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CLINICAL LIVER

Liver Phenotypes of European Adults Heterozygous or Homozygous for Pi*Z Variant of *AAT* (Pi*MZ vs Pi*ZZ genotype) and Noncarriers

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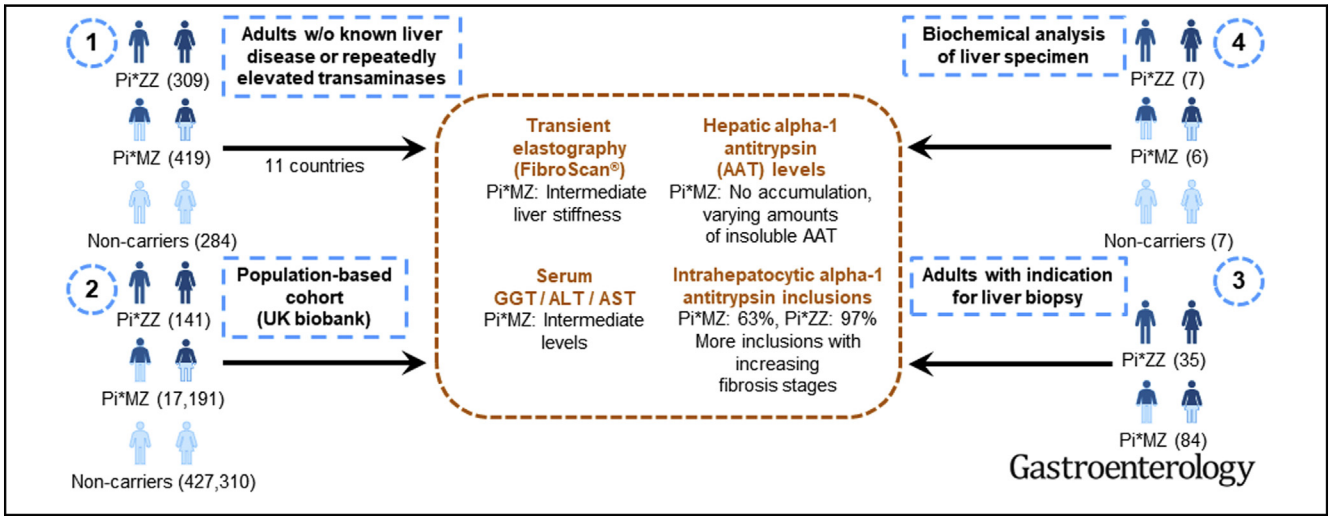

See editorial on page 433.

BACKGROUND & AIMS: Homozygosity for the Pi*Z variant of the gene that encodes the alpha-1 antitrypsin peptide (*AAT*), called the Pi*ZZ genotype, causes a liver and lung disease called alpha-1 antitrypsin deficiency. Heterozygosity (the Pi*MZ genotype) is a risk factor for cirrhosis in individuals with liver disease. Up to 4% of Europeans have the Pi*MZ genotype; we compared features of adults with and without Pi*MZ genotype among persons without preexisting liver disease. **METHODS:** We analyzed data from the European Alpha-1 Liver Cohort, from 419 adults with the Pi*MZ genotype, 309 adults with the Pi*ZZ genotype, and 284 individuals without the variant (noncarriers). All underwent a comprehensive evaluation; liver stiffness measurements (LSMs) were made by transient elastography. Liver biopsies were analyzed to define histologic and biochemical features associated with the Pi*Z variant. Levels of serum transaminases were retrieved from 444,642 participants, available in the United Kingdom biobank. **RESULTS:** In the UK biobank database, levels of serum transaminases were increased in subjects with the Pi*MZ genotype compared with noncarriers. In the Alpha-1 Liver Cohort, adults with Pi*MZ had lower levels of gamma-glutamyl transferase in serum and lower LSMs than adults with the Pi*ZZ variant, but these were higher than in noncarriers. Ten percent of subjects with the Pi*MZ genotype vs 4% of noncarriers had LSMs of 7.1 kPa or more (adjusted odds ratio, 4.8; 95% confidence interval, 2.0–11.8). Obesity and diabetes were the most important factors associated with LSMs $\geq$7.1 kPa in subjects with the Pi*MZ genotype. AAT inclusions were detected in liver biopsies of 63% of subjects with the Pi*MZ genotype, vs 97% of subjects with the Pi*ZZ genotype, and increased with liver fibrosis stages. Subjects with the Pi*MZ genotype did not have increased hepatic levels of AAT, whereas levels of insoluble AAT varied among individuals. **CONCLUSIONS:** Adults with the Pi*MZ genotype have lower levels of serum transaminases, fewer AAT inclusions in liver, and lower liver stiffness than adults with the Pi*ZZ genotype, but higher than adults without the Pi*Z variant. These findings should help determine risk of subjects with the Pi*MZ genotype and aid in counseling.

Keywords: FibroScan; SERPINA1; GGT; ALT.

Alpha-1 antitrypsin (AAT) is produced predominantly in hepatocytes, secreted into the bloodstream, and then transported to the organs where it fulfills its physiological functions as an anti-protease.[1] However, approximately 10% of Europeans carry a genetic variant in *SERPINA1* (the AAT gene), as more than 100 different mutations are known.[2] Mutations in *SERPINA1* typically interfere with its production/secretion.[1,3] Among the *SERPINA1* variants, a glutamate-to-lysine substitution at position 342, termed "Pi*Z," is the most clinically relevant one.[1,3] Compared with "Pi*Z," a glutamate-to-valine substitution at position 264, termed "Pi*S," is clinically less severe.[2]

Pi*Z in its homozygous form, known as "Pi*ZZ" genotype or severe AAT deficiency (AATD), confers a strong susceptibility to early-onset lung emphysema as well as liver disease, that together constitute the major causes of Pi*ZZ-related mortality.[1,3,4] Notably, Pi*ZZ subjects can be easily

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Abbreviations used in this paper: **AAT, alpha-1 antitrypsin; AATD, alpha-1 antitrypsin deficiency; ALD/NAFLD, alcoholic/nonalcoholic fatty liver disease; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CAP, controlled attenuation parameter; GGT, gamma-glutamyl transferase; IHC, immunohistochemistry; LSM, liver stiffness measurements; OR, odds ratio; PAS-D, periodic acid–Schiff–diastase; 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*ZZ, AAT genotype with homozygosity for the Pi*Z variant; *SERPINA1*, AAT gene; TE, transient elastography (FibroScan); ULN, upper limit of normal.**

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WHAT YOU NEED TO KNOW

BACKGROUND

Homozygosity for the Pi*Z variant in the gene that encodes alpha-1 antitrypsin (AAT) increases risk for liver fibrosis. We investigated the effects of heterozygosity (the Pi*MZ genotype) in different European cohorts.

FINDINGS

Adults with the Pi*MZ genotype have lower levels of serum transaminases, fewer AAT inclusions in liver, and lower liver stiffness than adults with the Pi*ZZ genotype, but higher than adults without the Pi*Z variant.

IMPACT

This information should improve counseling and management of Pi*MZ individuals.

LIMITATIONS

Many adults with the Pi*MZ genotype do not develop symptoms or have a known family history of AAT deficiency and, as a consequence, remain undetected — our findings might not apply to this population.

identified in the clinical routine due to their substantially reduced serum AAT levels.[1,3]

Two large cross-sectional studies demonstrated significant liver fibrosis (defined as fibrosis stage $\geq$2) in 20% to 35% of Pi*ZZ individuals[4,5] and in line with that, Pi*ZZ individuals carry an approximately 20 times increased risk to require liver transplantation.[6] The pathological hallmark of Pi*ZZ-related liver disease is the retention of misfolded, insoluble AAT in hepatocytes that leads to formation of roundish AAT inclusions.[2,7] These aggregates are rather indistinct in the routine hematoxylin and eosin labeling but appear brilliant red in periodic acid–Schiff–diastase (PAS-D) staining.[2,5] In addition, they can be visualized with immunohistochemistry against the Pi*Z variant.

Although the Pi*ZZ genotype is rare (prevalence ~1:3000 in Europeans), heterozygous Pi*Z carriage, termed "Pi*MZ" genotype, occurs in approximately 1:30 to 1:40 Europeans.[1] In contrast to Pi*ZZ, that is sufficient to cause end-stage liver disease on its own, the Pi*MZ genotype is primarily considered a disease-modifying factor in individuals with other liver diseases. Its relevance has been particularly well documented in individuals with cystic fibrosis as well as alcoholic/nonalcoholic fatty liver disease (ALD/NAFLD), in whom heterozygous Pi*Z presence greatly increased the odds to harbor cirrhosis.[8,9] These data were further strengthened by a large genome-wide association study, where the Pi*Z variant emerged as the variant conferring the highest odds ratio (OR) for ALD-/NAFLD-related cirrhosis,[10] while well-known variants in PNPLA3 (rs738409) or TM6SF2 (rs58542926) were either not significantly associated with ALD/NAFLD-related cirrhosis or displayed lower odds. Finally, Pi*Z has also been linked to clinically significant portal hypertension in chronic liver diseases.[11]

Despite this unambiguous evidence, the importance and natural history of the Pi*MZ genotype in the general population as well as factors promoting liver disease development in Pi*MZ subjects remain to be defined. Moreover, the histological and biochemical consequences of Pi*MZ carriage have not been systematically analyzed. This is of particular importance to guide the counseling of Pi*MZ individuals, who in most cases do not display any hepatic comorbidity. Therefore, we prospectively recruited the hitherto largest, multinational cohort of genetically diagnosed Pi*MZ individuals without previously known liver disease and assessed their liver phenotypes compared with Pi*ZZ subjects and noncarriers. In addition to this noninvasive evaluation, we collected liver specimens and conducted a comprehensive and systematic histological, immunohistochemical, and biochemical evaluation. Finally, we compared liver transaminases in a large population-based cohort of Pi*MZ subjects, Pi*ZZ individuals, and noncarriers. The ultimate goal of our study was to define the clinical, histological, and biochemical phenotype of Pi*MZ subjects needed for evidence-based patient management and counseling.

