SUPPLEMENT

Table of contents

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 plasma 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)**
Liver status					
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 $p < 0.05$ (*), $p < 0.01$ (**) and $p < 0.001$ (***).

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).

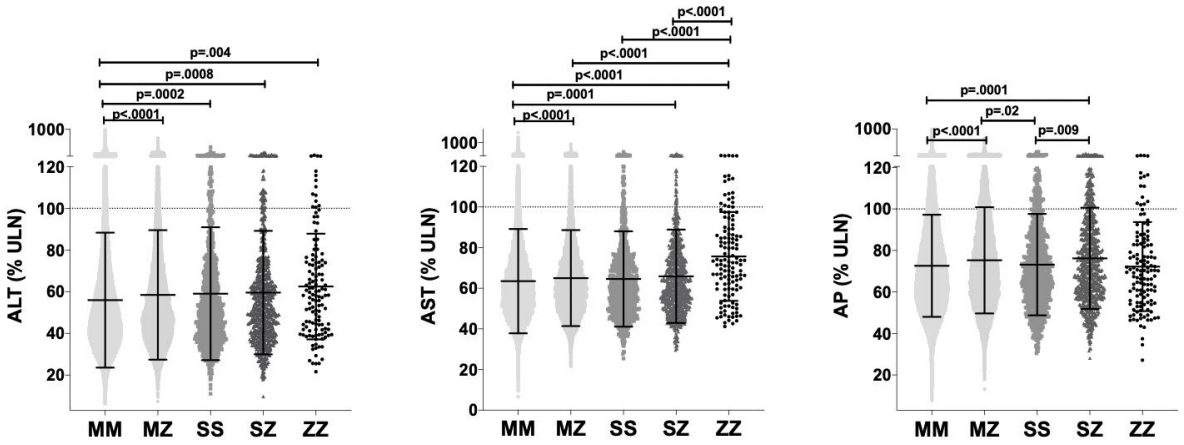
	Pi*SZ, LSM <7.1 kPa *n= 166*	**Pi*SZ, LSM ≥7.1 kPa** *n= 24*	***P value (univariable)***
Characteristics			
Age, median [IQR], y	52.0 [37.2–63.0]	53.5 [44.8–67.8]	0.335
Women, No. (%)	87 (52.4)	13 (54.2)	0.872
BMI, median [IQR], kg/m^2	25.1 [22.5–28.1]	27.8 [24.3–33.7]	**0.017**
Alcohol, median [IQR], 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).

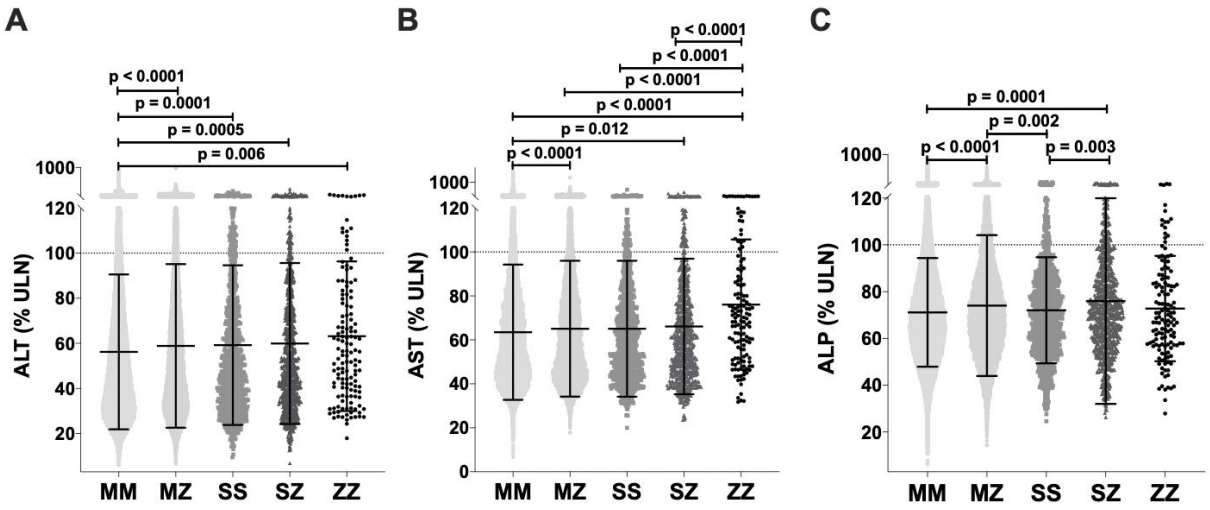
SI conversion factors: To convert ALT, AST, GGT, ALP, and GLDH to µkat/L, multiply values by 0.0167; to convert Bilirubin to µmol/L, multiply values by 17.104.

