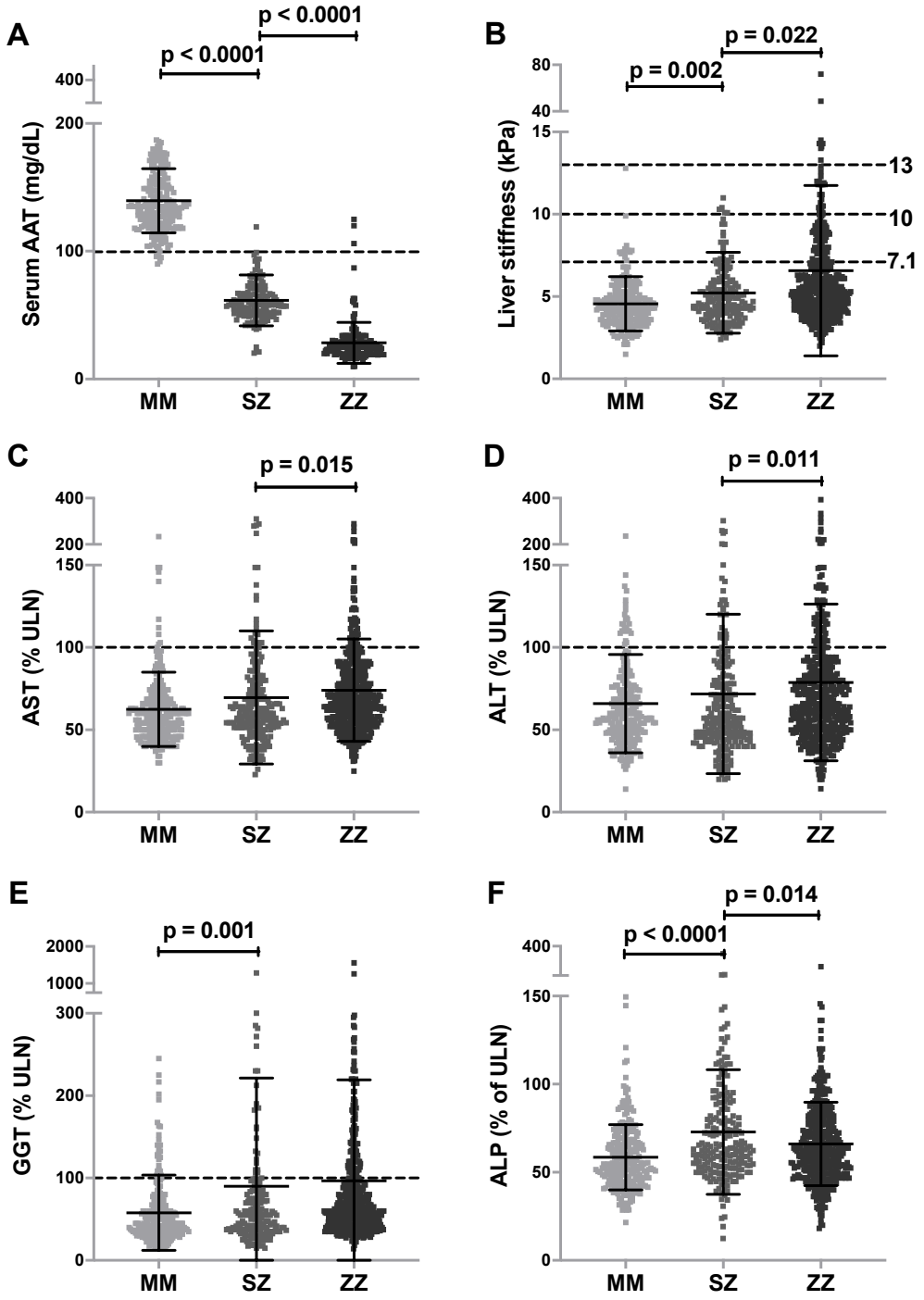
379 522 non-carriers, 15 462 Pi*MZ subjects, 919 Pi*SS individuals, 782 Pi*SZ subjects, and 132 Pi*ZZ individuals underwent laboratory analysis. P values were adjusted for age, sex, BMI, mean alcohol consumption, and presence of diabetes mellitus. Scatter plots of serum level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) are shown, all normalized to the sex-specific upper limit of normal (ULN). The presence of metabolic syndrome was based on the IDF (International diabetes federation) definition, which consists of central obesity (defined as waist circumference with ethnicity specific values) plus any two of the following four factors: (i) raised triglycerides ≥ 150 mg/dL (1.7 mmol/L) or specific treatment for this lipid abnormality; (ii) reduced HDL cholesterol < 40 mg/dL (1.03 mmol/L) in males or < 50 mg/dL (1.29 mmol/L) in females or specific treatment for this lipid abnormality; (iii) raised blood pressure systolic BP ≥ 130 or diastolic BP ≥ 85 mm Hg or treatment of previously diagnosed hypertension; (iv) raised fasting plasma glucose (FPG) ≥ 100 mg/dL (5.6 mmol/L), or previously diagnosed type 2 diabetes.