Methods

*Prospectively Recruited Pi*MZ Subjects, Pi*ZZ Individuals, and Pi*Z Noncarriers (Cohort 1)*

Recruitment of European alpha-1 liver cohort. The studied populations were selected from an ongoing, multicenter, patient registry on AATD-related liver disease with intended prospective and longitudinal follow-up ("European Alpha-1 Liver Cohort"; inclusion and exclusion criteria are listed later in this article). To establish this European AATD registry, subjects with known or presumed AATD were recruited at multiple sites across Europe via following approaches: (1) cooperation with established AATD networks, (2) cooperation with rare liver disease networks, and (3) a broad awareness campaign.

(1) In all participating countries, we cooperated with national and global patient advocacy groups, lung-centered AATD registries, as well as respiratory specialists with a particular interest in AATD. Hence, many subjects with known or presumed AATD were recruited as the aforementioned institutions informed their patients about our European liver-centered AATD registry, that offered free examinations in every participating country.

(2) The European commission started a campaign in 2016 to establish Europe-wide networks of health care providers for rare diseases. Subsequently, the European Reference Network (ERN) for hepatological diseases (ERN Rare-Liver; www.rare-liver.eu) was established and the University Hospital Aachen became the coordinating center for AATD-related liver disease. In addition, the European Association for the Study of the Liver provides registry grants for establishing Europe-wide consortia groups, and in 2017 the University Hospital Aachen became the coordinating center for AATD-related liver disease. Within these networks, patients with known or presumed AATD were referred from other health care providers to our study group or patients consulted us directly.

(3) The awareness campaigns were composed of (a) an AATD liver-related Web site (www.alpha1-liver.eu), (b) a telephone hotline for patients and physicians, (c) a presence on social media, (d) talks at patient meetings, (e) regular contributions to patient-centred periodicals and patient-centred Web sites, (f) information flyer handed out to patients on AAT augmentation therapy for their lung disease, and (g) presentations for physicians caring for patients with AATD.

Non-carriers had been recruited from genetically unrelated household members of subjects with an established diagnosis of AATD (33.5%) or as volunteers in liver education campaigns (66.5%). These campaigns were organized by the University Hospital Aachen (Germany) and were announced via local media to provide a free liver examination for the general population.

Study population and definition of subcohorts. This study population represents a cross-section of the baseline examinations of the European Alpha-1 Liver Cohort. All participants of this study population were prospectively recruited from April 1, 2015, to May 1, 2019, and fulfilled the study-specific inclusion and exclusion criteria (Figure 1*A*). In total, 1012 adults of self-reported European ancestry were recruited in the same manner from 11 European countries (Austria, Belgium, Czech Republic, Denmark, Germany, Italy, Poland, Portugal, Spain, Switzerland, and the Netherlands). The inclusion criteria were (1) age $\geq$18 years, (2) no known pregnancy, and (3) the ability to provide a written informed consent. The main exclusion criteria were (1) the presence of known liver disease; (2) the presence of concomitant, yet unknown, liver disease picked up during our systematic workup (see supplement for details); (3) repeatedly elevated liver enzymes in medical history; (4) nonvalid assessment of liver stiffness using vibration-controlled transient elastography (TE/VCTE; FibroScan, Echosens, Paris, France); (5) presence of confounders of reliable TE assessment (eg, nonfasting or cholestasis); or (6) non-European descent.

*Pi*ZZ subjects* (n = 309) were defined as individuals with homozygous carriage of the *SERPINA1* "Pi*Z" variant (rs28929474, also known as p.E342K or Glu342Lys), that is, they had the Pi*ZZ genotype. A major part of this subcohort was described previously.[4] To allow an optimal comparison with the studied subcohorts, only Pi*ZZ individuals without current AAT augmentation therapy were included; 48% of the presented subcohort was recruited in Germany.

*Pi*MZ subjects* (n = 419) were defined as individuals with heterozygous carriage of the "Pi*Z" variant without Pi*S (rs17580) carriage, that is, they had the Pi*MZ genotype. Although most Pi*ZZ individuals had a previously established diagnosis of AATD,[4] most Pi*MZ individuals did not, as they usually neither develop liver-related nor lung-related symptoms. Indeed, most Pi*MZ subjects had a family member with an established AATD diagnosis but had not themselves been genotyped until recruitment into our registry. Only participants with a proven Pi*MZ genotype regardless of the AAT serum level were included. 74% of Pi*MZ subjects were recruited in Germany.

Non-carriers (n = 284) were defined as individuals without signs of AATD. In all participants, the AAT serum level was determined by nephelometry and genotyping for the 2 most relevant mutations of *SERPINA1* (ie, the "Pi*Z" variant and the "Pi*S" variant) was carried out as described.[4] Whenever considered appropriate, the samples were additionally sent to the corresponding national AAT reference laboratory for further phenotyping via isoelectric focusing as well as for further genetic analyses according to established recommendations.[12] Hence, noncarriers had a normal AAT serum level (>110 mg/dL) and were neither carriers of the Pi*Z nor the Pi*S variant. A major part of this subcohort was described previously[4]; 90% of the noncarrier subcohort at hand was recruited in Germany.

Assessment of liver disease and ethical statement. Details are given in the supplement.

Analysis in the Population-based UK Biobank (Cohort 2)

A total of 487,831 participants were genotyped for both the Pi*Z and Pi*S mutation; 43,330 participants were excluded because of Pi*S carriage (either heterozygous/homozygous or compound heterozygosity with Pi*Z). Further details are given in the Supplement.

Histological Analysis of Liver Biopsies (Cohort 3)

During the course of the study, we collected percutaneous liver biopsies from a total of 84 Pi*MZ and 35 Pi*ZZ individuals. Further details are given in the Supplement.

Biochemical Analysis of Liver Specimens (Cohort 4)

Tissue samples for protein analyses were obtained from Pi*MZ (n = 6) and Pi*ZZ subjects (n = 7) as well as noncarriers (n = 7) who underwent clinically indicated liver biopsies or liver resection (Figure 1*C*). Further details are given in the Supplement.

Statistical Analysis

Details are given in the Supplement.

Results

*Liver-related Parameters in Pi*MZ Subjects (Cohort 1)*

We analyzed the liver phenotype of 419 Pi*MZ subjects, 309 Pi*ZZ individuals, as well as 284 Pi*Z noncarriers, all without previously known or concomitant liver disease (Figure 1*A*). All 3 groups showed a comparable sex distribution and similar rates of diabetes mellitus (Table 1). Pi*MZ subjects were younger than the other 2 groups, whereas Pi*ZZ individuals displayed the lowest body mass indices (BMI) (Table 1). Relevant alcohol consumption (12–40 g/d for women / 24–60 g/d for men) was rare in all 3 groups but more frequent in noncarriers (Table 1). As expected, Pi*MZ subjects showed intermediate AAT serum levels (87 ± 20 mg/dL vs 139 ± 27 mg/dL in noncarriers vs 30 ± 25 mg/dL in Pi*ZZ, all $P < .0001$, Table 1, Figure 2*A*). The previously proposed cutoff AAT level of 110 mg/dL differentiated well between AATD subjects (Pi*MZ/Pi*ZZ) and noncarriers (positive likelihood ratio 9.7, negative likelihood ratio 0.1).

Alanine aminotransferase (ALT), bilirubin, and alkaline phosphatase (ALP) levels did not show major differences among genotypes. Mean aspartate aminotransferase (AST)

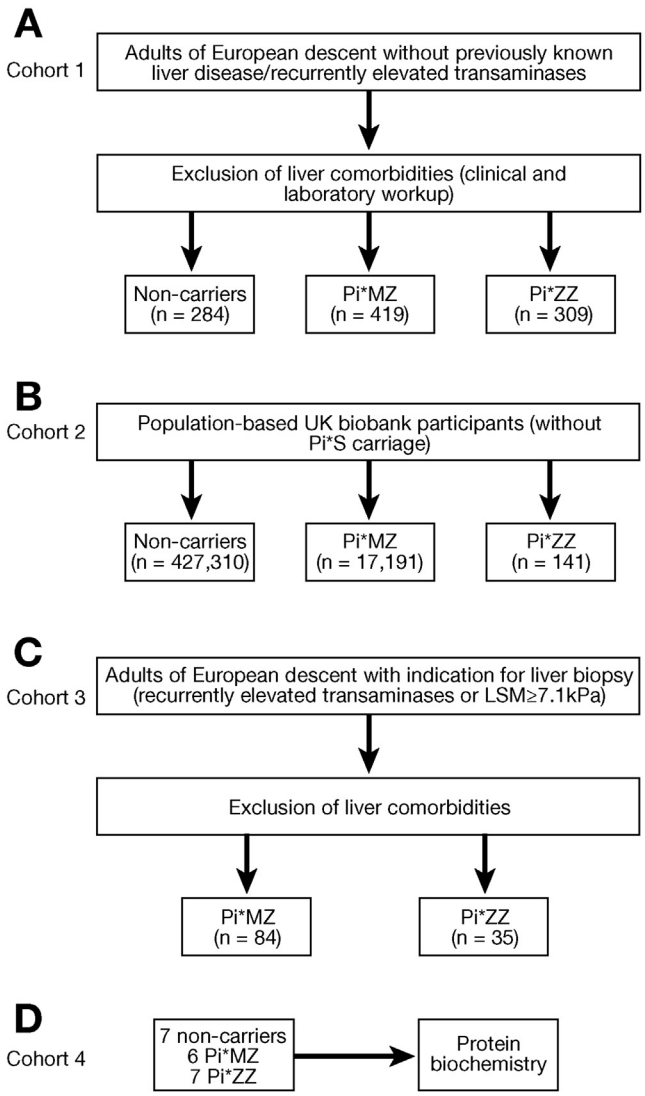


Figure 1. Overview of analyzed subpopulations. (*A*) Cohort 1: Prospectively recruited individuals as part of the "European Alpha-1 Liver" registry cohort. (*B*) Cohort 2: Population-based study analyzing participants with Pi*Z but without Pi*S carriage. (*C*) Cohort 3: Histologic analysis of Pi*MZ and Pi*ZZ individuals with an indication for liver biopsy. (*D*) Cohort 4: Biochemical analysis of liver specimen. Pi*Z, most relevant mutation of *SERPINA1*; Pi*S, second most relevant mutation of *SERPINA1*; Pi*MZ, heterozygosity for the Pi*Z variant; Pi*ZZ, homozygosity for the Pi*Z variant.

values were higher in Pi*ZZ vs Pi*MZ participants but were comparable between noncarriers and Pi*MZ individuals (Supplementary Table 1; Figure 2*C*). Gamma-glutamyl transferase (GGT) was the only parameter that clearly differed among the 3 genotypes, that is, the values were the lowest in noncarriers and highest in Pi*ZZ subjects (Supplementary Table 1; Figure 2*D*). Surrogate markers for advanced chronic liver disease such as platelet count, albumin, and International Normalized Ratio were within normal limits in most study participants (Supplementary Table 1). In line with previous observations, Pi*ZZ subjects had lower triglyceride levels than both noncarriers and Pi*MZ individuals (Supplementary Table 1).