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



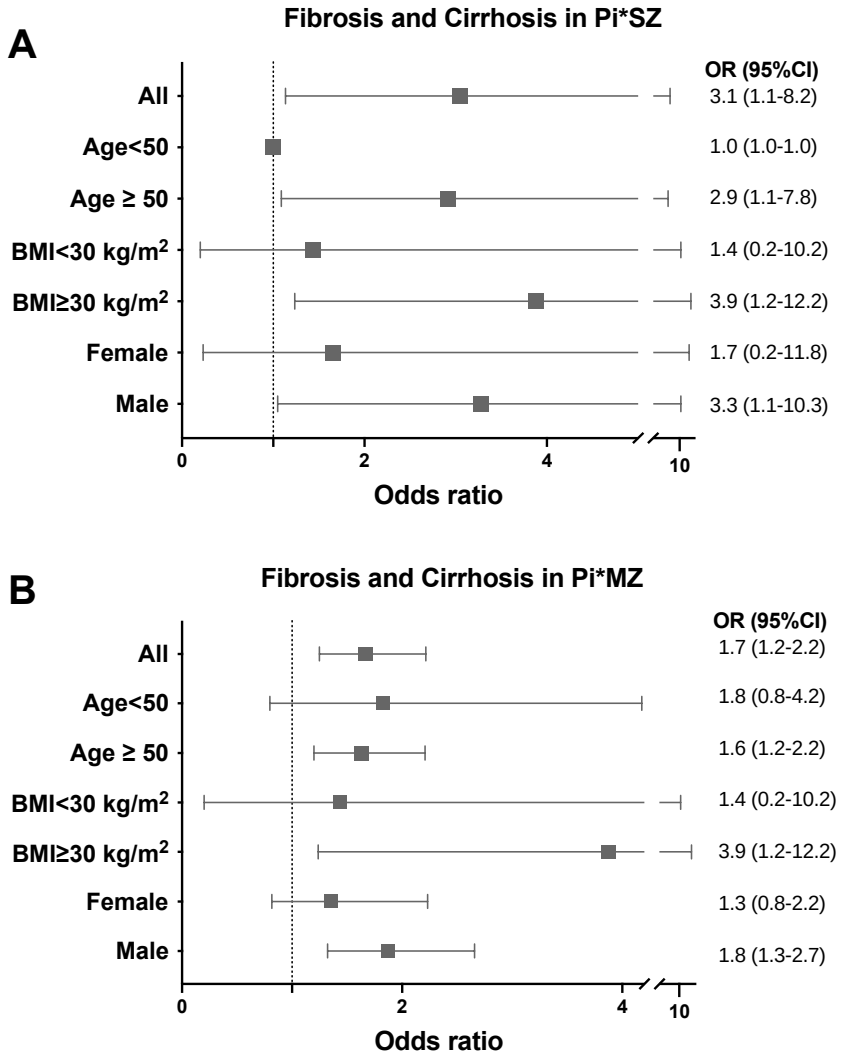
420 196 non-carriers, 16 886 Pi*MZ subjects, 1007 Pi*SS individuals,856 Pi*SZ subjects, and 137 Pi*ZZ individuals underwent a laboratory analysis. P values were adjusted for age, sex, BMI, mean alcohol consumption, and presence of diabetes mellitus. Scatter plots of serum level of alanine aminotransferase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