Supplementary figure 3: Factors associated with fibrosis and cirrhosis in individuals heterozygous for both Pi*S and Pi*Z (Pi*SZ) or heterozygous for Pi*Z (Pi*MZ) compared to non-carriers (Cohort 1).



Unadjusted odds ratios (OR) with their corresponding 95% confidence intervals (CI) are shown for fibrosis and cirrhosis in different subgroups of Pi*SZ (A) and Pi*MZ (B) individuals. If in one group no cases were available, the corresponding odds ratio is displayed as 1[1;1]. Abbreviations: BMI, body mass index.

Supplementary figure 4: Liver-related parameters and alpha-1 antitrypsin (AAT) concentrations in individuals heterozygous for both Pi*S and Pi*Z (Pi*SZ), and homozygous for the Pi*Z variant (Pi*ZZ) compared to non-carriers (Cohort 2).

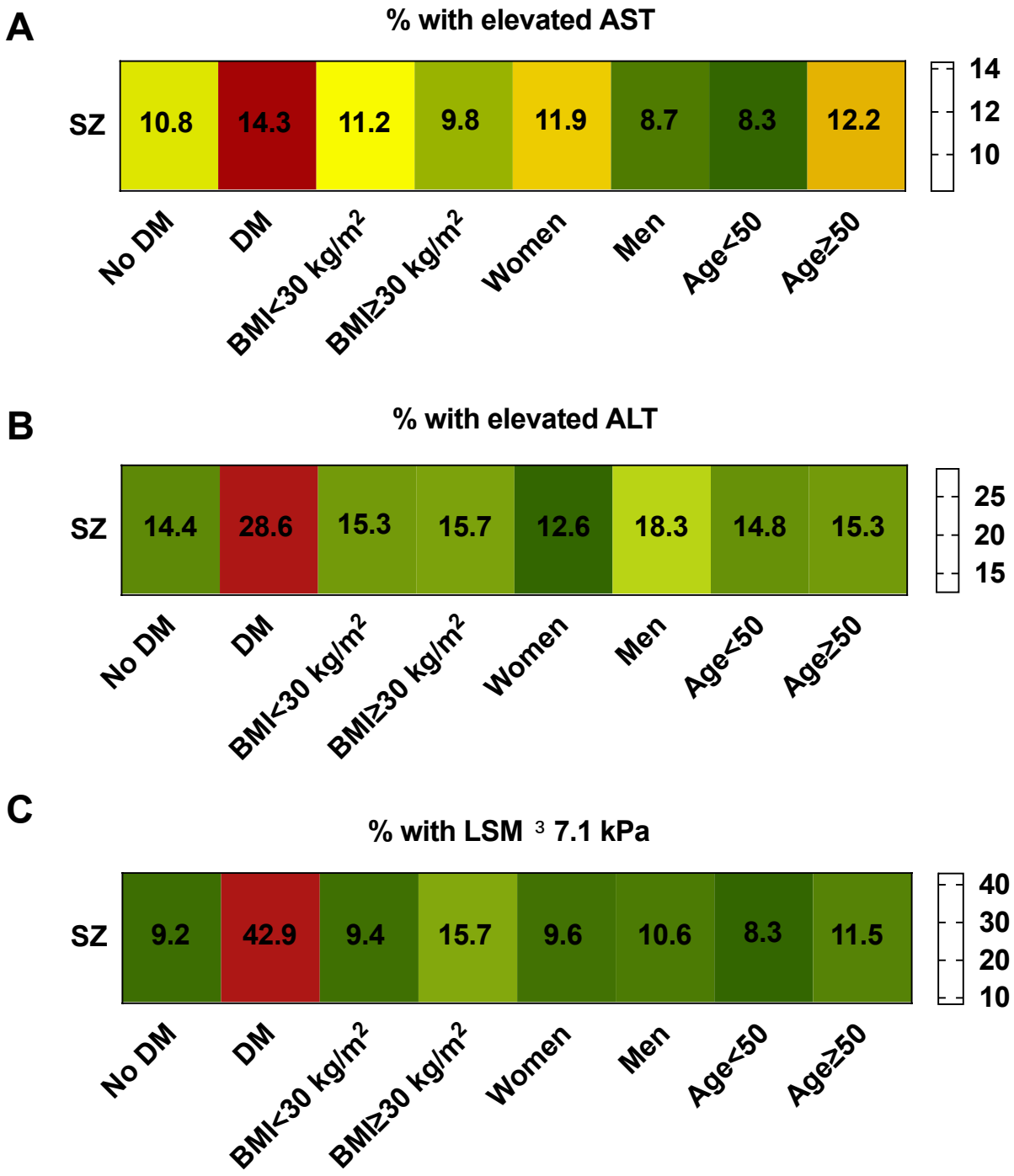


279 non-carriers, 239 Pi*SZ subjects, and 586 Pi*ZZ individuals underwent laboratory analysis and non-invasive transient elastography measurement. AAT serum levels of individuals, who did not receive AAT augmentation therapy, are shown. P values were adjusted for age, sex, BMI, diabetes mellitus, and mean alcohol consumption.

A) Scatter plot of the alpha-1 antitrypsin serum concentration. B) Scatter plot of liver stiffness assessed via transient-elastography (dotted lines representing cut-off levels of fibrosis stage: 7.1 kPa showing fibrosis stage ≥2, 10.0 kPa suggestive of fibrosis stage ≥3, and 13.0 kPa suggestive of fibrosis stage 4 (=cirrhosis)).