To further account for the age difference between the groups, we separately assessed all parameters in participants who were ≥50 years old. Among them, Pi*MZ individuals displayed higher ALT, AST, GGT, and ALP levels than noncarriers (Supplementary Table 2). Compared with older Pi*MZ subjects, older Pi*ZZ individuals had elevated AST, GGT, and bilirubin levels, whereas platelets, and triglyceride values were lower (Supplementary Table 2).

*Liver Fibrosis and Steatosis in Prospectively Recruited Pi*MZ Subjects (Cohort 1)*

To noninvasively determine the degree of liver fibrosis and steatosis, all participants from the registry cohort were evaluated with TE. Pi*MZ individuals had intermediate LSM values, that is, LSM values were higher than in noncarriers (5.5 ± 6.0 kPa vs 4.5 ± 1.3 kPa, $P = .007$), but lower than in Pi*ZZ subjects (5.5 ± 6.0 kPa vs 7.0 ± 6.9 kPa; $P = .004$, Table 1; Figure 2*E*). Ten percent of Pi*MZ individuals showed LSM values ≥7.1 kPa (ie, values suggesting fibrosis stage of at least 2) compared with 4% of noncarriers (adjusted OR = 4.8 [2.0–11.8], $P = .001$; Table 1) and 25% of Pi*ZZ subjects (adjusted OR = 0.3 [0.1–0.9], $P < .0001$; Table 1). The difference between Pi*ZZ, Pi*MZ, and noncarriers was even more pronounced for those with LSM ≥10 kPa suggesting at least fibrosis stage 3 (11% vs 4% vs 1%; all $P < .01$; Table 1). Notably, the differences in LSM remained significant when only participants ≥50 years old were considered (Supplementary Table 2). To further corroborate the association of increased LSM in Pi*MZ subjects, we constructed multiple logistic regression models accounting for potential confounders. In all of them, Pi*MZ individuals had significantly higher odds for LSM ,≥7.1 kPa as well as LSM ≥10 kPa than noncarriers (Supplementary Table 3).

To determine cofactors associated with liver fibrosis, we compared Pi*MZ individuals with Pi*ZZ subjects who both had LSM ≥7.1 kPa. Although largely the same risk factors were identified in both groups (ie, higher age, male sex, and higher BMI), Pi*MZ individuals with LSM ≥7.1 kPa were clearly more obese or diabetic than their Pi*ZZ counterparts (Supplementary Table 4).[4]

The simultaneously assessed controlled attenuation parameter (CAP), which is an established surrogate of hepatic steatosis, showed higher mean values in Pi*ZZ individuals than in Pi*MZ subjects (258 ± 59 dB/m vs 245 ± 61 dB/m, $P < .0001$), but no difference between Pi*MZ subjects and noncarriers (Table 1; Figure 2*F*).

*Factors Associated With Significant Liver Fibrosis in Pi*MZ Subjects (Cohort 1)*

To identify the demographic and laboratory parameters associated with significant liver fibrosis in Pi*MZ participants, we compared the subgroups with LSM <7.1 and ≥7.1 kPa. Age ≥50 years (OR = 2.3 [1.2–4.5]), BMI ≥30 kg/m^2 (OR = 3.8 [1.9–7.5]), and CAP ≥248 dB/m suggesting steatosis (OR = 3.1 [1.4–6.5]) conferred higher odds of having LSM ≥7.1 kPa (Supplementary Table 5; Supplementary Figure 1). Notably, diabetic Pi*MZ

Table 1. Characteristics of Pi*MZ Subjects Compared With Pi*ZZ Individuals and Noncarriers of the Pi*Z and Pi*S variant of *SERPINA1* (Cohort 1)

	Noncarriers n=284	Pi*MZ n=419	Pi*ZZ n=309	*P* value Pi*MZ vs noncarriers (uni variable)	*P* value Pi*MZ vs noncarriers (multivariable)	*P* value Pi*MZ vs Pi*ZZ (univariable)	*P* value Pi*MZ vs Pi*ZZ (multivariable)
Characteristics							
Age (*y*)	51±15	45±16	50±15	**<.0001**		**<.0001**	
Women (%)	52	56	49	.30		.054	
BMI (kg/m^2)	26.0±5.2	26.0±4.6	24.9±4.5	.94		**.002**	
Mean alcohol consumption (g/d)	7.1±9.7	4.4±.6.8	4.2±7.3	**<.0001**		.77	
AAT serum level (mg/dL)	139±27	87±20	30±25	**<.0001**	**<.0001**	**<.0001**	**<.0001**
Modifiable risk factors							
BMI ≥30 kg/m^2 (%)	17	18	10	.77		**.002**	
Diabetes mellitus (%)	5	4	4	.66		.83	
Relevant alcohol intake[a] (%)	13	7	7	**.007**		.95	
Liver status							
Liver stiffness (kPa)	4.5±1.3	5.5±6.0	7.0±6.9	**.007**	**.005**	**.002**	**.0004**
Liver stiffness ≥7.1 kPa (%)	4	10	25	**.005**	**.002**	**<.0001**	**<.0001**
Liver stiffness ≥10.0 kPa (%)	1	4	11	**.005**	**.010**	**.0001**	**.001**
CAP (dB/m)	244±60	245±61	258±59	.84	.094	**.008**	**.0002**
CAP ≥248 dB/m (%)	49	49	57	.97	.20	.051	**.020**
CAP ≥280 dB/m (%)	27	29	33	.52	.13	.37	.14

NOTE. Quantitative measures are expressed as means and standard deviations or as relative frequencies (%). The cutoffs for liver stiffness were selected based on etiology-unspecific recommendations, that is, ≥7.1 kPa suggesting the presence of significant fibrosis (fibrosis stage ≥2) and ≥10.0 kPa suggesting the presence of advanced fibrosis (fibrosis stage ≥3). The cutoffs for controlled attenuation parameter (CAP) were selected based on etiology-unspecific recommendations, that is, ≥248 dB/m suggesting the presence of steatosis (steatosis grade ≥1) and ≥280 dB/m suggesting the presence of severe steatosis (steatosis grade =3). All multivariable analyses were adjusted for age, sex, BMI, diabetes, and mean alcohol consumption.

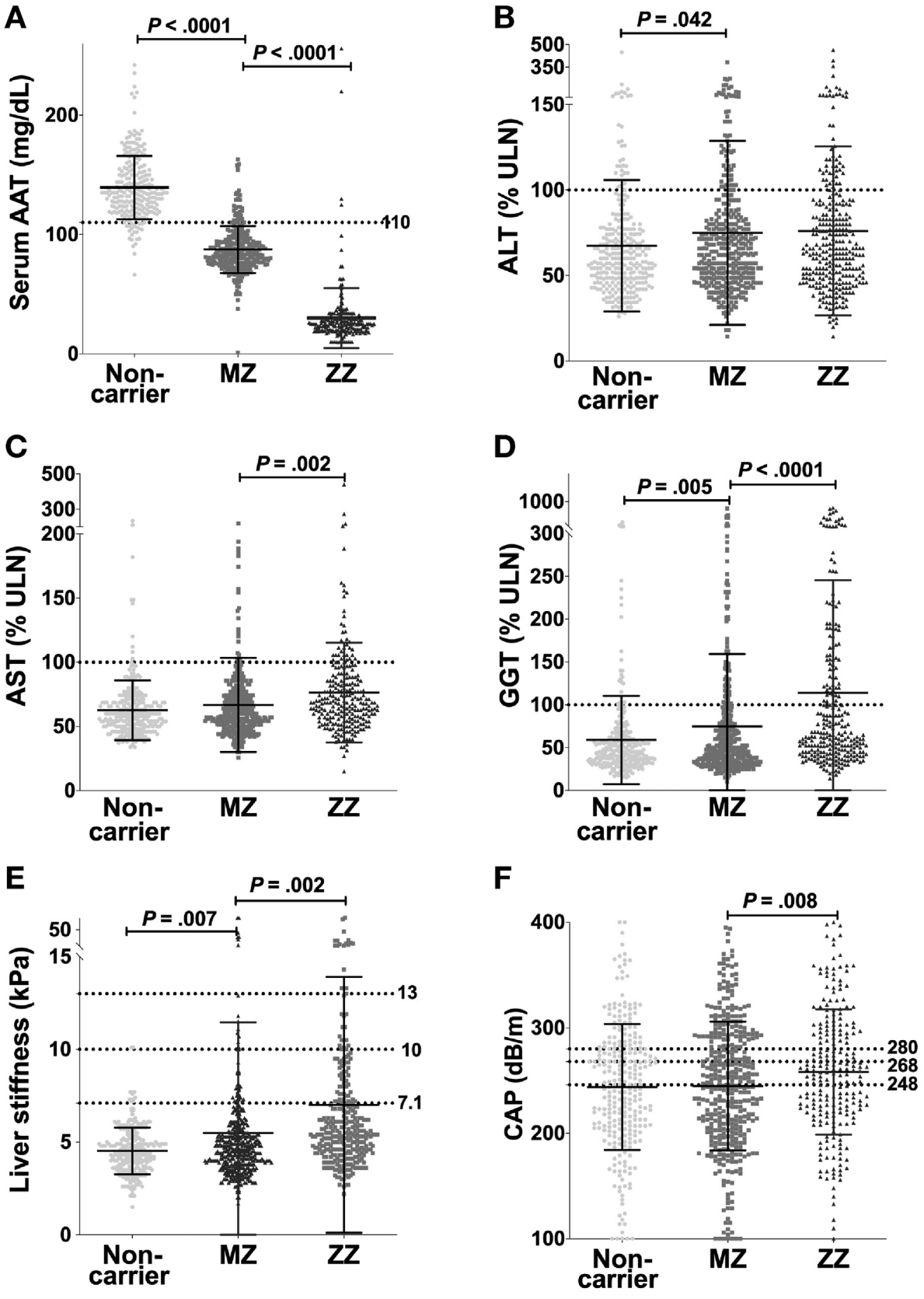
[a]Alcohol intake >12 g/d women, >24 g/d men (Individuals with alcohol consumption >40 g/d for females or >60 g/d for males had been excluded a priori). *P* values <.05 are indicated in bold font.

individuals displayed particularly high odds to have LSM ≥7.1 kPa (OR = 8.1 [2.9–23.2]). Interestingly, male sex did not confer a significantly increased risk of LSM ≥7.1 kPa (OR = 1.8 [1.0–3.4] (Supplementary Figure 1). Among laboratory parameters, elevated GGT (OR = 2.6 [1.2–5.3]) and reduced platelets (OR = 4.2 [1.4–12.6]) were the parameters most clearly associating with LSM ≥7.1 kPa (Supplementary Table 5). Basically the same risk factors and ORs were seen for LSM ≥10 kPa (Supplementary Figure 1).