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



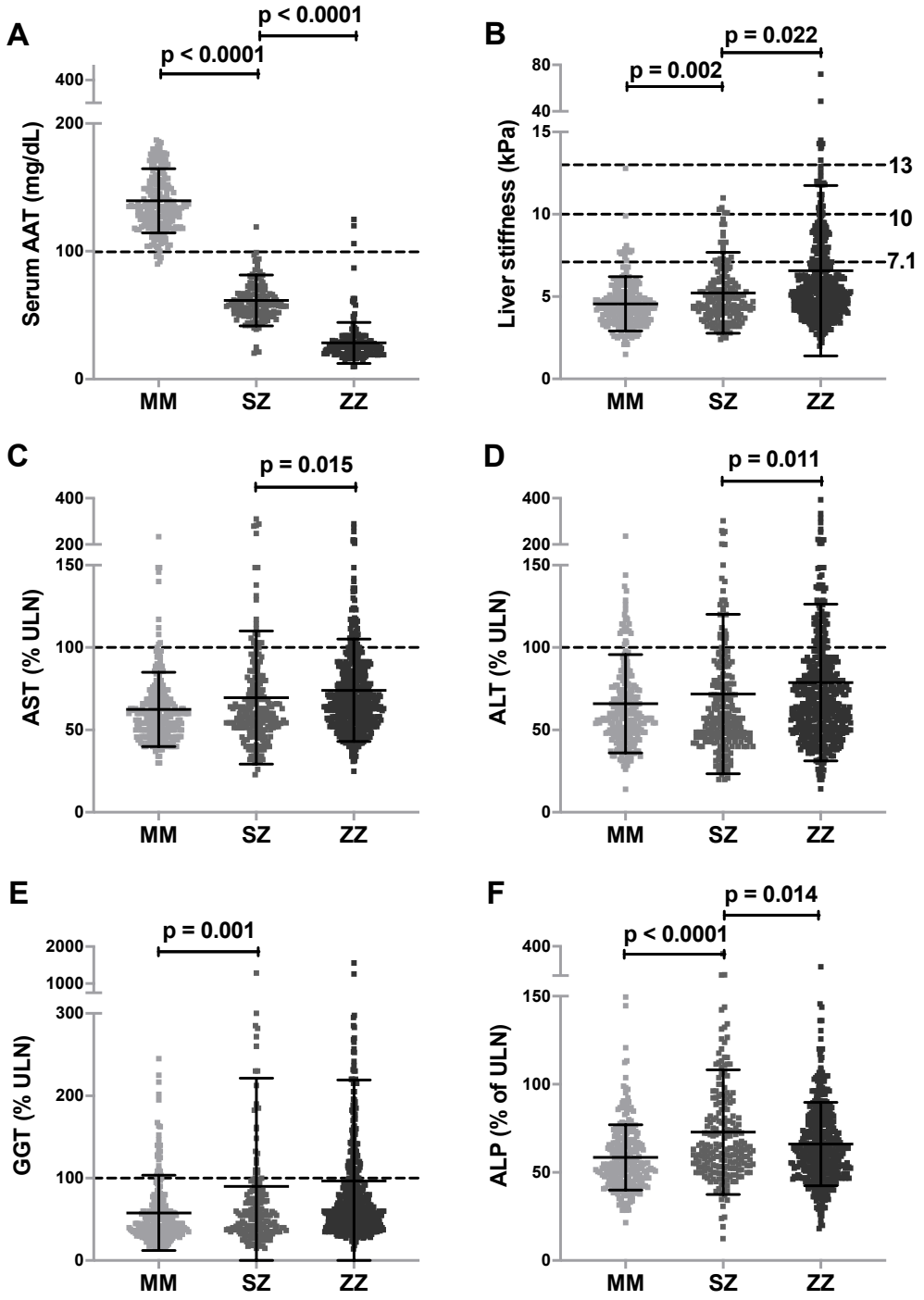
379 522 non-carriers, 15 462 Pi*MZ subjects, 919 Pi*SS individuals, 782 Pi*SZ subjects, and 132 Pi*ZZ individuals underwent laboratory analysis. P values were adjusted for age, sex, BMI, mean alcohol consumption, and presence of diabetes mellitus. Scatter plots of serum level of alanine aminotransferase (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).