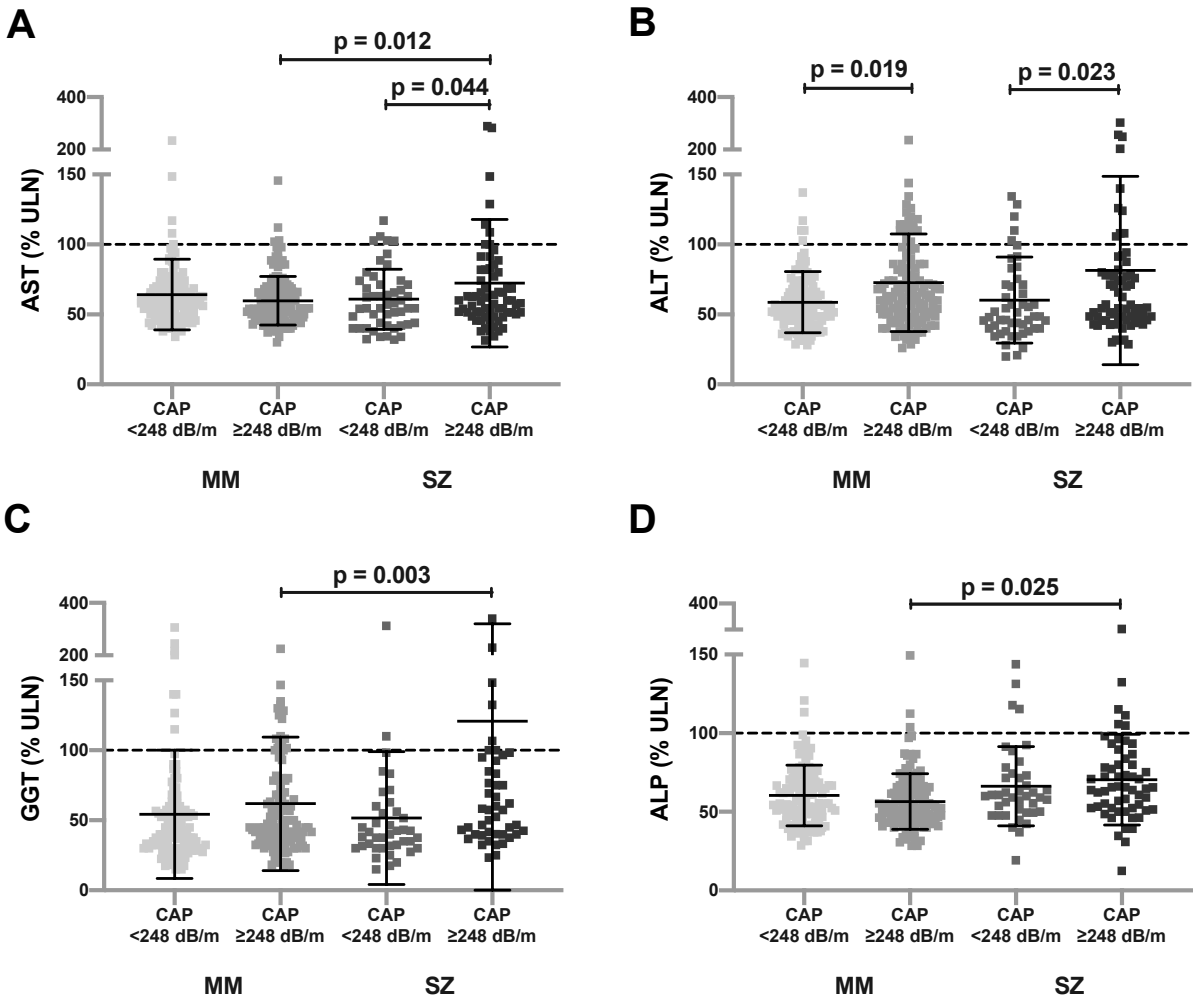
C-F) Scatter plots of serum level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), and alkaline phosphatase (ALP), all normalized to the sex-specific upper limit of normal (ULN) (marked as dotted line).

Supplementary figure 5: Rate of Pi*SZ individuals with elevated AST, ALT, and liver stiffness measurement indicating significant fibrosis in different subpopulations (Cohort 2).



Relative frequencies (%) are shown and visualized by a color coding (right panel). Abbreviations: BMI, body mass index (kg/m²); DM, diabetes mellitus; LSM, liver stiffness measurement.

Supplementary figure 6: Liver-related parameters in individuals heterozygous for both Pi*S and Pi*Z (Pi*SZ) and non-carriers divided into subgroups with and without elevated CAP (Cohort 2).



Individuals were divided into subgroups with controlled attenuation parameter (CAP) <248 dB/m and ≥248 dB/m. CAP ≥248 dB/m was used as a surrogate marker for the presence of steatosis grade ≥1. 136 non-carriers and 67 Pi*SZ individuals with CAP≥248 dB/m and 143 non-carriers and 172 Pi*SZ participants with CAP <248 dB/m underwent a laboratory analysis. P values were adjusted for age, sex, BMI, diabetes mellitus, and mean alcohol consumption.
Scatter plot of serum levels of aspartate aminotransferase (AST, A), alanine aminotransferase (ALT, B), gamma glutamyltransferase (GGT, C), and alkaline phosphatase (ALP, D) are shown, all normalized to the sex-specific upper limit of normal (ULN) (marked as dotted line).