To further study the importance of obesity as the major modifiable risk factor, we compared obese and nonobese individuals (Supplementary Figure 2). Although obese noncarriers displayed higher LSM and GGT values than their nonobese counterparts, the obese Pi*MZ subjects had the highest values of all subgroups. In particular, obese Pi*MZ subjects had approximately 4 times the odds of LSM ≥7.1 kPa compared with nonobese Pi*MZ individuals (unadjusted OR = 3.8 [1.9–7.5]; Supplementary Table 5). To further corroborate this finding, we included Pi*MZ genotype carriage, obesity, and their interaction term (ie, Pi*MZ × obesity) in a logistic regression model on the status of LSM ≥7.1 kPa. Although both Pi*MZ and obesity made significant contributions in the main effects model ($P = .006$ and $P < .0001$, respectively), additionally including the interaction term (Pi*MZ x obesity) yielded a nonsignificant testing result ($P = .33$). This indicated independent and additive contributions (on the logit scale) by both Pi*MZ and obesity. The same analysis for BMI (continuous) instead of obesity (BMI dichotomized by 30 kg/m^2) revealed comparable results.

With regard to serum GGT, the most prominently elevated liver-related blood parameter in Pi*MZ subjects, we compared Pi*MZ individuals with GGT within and above the sex-specific upper limit of normal (ULN) (Supplementary Table 6). Elevated GGT was associated with obesity; elevated ALT, AST, ALP, and triglycerides; as well as increased LSM and CAP. To further corroborate the higher odds of Pi*MZ subjects to display increased GGT, we constructed multiple logistic regression models accounting for potential confounders. In all of them, Pi*MZ individuals had significantly higher odds for elevated GGT than noncarriers (Supplementary Table 7).

Finally, to facilitate patient counseling, we determined the frequency of LSM ≥7.1 kPa in clinically relevant Pi*MZ subpopulations (Figure 3). Among the analyzed parameters, the presence of diabetes and decreased platelet counts (< 150 G/L) conferred >20% chance to have LSM ≥7.1 kPa, whereas male sex as well as elevated AST, ALT, or GGT levels were associated with a similarly high risk only in Pi*MZ individuals who were ≥50 years old (Figure 3).


A
P < .0001
P < .0001
Serum AAT (mg/dL)
200
100
0
110
Non-carrier
MZ
ZZ
B
P = .042
ALT (% ULN)
500
350
150
100
50
0
Non-carrier
MZ
ZZ
C
P = .002
AST (% ULN)
500
300
200
150
100
50
0
Non-carrier
MZ
ZZ
D
P = .005
P < .0001
GGT (% ULN)
1000
300
250
200
150
100
50
0
Non-carrier
MZ
ZZ
E
P = .007
P = .002
Liver stiffness (kPa)
50
15
10
5
0
13
10
7.1
Non-carrier
MZ
ZZ
F
P = .008
CAP (dB/m)
400
300
200
100
280
268
248
Non-carrier
MZ
ZZ

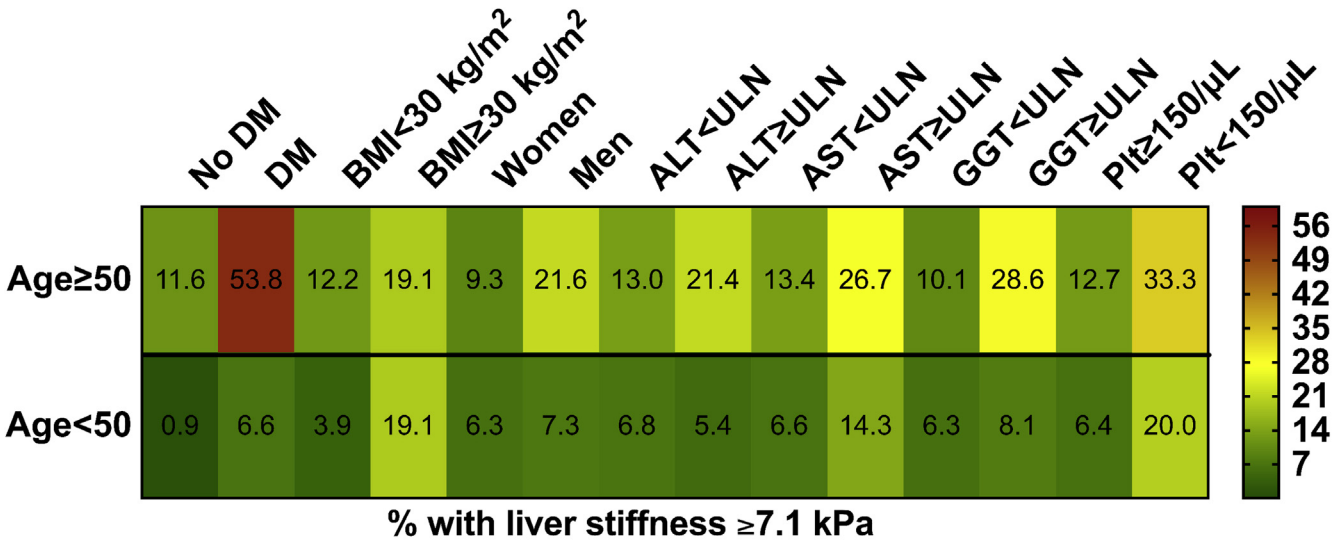


Figure 3. Rate of Pi*MZ individuals with significant liver fibrosis (LSM ≥7.1 kPa) among the highlighted subpopulations (Cohort 1). Relative frequencies (%) are shown and visualized by a color coding (*right*). DM, diabetes mellitus; Plt, platelets.

Liver Transaminases in the Population-based UK Biobank (Cohort 2)

To validate the findings from the cross-sectional analysis from the aforementioned registry cohort, and to provide largely unbiased population-based data, we turned to the UK biobank cohort (Figure 1*B*). Pi*MZ individuals were older and less likely to have diabetes compared with noncarriers (Table 2). To account for these differences, we applied multivariable analyses adjusting for age, sex, BMI, presence of diabetes, and mean alcohol consumption. Compared with noncarriers, Pi*MZ subjects had higher ALT serum levels (58.9 ± 32.1 vs 56.3 ± 33.2 (% of ULN), adjusted $P = 1.6*10^{-30}$) and more frequently ALT levels above the ULN (7.5% vs 6.6%, adjusted $P = 9.1*10^{-9}$; Table 2). Likely due to the low numbers of Pi*ZZ subjects, the differences between Pi*MZ and Pi*ZZ individuals did not reach statistical significance (Table 2). Regarding serum AST levels, Pi*MZ subjects had intermediate levels compared with noncarriers and Pi*ZZ individuals (Pi*MZ vs noncarriers: 65.3 ± 24.3 vs 63.8 ± 26.7 (% of ULN), adjusted $P = 1.9*10^{-14}$; Pi*MZ vs Pi*ZZ: 65.3 ± 24.3 vs 77.0 ± 29.0 (% of ULN), adjusted $P = 4.0*10^{-10}$; Table 2). Accordingly, Pi*MZ individuals had an intermediate proportion of individuals with serum AST above the ULN (Pi*MZ vs noncarriers: 5.2% vs 4.6%, adjusted $P = 1.9*10^{-4}$; Pi*MZ vs Pi*ZZ; 5.2% vs 15.9%, adjusted $P = 1.6*10^{-8}$; Table 2).

*Histological and Biochemical Phenotype of Pi*MZ Livers (Cohorts 3 and 4)*

To characterize the histological phenotype of Pi*MZ status, we compared 84 liver biopsies from Pi*MZ with 35 biopsies from Pi*ZZ individuals (Figure 1*C*). Although the presence of comorbidities other than NAFLD was excluded by systematic workup, we decided to keep individuals with NAFLD in the analysis as NAFLD is a widespread entity seen in a substantial part of the Caucasian population. Definite nonalcoholic steatohepatitis (defined as NAS score ≥5) was rare in both groups (Table 3). Pi*MZ subjects had higher BMI (31.4 ± 6.5 vs 25.9 ± 7.3 kg/m^2; $P < .0001$) and a higher prevalence of diabetes (31% vs 3%; $P = .001$), whereas Pi*ZZ individuals were more frequently male (83% vs 59%, $P = .013$, Table 3). Compared with the prospectively recruited Pi*MZ participants (cohort 1), biopsied Pi*MZ individuals (cohort 3) were older (55 ± 16 vs 45 ± 16 years; $P < .0001$), more frequently male (56% vs 44%; $P < .0001$), and had higher BMI (31.4 ± 6.5 vs 26.0 ± 4.6 kg/m^2; $P < .0001$; Supplementary Table 8).