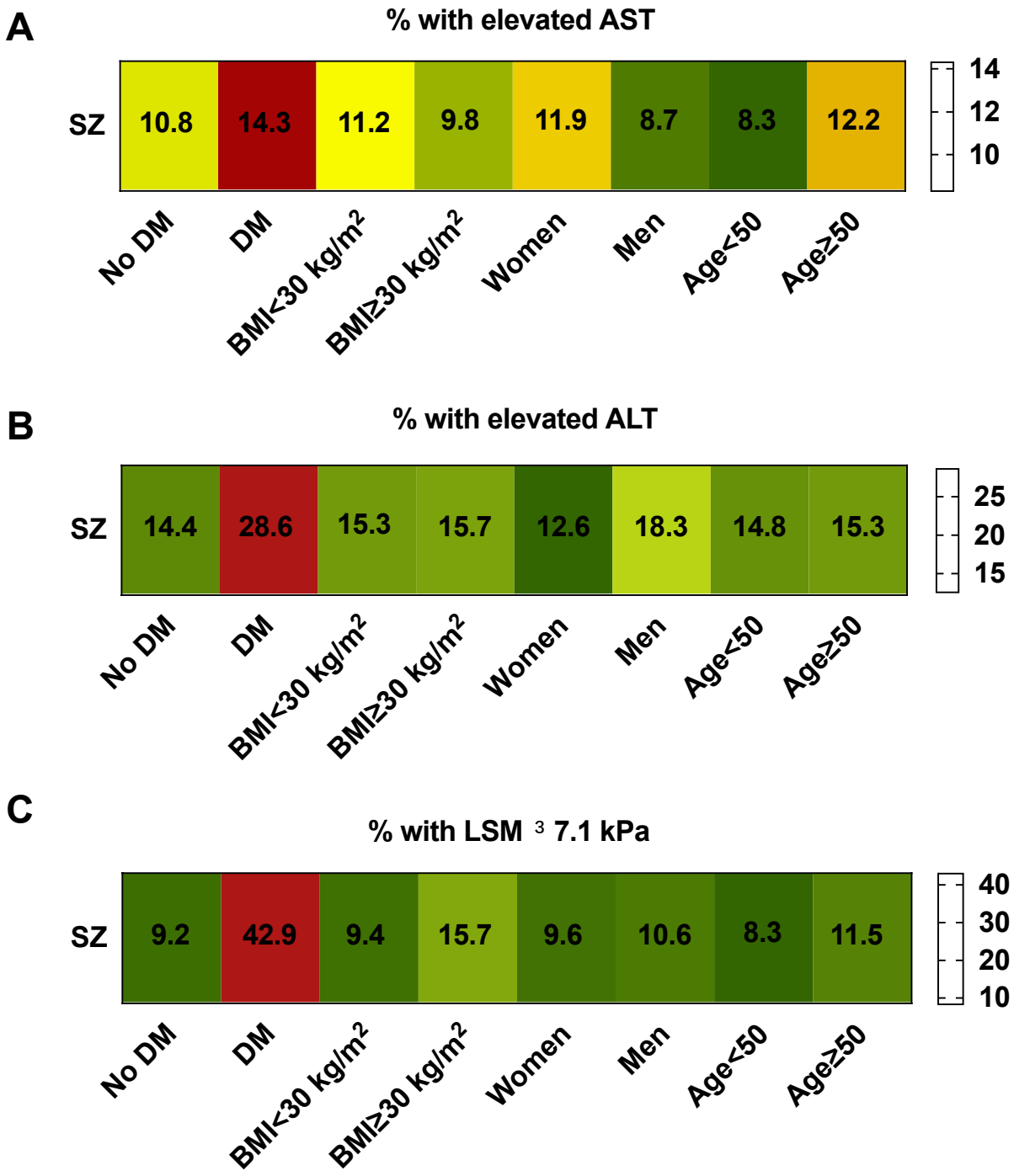


279 non-carriers, 239 Pi*SZ subjects, and 586 Pi*ZZ individuals underwent laboratory analysis and non-invasive transient elastography measurement. AAT serum levels of individuals, who did not receive AAT augmentation therapy, are shown. P values were adjusted for age, sex, BMI, diabetes mellitus, and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