Histological assessment revealed no differences in steatosis and inflammation, whereas Pi*MZ biopsies showed lower fibrosis stages (2.2 ± 1.3 vs 2.9 ± 1.0, $P = .007$) and lower proportion of perisinusoidal fibrosis (69.1% vs 97.1%, $P = .002$). Hematoxylin and eosin staining, as the routine histological assessment method, revealed inclusion bodies in >50% of Pi*ZZ individuals but only in 10.7% of

Figure 2. AAT concentrations and liver-related parameters in subjects heterozygous (Pi*MZ/MZ) and homozygous (Pi*ZZ/ZZ) for the Pi*Z variant of *SERPINA1* compared with noncarriers (Cohort 1). A total of 419 Pi*MZ individuals, 309 Pi*ZZ subjects, and 284 noncarriers were analyzed. The shown *P* values were adjusted for age, sex, BMI, diabetes mellitus, and mean alcohol consumption. (*A*) Scatter plot of the absolute serum AAT concentrations. The *dotted line* represents the 110 mg/dL cutoff level, which was previously suggested to discriminate between Pi*MZ individuals and noncarriers.[42,43] (*B–D*) Scatter plots of serum ALT, AST, and GGT levels, each normalized to the sex-specific ULN, marked with a *dotted line*. (*E*) Scatter plot of liver stiffness measurements determined by TE. The *dotted lines* represent the following etiology-unspecific cutoff levels[4,15]: 7.1 kPa (suggestive of fibrosis stage ≥2), 10.0 kPa (suggestive of fibrosis stage ≥3), and 13.0 kPa (suggestive of fibrosis stage 4). F) Scatter plot of CAP, a surrogate parameter of liver steatosis determined by TE. The *dotted lines* indicate the following etiology-unspecific cutoff levels[44]: 248 dB/m (suggestive of steatosis grade ≥1), 268 dB/m (suggestive of steatosis grade ≥2), and 280 dB/m (suggestive of steatosis grade 3).

Table 2. Characteristics of Individuals From the Population-based UK Biobank Who Neither Carry the Pi*Z Nor the Pi*S Variant (NC), Who Carry 1 Pi*Z allele but No Pi*S Allele (MZ), and Who Carry 2 Pi*Z Alleles (ZZ) (Cohort 2)

	Pi*Z/Pi*S noncarriers n=427,310	Pi*Z carrier, Pi*S noncarrier n=17,191	Pi*ZZ n=141	*P* value NC vs MZ (univariable)	*P* value MZ vs ZZ (univariable)	*P* value NC vs MZ (multivariable)	*P* value MZ vs ZZ (multivariable)
Age (*y*)	56.5±8.1	56.9±8.1	56.4±7.9	**$4.5*10^{-10}$**	.17		
Men (%)	46	45	52	.31	.13		
Diabetes mellitus (%)	5	4	2	**$2.8*10^{-7}$**	.58		
BMI (kg/m^2)	27.4±4.8	27.3±4.7	26.8±4.9	**$2.2*10^{-5}$**	.27		
Alcohol (units/d)	1.28±1.60	1.32±1.41	1.15±0.86	.49	.65		
ALT (% of ULN)	56.3±33.2	58.9±32.1	62.9±25.9	**$1.2*10^{-22}$**	.16	**$1.6*10^{-30}$**	.077
ALT ≥ULN (%) [a]	6.6	7.5	11.4	**$3.0*10^{-6}$**	.09	**$9.1*10^{-9}$**	.051
AST (% of ULN)	63.8±26.7	65.3±24.3	77.0±29.0	**$7.2*10^{-13}$**	**$4.0*10^{-8}$**	**$1.9*10^{-14}$**	**$4.0*10^{-10}$**
AST ≥ULN (%) [b]	4.6	5.2	15.9	**.001**	**$2.8*10^{-8}$**	**$1.9*10^{-4}$**	**$1.6*10^{-8}$**

NOTE. Quantitative measures are expressed as means and standard deviations or as relative frequencies (%).
[a]MZ vs noncarriers: Unadjusted OR = 1.129 [1.054–1.210], adjusted OR = 1.189 [1.121–1.261].
[b]MZ vs noncarriers: Unadjusted OR = 1.148 [1.083–1.216], adjusted OR = 1.142 [1.065–1.225]; MZ vs ZZ: Unadjusted OR = 0.286 [0.178–0.459], adjusted OR = 0.250 [0.154–0.404]. *P* values <.05 are indicated in bold font. One unit equals 8 g alcohol.

Pi*MZ subjects ($P < .0001$; Table 3, Figure 4*A* and *B*). PAS-D–positive inclusions were detected in all but 2 Pi*ZZ individuals, but only in 40.5% of Pi*MZ subjects ($P < .0001$, Table 3, Figure 4*A* and *B*). Immunohistochemistry (IHC) constituted the most sensitive method for detection of AAT inclusions (Figure 4*A*) and visualized aggregates in all but 1 Pi*ZZ individual (97%) and in 63% of Pi*MZ subjects ($P < .0001$, Table 3, Figure 4*A* and *B*). When using IHC, 74% of Pi*ZZ individuals showed inclusions in more than 20 hepatocytes per field, whereas only 37% of Pi*MZ subjects displayed such abundant aggregates ($P < .0001$, Table 3). Accordingly, Pi*MZ individuals had lower mean inclusion scores (1.6 ± 1.3 vs 2.6 ± 0.8, $P < .0001$, Table 3).

Because data from Pi*ZZ subjects indicate that the amount of AAT inclusions increases during, or may even promote, the progression of liver disease,[5] we assessed the abundance of inclusions throughout different fibrosis stages. In IHC analysis, all cirrhotic Pi*MZ individuals displayed many inclusions, whereas most noncirrhotic Pi*MZ subjects had no or only rare inclusions. Overall, in Pi*MZ individuals, higher fibrosis stages were clearly associated with higher inclusions scores (Figure 4*C*). Although the differences were less obvious in Pi*ZZ subjects, most Pi*ZZ individuals with no or mild fibrosis had no or only few inclusions, whereas most Pi*ZZ subjects with significant liver fibrosis/cirrhosis had many AAT aggregates (Figure 4*C*). With regard to other demographic parameters and/or histological features, AAT inclusions were more numerous in ≥50-year-old Pi*MZ individuals, whereas no obvious relationship with sex, BMI, and presence of steatosis or inflammation was noted (Supplementary Figure 3).

To quantify hepatic AAT levels, we performed immunoblotting of total tissue lysates (Figure 1*D*). Notably, we did not observe a significant accumulation of AAT in Pi*MZ individuals, whereas Pi*ZZ subjects displayed markedly elevated hepatic AAT levels (Figure 4*D*). Furthermore, no insoluble AAT was seen in noncarriers, whereas the amounts of insoluble AAT strongly varied among the Pi*MZ individuals. Although some of them did not show any insoluble AAT, others had levels comparable or even higher than the amount of soluble AAT (Figure 4*E*).

Collectively, our data indicate that the presence of AAT inclusions cannot be used for histological diagnosis of Pi*MZ subjects and that the amounts of inclusions strongly differ between both the individuals and the disease stages.

Discussion

Our study represents the first systematic evaluation of liver disease burden in Pi*MZ individuals. By combining the multinational registry cohort and the population-based cohort, we were able to demonstrate that Pi*MZ subjects display an intermediate liver phenotype compared with noncarriers of Pi*Z and Pi*S as well as Pi*ZZ individuals.

Among serum levels of liver enzymes in the registry cohort, GGT was most strongly elevated and significantly higher in Pi*MZ individuals compared with noncarriers. This is not surprising because GGT also constitutes the most commonly elevated liver enzyme in Pi*ZZ subjects[4]; however, only borderline or no difference was seen in AST and ALT levels. This was unexpected, given that Pi*MZ subjects harbored substantially higher AST/ALT levels than noncarriers in a large genome-wide study[10] and another genome-wide study confirmed the higher ALT values.[13] To clarify this discrepancy, we turned to a large population-based cohort and found elevated AST and ALT values in Pi*MZ individuals compared with noncarriers. The limited size of our registry cohort, the different recruitment strategy, the exclusion of individuals with repeatedly elevated transaminases, and/or the overrepresentation of younger and nonobese individuals may explain the fact, that no consistent differences in transaminase levels were seen.

A particular strength of our registry cohort was the noninvasive evaluation of liver fibrosis via LSM in all participants. Although LSM and CAP using TE was not validated

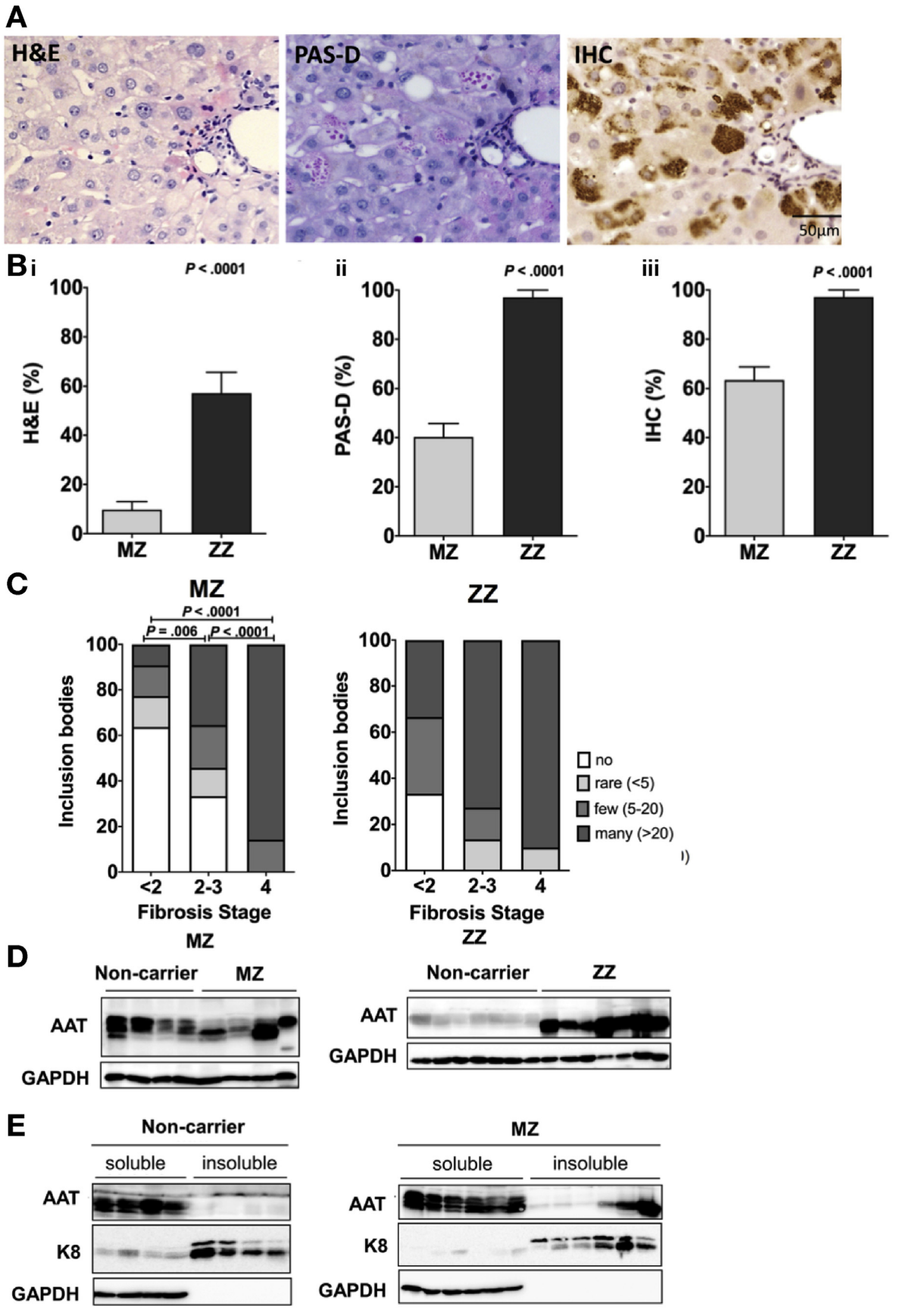
Table 3. Characteristics of Pi*MZ and Pi*ZZ Individuals Who Received a Liver Biopsy (Cohort 3)

	Pi*MZ n=84	Pi*ZZ n=35	*P* value (univariable)	*P* value (multivariable)
Characteristics				
Age (*y*)	55±16	53±12	.44	
Women (%)	44	17	**.006**	
BMI (kg/m^2)	31.4±6.5	25.9±7.3	**<.0001**	
Mean alcohol consumption (g/d)	5.1±10.3	4.1±6.6	.63	
AAT augmentation therapy (%)	N/A	34	N/A	
Modifiable risk factors				
BMI ≥30 kg/m^2 (%)	61	24	**<.0001**	
Diabetes mellitus (%)	31	3	**.001**	
Relevant alcohol intake[a] (%)	5	3	.32	
Steatosis				
Steatosis grade	1.4±0.9	1.2±0.9	.15	.78
Steatosis grade 0 (%)	15.5	25.7	.19	.96
Steatosis grade 1 (%)	40.5	34.3	.53	.73
Steatosis grade 2 (%)	28.5	37.1	.36	.17
Steatosis grade 3 (%)	15.5	2.9	.052	.15
Fibrosis				
Fibrosis stage (Kleiner)	2.2± 1.3	2.9±1.0	**.007**	**<.001**
Fibrosis stage 0	13.1	2.9	.093	**.007**
Fibrosis stage 1	13.1	5.7	.24	.59
Fibrosis stage 2	28.6	20.0	.33	.40
Fibrosis stage 3	28.6	42.9	.13	.14
Fibrosis stage 4	16.7	28.6	.14	.13
Perisinusoidal fibrosis (%)	69.1	97.1	**.002**	**<.0001**
Inflammation				
Lobular inflammation (%)	40.5	41.2	.94	.95
Portal inflammation (%)	72.8	61.8	.24	.55
Hepatocyte ballooning (%)	33.3	11.8	**.049**	.43
Inflammatory foci (%)	41.9	14.7	**.007**	.10
NAS score	2.4±1.7	1.5±1.2	**.004**	.32
0–1 (%)	35.7	55.9	**.031**	.30
2–4 (%)	54.8	41.2	.14	.28
≥5 (%)	9.5	2.9	.21	.82
AATD-related findings				
Inclusion bodies in HE (%)	10.7	57.1	**<.0001**	**<.0001**
Inclusion bodies in PAS-D (%)	40.5	94.3	**<.0001**	**<.0001**
Inclusion bodies in immunohistochemistry (%)	63.1	97.1	**<.0001**	**<.0001**
Inclusion bodies in >5 hepatocytes (%)	54.7	85.7	**<.0001**	**<.0001**
Inclusion bodies in >20 hepatocytes (%)	36.9	74.3	**<.0001**	**<.0001**
Inclusion body score	1.6±1.3	2.6±0.8	**<.0001**	**<.0001**

NOTE. Quantitative measures are expressed as means and standard deviations or as relative frequencies (%). Fibrosis stage and steatosis grade are based on Kleiner scoring system. Disease activity was assessed with Nonalcoholic Fatty Liver Disease Activity Score (NAS). The abundance of inclusion bodies was evaluated as described[5] and was based on appearance in immunohistochemistry staining with a specific antibody for mutated Pi*Z protein.[41] All multivariable analyses were adjusted for age, sex, BMI, diabetes, and mean alcohol consumption.
HE, hematoxylin and eosin staining; N/A, not applicable.
[a]Alcohol intake >12 g/d women, >24 g/d men (Individuals with alcohol consumption >40 g/d for females or >60 g/d for males had been excluded a priori). *P* values <.05 are indicated in bold font.

in Pi*MZ subjects per se, TE was recently validated in Pi*ZZ subjects[5,11,14] and was extensively validated in both the general population as well as various liver disease entities.[15–18] LSM demonstrated 5- and 9-times increased odds for significant and advanced liver fibrosis in Pi*MZ individuals vs noncarriers, respectively (Supplementary Table 3). The latter OR is comparable with the reported odds of Pi*MZ subjects to develop cirrhosis when suffering from cystic fibrosis, ALD, or NAFLD.[8,9] In contrast, other studies reported a lower, but still significant, enrichment of Pi*MZ individuals in patients with ALD/NAFLD-associated cirrhosis or clinically significant portal hypertension as well as in patients who underwent liver transplantation.[10,11,19]

Although the Pi*MZ status constitutes an undisputed risk factor for significant liver fibrosis and although a


A
H&E
PAS-D
IHC
50μm
B i
P < .0001
H&E (%)
MZ
ZZ
ii
P < .0001
PAS-D (%)
iii
P < .0001
IHC (%)
C
MZ
P < .0001
P = .006
P < .0001
Inclusion bodies
Fibrosis Stage
ZZ
no
rare (<5)
few (5-20)
many (>20)
D
Non-carrier
MZ
ZZ
AAT
GAPDH
E
soluble
insoluble
K8

growing body of evidence convincingly shows that Pi*MZ individuals are susceptible to developing liver fibrosis in coexisting liver disease ("second hit"), understanding the additional factors is of utmost importance. To delineate them, we focused on individuals without concomitant liver disease. Comparable to other entities of liver disease, the most prominent risk factors associating with LSM ≥7.1 kPa in Pi*MZ subjects were presence of obesity and diabetes mellitus. Moreover, age ≥50 years was a comparably weak disease modifier. However the association was not unexpected, because older Pi*ZZ subjects have a higher risk to suffer advanced liver fibrosis than younger Pi*ZZ individuals.[4,20,21] Although a rising prevalence of liver fibrosis in the aging population is well documented,[22,23] aging may be particularly applicable to genetic liver disease, where the duration of the exposure to the etiologic factor (ie, misfolded AAT in hepatocytes) increases steadily with age. In addition, aging entails an increased proteotoxic stress[7,24] and decreased autophagic capacity[25] and because of that, might be of particular relevance in AATD.[24] In that respect, the amount of AAT inclusions was higher in ≥50 year-old Pi*MZ individuals (Supplementary Figure 3), whereas inclusions were rarely seen in neonatal AATD-related liver disease.[2] Obesity was not only a comparably strong, but also an independent and additive risk factor for LSM ≥7.1 kPa in Pi*MZ subjects. This disease-promoting role of obesity is not surprising, because it is known to promote liver fibrogenesis in other etiologies and because it has been shown to amplify the effect of other genetic variants.[26,27] Moreover, obesity was also associated with advanced liver fibrosis in individuals with the Pi*ZZ genotype.[4] However, compared with Pi*ZZ individuals, Pi*MZ subjects appear to need a "stronger" metabolic second hit to develop significant liver fibrosis (Supplementary Table 4). Finally, among the described contributing factors, the presence of diabetes conferred 8 times higher odds to display LSM ≥7.1 kPa (Supplementary Figure 1). These results are reminiscent of the situation in Pi*ZZ individuals, where the presence of diabetes also strongly associates with significant liver fibrosis.[5] Although the exact relationship between diabetes and AATD remains to be determined, insulin resistance results in an increased lipolysis that likely augments the endoplasmic reticulum stress occurring in AATD livers.[28] In terms of patient and family counseling regarding modifiable risk factors, Pi*MZ individuals should be advised that obesity and insulin resistance/diabetes markedly increase their risk to develop significant liver disease.

Besides an identification of disease-promoting factors, we also assessed routine laboratory parameters, which might be useful to facilitate the clinical management of this large patient group. Among them, elevated GGT levels and decreased platelet counts were particularly indicative of LSM ≥7.1 kPa (Supplementary Figure 1). This is not surprising because GGT has been shown to predict the existence of significant liver fibrosis in Pi*ZZ subjects.[4,5] As a likely underlying mechanism, GGT is an established marker of metabolic liver disease and plays an important role in defense against oxidative stress.[29,30] The usefulness of platelet count is well in line with its ability to predict liver fibrosis and portal hypertension in general and significant liver fibrosis in Pi*ZZ subjects in particular.[4,31]

To better understand the consequences of Pi*MZ status, we performed a detailed histological, immunohistochemical, and biochemical analysis. We demonstrated that occurrence of AAT aggregates is not an obligate/mandatory feature of Pi*MZ genotype and that in contrast to Pi*ZZ individuals, it is seen only in a minority of Pi*MZ subjects with no or mild liver fibrosis.[5] Hence, PAS-D or Pi*Z IHC staining neither allows histological diagnosis of Pi*MZ individuals nor is it useful to discriminate Pi*MZ from Pi*ZZ subjects. This finding is important both for scientific and routine clinical evaluation of these individuals as many centers solely relied on liver histology yet. For example, multiple studies used the presence of AAT globules as a method to screen for Pi*MZ status.[32–35] Such studies likely miss a significant portion of Pi*MZ individuals and may enrich for heterozygotes with a more severe liver pathology. Therefore, genetic approaches (ideally in combination with AAT phenotyping) are recommended for a reliable detection of Pi*MZ status.