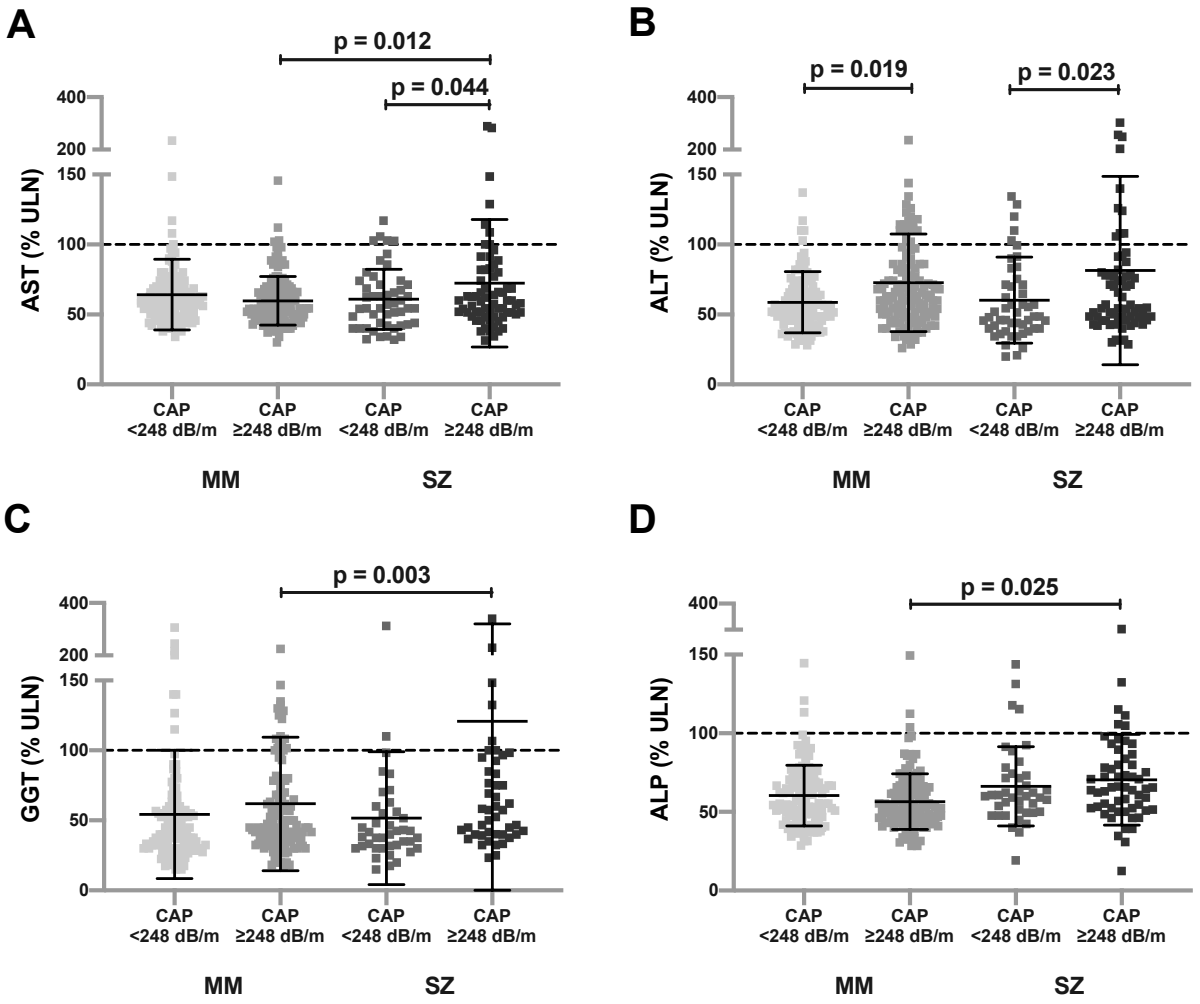
C-F) Scatter plots of serum level of aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma glutamyltransferase (GGT), and alkaline phosphatase (ALP), all normalized to the sex-specific upper limit of normal (ULN) (marked as dotted line).

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).