Our histological/biochemical examination revealed that the amount of accumulated AAT displays strong interindividual differences but increases with progression of liver disease. The former finding is reminiscent of the situation in Pi*ZZ subjects[5] and might be, at least in part, due to interindividual variability in AAT expression and degradation.[2] Given the correlation between the expression of Pi*Z and the development of liver disease seen in transgenic mice,[36] as well as the fact that Pi*ZZ individuals with manifest liver disease frequently display a delayed AAT degradation,[37] it is tempting to speculate that abundant AAT aggregates identify patients at risk for

Figure 4. Comparison of the histological and biochemical features of heterozygotes (MZ), homozygotes (ZZ), and noncarriers of the Pi*Z variant (Cohorts 3 and 4). (*A*) Histological appearance of AAT aggregates in corresponding liver tissue sections that were stained with hematoxylin and eosin (H&E), PAS-D, or with IHC using an antibody specific for the Pi*Z variant of *SERPINA1*.[41] (*B*) Rate of detection of inclusion bodies in Pi*MZ and Pi*ZZ subjects when using the staining approaches described previously. (*C*) Amounts of inclusion bodies per field in biopsy samples of Pi*MZ/Pi*ZZ individuals with different fibrosis stages, based on IHC. The abundance of inclusion bodies was scored as described by Clark et al[5]: no, no inclusions visible; rare, inclusions in up to 5 cells; few, inclusion bodies in 5 to 20 cells; and many, inclusions in more than 20 cells. (*D*) AAT protein levels were assessed by immunoblotting in total lysates obtained from liver biopsies of Pi*MZ subjects, Pi*ZZ individuals, and noncarriers. GAPDH was used as a loading control. (*E*) AAT solubility in 1% Triton-X containing-buffer was determined in liver samples from Pi*MZ individuals and noncarriers. GAPDH and keratin 8 (K8) were used as loading controls for soluble and insoluble pools, respectively. Univariate *P* values are shown in all panels.

rapid liver disease progression. However, AAT accumulation may be both a sign and a culprit of advancing liver disease. With regard to the former, *SERPINA1* represents a stress-inducible gene and a stress-mediated increase in AAT production promoted the development of liver injury in transgenic mice overexpressing the Pi*Z variant.[2,4,38] Therefore, hepatotoxic stress in Pi*MZ subjects with advanced liver disease may result in a vicious cycle with elevated Pi*Z production and a consequent aggravation of liver damage. To support this hypothesis, a recent study demonstrated that Pi*MZ individuals with cirrhosis were at increased risk of hepatic decompensation, as compared with cirrhotic noncarriers.[19]

A potential limitation is the cross-sectional design of the multinational registry cohort which cannot deduce cause-and-effect relationships and cannot fully exclude selection bias. The only population-based cohort aiming at Pi*ZZ individuals is from a birth screening program in ~200,000 Swedish newborns: the 87 participants who remained in the follow-up are now approximately 45 years old,[39,40] which is below the typical manifestation age of AATD-related liver disease. Moreover, despite our broad awareness campaign basically involving all stakeholders dealing with AATD individuals, there still is a "hidden" population that we were unable to account for, as many Pi*MZ individuals neither develop symptoms nor have a known family history of AATD (ie, as they remain undetected they do not hear about our Europe-wide initiative). However, several analyses accounting for relevant confounders as well as the observations in the population-based UK biobank and large genome-wide studies suggest that the presented observations hold true. Despite that, a validation of the observed findings in prospective screening cohorts is warranted.

Another challenging issue in studies analyzing the natural history of rare genetic variants is the exact composition of control cohorts. In the registry cohort, the noncarriers were examined in exactly the same manner as AATD individuals and were recruited both from the general population (two-thirds) as well as from unrelated household members of AATD participants (one-third). The unrelated household members share unknown environmental factors with AATD subjects, which cannot be accounted for in statistical analyses. Importantly, the results from the population-based UK biobank (having the least possibility of selection bias) underline that the Pi*MZ status is independently associated with higher serum transaminases.

In summary, our study involving 4 large and multinational cohorts defines the clinical, histological, and biochemical liver phenotype of adult Pi*MZ subjects as well as risk factors for significant liver fibrosis. This comprehensive phenotyping supports the emerging concept that Pi*MZ individuals are at risk of developing liver disease, especially in the presence of the discovered risk factors (eg, obesity and diabetes). This is particularly relevant, because only a small portion of the studied Pi*MZ population displays clinically relevant liver disease, but it is indispensable to recognize them. A long-term, prospective monitoring of Pi*MZ subjects will be necessary to determine the rate of disease progression and the occurrence of complications of advanced chronic liver disease.

Supplementary Material

Note: To access the supplementary material accompanying this article, visit the online version of *Gastroenterology* at www.gastrojournal.org, and at https://doi.org/10.1053/j.gastro.2020.04.058.

References

1. Greene CM, Marciniak SJ, Teckman J, et al. alpha1-Antitrypsin deficiency. Nat Rev Dis Primers 2016; 2:16051.
2. Strnad P, McElvaney NG, Lomas D. alpha1-Antitrypsin Deficiency. N Engl J Med 2020;382:1443–1455.
3. Silverman EK, Sandhaus RA. Clinical practice. Alpha1-antitrypsin deficiency. N Engl J Med 2009;360:2749–2757.
4. Hamesch K, Mandorfer M, Pereira VM, et al. Liver fibrosis and metabolic alterations in adults with alpha1 antitrypsin deficiency caused by the Pi*ZZ mutation. Gastroenterology 2019;157:705–719.e18.
5. 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;69:1357–1364.
6. Adam R, Karam V, Delvart V, et al. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). J Hepatol 2012;57:675–688.
7. Kuscuoglu D, Janciauskiene S, Hamesch K, et al. Liver - master and servant of serum proteome. J Hepatol 2018; 69:512–524.
8. Bartlett JR, Friedman KJ, Ling SC, et al. Genetic modifiers of liver disease in cystic fibrosis. Jama 2009; 302:1076–1083.
9. **Strnad P, Buch S, Hamesch K**, et al. Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. Gut 2019;68:1099–1107.
10. Abul-Husn NS, Cheng X, Li AH, et al. A protein-truncating HSD17B13 variant and protection from chronic liver disease. N Engl J Med 2018;378:1096–1106.
11. Mandorfer M, Bucsics T, Hutya V, et al. Liver disease in adults with alpha1-antitrypsin deficiency. United European Gastroenterol J 2018;6:710–718.
12. Greulich T, Ottaviani S, Bals R, et al. Alpha1-antitrypsin deficiency - diagnostic testing and disease awareness in Germany and Italy. Respir Med 2013;107:1400–1408.
13. Prins BP, Kuchenbaecker KB, Bao Y, et al. Genome-wide analysis of health-related biomarkers in the UK Household Longitudinal Study reveals novel associations. Sci Rep 2017;7:11008.
14. 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; 71:1272–1274.

15. Friedrich-Rust M, Poynard T, Castera L. Critical comparison of elastography methods to assess chronic liver disease. Nat Rev Gastroenterol Hepatol 2016;13:402–411.
16. Tapper EB, Lok ASF. Use of liver imaging and biopsy in clinical practice. N Engl J Med 2017;377:2296–2297.
17. Poynard T, Peta V, Pollo-Flores P, et al. Screening studies of transient elastography and FibroTest in the general population. Lancet Gastroenterol Hepatol 2017;2:246.
18. Tapper EB, Challies T, Nasser I, et al. The performance of vibration controlled transient elastography in a US cohort of patients with nonalcoholic fatty liver disease. Am J Gastroenterol 2016;111:677–684.
19. Schaefer B, Mandorfer M, Viveiros A, et al. Heterozygosity for the alpha-1-antitrypsin Z allele in cirrhosis is associated with more advanced disease. Liver Transpl 2018;24:744–751.
20. Bowlus CL, Willner I, Zern MA, et al. Factors associated with advanced liver disease in adults with alpha1-antitrypsin deficiency. Clin Gastroenterol Hepatol 2005;3:390–396.
21. Tanash HA, Piitulainen E. Liver disease in adults with severe alpha-1-antitrypsin deficiency. J Gastroenterol 2019;54:541–548.
22. Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Lancet 1997;349:825–832.
23. Roake CM, Artandi SE. Control of cellular aging, tissue function, and cancer by p53 downstream of telomeres. Cold Spring Harb Perspect Med 2017;7:a026088.
24. Labbadia J, Morimoto RI. The biology of proteostasis in aging and disease. Annu Rev Biochem 2015;84:435–464.
25. Leidal AM, Levine B, Debnath J. Autophagy and the cell biology of age-related disease. Nat Cell Biol 2018;20:1338–1348.
26. Williams R, Aspinall R, Bellis M, 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;384:1953–1997.
27. Stender S, Kozlitina J, Nordestgaard BG, et al. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nat Genet 2017;49:842–847.
28. Petersen MC, Shulman GI. Mechanisms of insulin action and insulin resistance. Physiol Rev 2018;98:2133–2223.
29. Kunutsor SK. Gamma-glutamyltransferase-friend or foe within? Liver Int 2016;36:1723–1734.
30. Fall T, Hagg S, Magi R, et al. The role of adiposity in cardiometabolic traits: a Mendelian randomization analysis. PLoS Med 2013;10:e1001474.
31. Berzigotti A, Seijo S, Arena U, et al. Elastography, spleen size, and platelet count identify portal hypertension in patients with compensated cirrhosis. Gastroenterology 2013;144:102–111.e1.
32. Fischer HP, Ortiz-Pallardo ME, Ko Y, et al. Chronic liver disease in heterozygous alpha1-antitrypsin deficiency PiZ. J Hepatol 2000;33:883–892.
33. Goltz D, Hittetiya K, Vossing LM, et al. Alpha(1)-antitrypsin PiMZ heterozygosity has an independent aggravating effect on liver fibrosis in alcoholic liver disease. Virchows Arch 2014;465:539–546.
34. Elzouki AN, Verbaan H, Lindgren S, et al. Serine protease inhibitors in patients with chronic viral hepatitis. J Hepatol 1997;27:42–48.
35. Hodges JR, Millward-Sadler GH, Barbatis C, et al. Heterozygous MZ alpha 1-antitrypsin deficiency in adults with chronic active hepatitis and cryptogenic cirrhosis. N Engl J Med 1981;304:557–560.
36. Carlson JA, Rogers BB, Sifers RN, et al. Accumulation of PiZ alpha 1-antitrypsin causes liver damage in transgenic mice. J Clin Invest 1989;83:1183–1190.
37. Wu JC, Merlino G, Fausto N. Establishment and characterization of differentiated, nontransformed hepatocyte cell lines derived from mice transgenic for transforming growth factor alpha. Proc Natl Acad Sci U S A 1994;91:674–678.
38. Rudnick DA, Shikapwashya O, Blomenkamp K, et al. Indomethacin increases liver damage in a murine model of liver injury from alpha-1-antitrypsin deficiency. Hepatology 2006;44:976–982.
39. Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med 1976;294:1316–1321.
40. 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;14:525–530.
41. Fu DA, Campbell-Thompson M. Immunohistochemistry staining for human alpha-1 antitrypsin. Methods Mol Biol 2017;1639:139–143.
42. Kok KF, Willems HL, Drenth JP. The cut-off value of 100 mg/dl is insufficient to detect heterozygous alpha-1 antitrypsin-deficient liver disease patients. Liver Int 2010;30:491–492.
43. Ferrarotti I, Poplawska-Wisniewska B, Trevisan MT, et al. How can we improve the detection of alpha1-antitrypsin deficiency? PLoS One 2015;10:e0135316.
44. **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;66:1022–1030.

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CRediT Authorship Contributions

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Conflict of interest

The authors disclose no conflicts.

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Supplementary Methods

*Prospectively Recruited Pi*MZ Subjects, Pi*ZZ Individuals, and Pi*Z Noncarriers (Cohort 1)*

Assessment of Liver Disease. All comers fulfilling the inclusion criteria have been examined and all examinations (questionnaires, physical examination, blood collection, and TE) were performed on the same day. Each participant completed standardized questionnaires (eg, demographic parameters or concomitant diseases). In all participants, the presence of a previously existing liver disease was excluded by a personal interview (eg, no established diagnosis of chronic liver disease, no repeatedly elevated liver enzymes, and no history of liver resection or liver transplant) as well as by physical examination. The only exception was known NAFLD without histologically proven nonalcoholic steatohepatitis as NAFLD is a widespread entity seen in a substantial part of Caucasian population and as steatosis is associated with Pi*ZZ-related liver disease.[1] For each participant, drinking habits were evaluated in a face-to-face conversation, determining the average weekly number of alcoholic beverages. Consequently, the amount of alcohol consumed per week was calculated and used for further analysis. Participants with excessive mean alcohol consumption (>40 g/d women, >60 g/d men) were excluded (5 Pi*ZZ, 4 Pi*MZ, 1 noncarrier). All participants underwent blood sampling. The obtained venous blood was centrifuged, aliquoted, and stored at −80oC. Laboratory workup was performed as described[1] to exclude liver comorbidities. It consisted of (1) a serologic search for chronic infections with hepatitis B and C virus (1 Pi*MZ and 2 Pi*ZZ individuals were excluded); (2) serum ferritin levels and transferrin saturation to screen for the presence of hereditary hemochromatosis (1 Pi*ZZ subject with an otherwise unexplained significant increase in both ferritin [>500 ng/mL] and transferrin saturation [>45%] was excluded); and (3) screen for autoimmune hepatitis in individuals with elevated serum transaminase levels (no exclusions).

TE was used as the most established method for noninvasive assessment of liver fibrosis and LSM using TE was recently validated in 2 biopsy-proven cohorts of adult Pi*ZZ individuals.[2,3] TE was performed using the M or XL probe following established recommendations.[4–6] Experienced examiners performed all TE measurements and only subjects with at least 10 successful measurements and an interquartile range ≤30% of the median LSM were considered for the analysis. Known confounders of reliable LSM were excluded in all participants. Among others, participants without the required fasting period were excluded (2 Pi*MZ, 2 Pi*ZZ, 3 noncarriers) as well as with serum ALT or AST activities >5 times the sex-specific ULN or ALP >2 times the sex-specific ULN at the time of recruitment (1 Pi*MZ, 2 Pi*ZZ, 2 noncarriers). CAP was evaluated as a surrogate for hepatic steatosis.[7] We made use of the TE cutoffs used in the hitherto largest Pi*ZZ cohort,[1] which are in line with etiology-unspecific recommendations.[4,7] For LSM, the cutoff of 7.1 kPa was considered suggestive of significant liver fibrosis (ie, fibrosis stage ≥2) and the cutoff of 10.0 kPa suggestive of advanced liver fibrosis (ie, fibrosis stage ≥3). For CAP, the cutoff of 248 dB/m was considered suggestive of mild steatosis (ie, steatosis grade ≥1) and the cutoff of 280 dB/m suggestive of severe steatosis (ie, steatosis grade =3).

Ethical Statement. Ethical approval was provided by the institutional review board of Aachen University (EK 173/15) as well as by the institutional ethics committees at each study center. All participants issued a written informed consent form and were treated following the ethical guidelines of the Helsinki Declaration (Hong Kong Amendment) as well as Good Clinical Practice (European guidelines). The study was listed at ClinicalTrials.gov (NCT029292940).

Analysis in the Population-based UK Biobank (Cohort 2)

The "UK biobank" (UKB) is a population-based study comprising 502,617 individuals from the general population whose genetic, demographic, and clinical data were collected between 2006 and 2010 in the United Kingdom. A total of 487,831 UKB participants were genotyped for both the Pi*Z and Pi*S mutation; 43,330 participants were excluded because of Pi*S carriage (either heterozygous/homozygous or compound heterozygosity with Pi*Z). Our study has been approved by the UKB Access Committee (Project #47527).

Histological Analysis of Liver Biopsies (Cohort 3)

Among the total of 84 Pi*MZ and 35 Pi*ZZ individuals, 36 Pi*MZ and all Pi*ZZ subjects were recruited as individuals with a known AAT deficiency genotype, who had a medical indication for liver biopsy (ie, elevated LSM ≥7.1 kPa or repeatedly elevated transaminases). Presence of a liver comorbidity except NAFLD was excluded as described for the prospectively recruited participants. The remaining 48 Pi*MZ subjects were gathered from cohorts of biopsied individuals with cryptogenic (n = 13) or NAFLD-related liver disease (n = 35). All individuals of these cohorts were genotyped for the Pi*S and Pi*Z variant and only Pi*MZ individuals were chosen for histologic analysis. The analyzed biopsies were from following centers: Aachen (20 Pi*MZ, 32 Pi*ZZ), Hannover (3 Pi*MZ), Munich (2 Pi*MZ), Salzburg (20 PiMZ, 2 Pi*ZZ), Vienna (13 Pi*MZ), Innsbruck (3 Pi*MZ), Newcastle (11 Pi*MZ), Madeira (1 Pi*ZZ), and Sydney (12 Pi*MZ). Only specimens with at least 10 portal triads were used for analysis (4 Pi*MZ excluded). For each specimen, the whole sample was analyzed.

Formalin-fixed and paraffin-embedded specimens were cut onto 5-μm-thick sections and stained with hematoxylin and eosin, PAS-D, and Sirius red staining. In addition, IHC labeling with a Pi*Z-specific antibody was carried out.[8] All specimens were assessed by a certified hepatopathologist (H.D.) in a blinded fashion. The presence of hepatic inflammation and liver fibrosis was quantified according to Kleiner's score.[9] Disease activity was further evaluated according to the NAFLD Activity Score (NAS).[9] The amount of

AAT inclusion bodies per field was assessed after IHC staining and was classified as described by Clark et al[2]: 0, none; 1, rare (<5 inclusions); 2, few (5–20 inclusions); 3, many (>20 inclusions).

Biochemical Analysis of Liver Biopsies (Cohort 4)

A total of 20 liver samples were studied (6 Pi*MZ, 7 Pi*ZZ, 7 noncarriers). Only 1 noncarrier had liver metastasis and this individual was neither part of the prospectively recruited cohort (no. 1) nor the biopsy cohort (no. 3). Only nontumor tissue was used for biochemical analysis. Written informed consent to use excess biopsy material for research purposes was obtained from all participants.

For total liver lysates, liver specimens were homogenized in 4× reducing Laemmli buffer (125 mM Tris-HCl [pH 6.8]; 4% [wt/vol] sodium dodecyl sulfate [SDS]; 20% [vol/vol] glycerol; 4% [vol/vol] β-Mercaptoethanol; 0.01% [wt/vol] bromophenol blue). To obtain soluble and insoluble proteins, liver tissues were homogenized in nonionic, 1% Triton-X containing buffer (5 mM EDTA/PBS, pH 7.4, supplemented with phosphatase and proteinase inhibitors) and centrifuged at 3174 G for 15 minutes at 4°C. Supernatant (soluble proteins) and pellet (insoluble proteins) were then supplemented with 4× reducing Laemmli buffer. The isolated proteins were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) followed by transfer to polyvinylidene difluoride membranes. The membranes were incubated with AAT (Sigma, A0409, St Louis, MO), keratin-8 (K8, Troma I, St Louis, MO), or glyceraldehyde-3-phosphate dehydrogenase (GAPDH; NovusBio, NB300–221, Nordenstadt, Germany) primary antibodies and species-specific horseradish peroxidase–coupled secondary antibodies (Invitrogen, Waltham, MA). The resulting signals were visualized by an enhanced chemiluminescence detection kit (GE Healthcare/Amersham Biosciences, Little Chalfont, UK) and images were taken with the luminescent image analyzer ImageQuant LAS4000 (GE Healthcare, Little Chalfont, UK).

Statistical Analysis

All categorical variables were described as absolute (n) and relative (%) frequencies and the corresponding contingency tables were analyzed with χ^2 tests. Continuous variables were displayed as stated (mainly mean ± standard deviation) and were analyzed by unpaired, 2-tailed *t*-tests as well as by a multivariable linear model to account for relevant confounders. Distributions among groups were assessed by univariable and forward-stepwise multiple logistic regression analyses to calculate ORs. ORs were presented with their corresponding 95% confidence intervals given in brackets. Multivariable logistic regression was performed to test for independent associations (including age, sex, BMI, diabetes, mean alcohol consumption, controlled attenuation parameter [CAP], and AAT serum level). Unless stated otherwise, the model adjusting for age, sex, BMI, diabetes, and mean alcohol consumption was used throughout the manuscript. Nominal *P* values were given for all statistical tests. Bonferroni's correction was used to account for multiple testing of covariates. Differences were considered to be statistically significant when $P < .05$. The data were analyzed using SPSS Statistics version 23 (IBM Corp, Armonk, NY) and Prism version 5 (GraphPad, LaJolla, CA).

Supplementary References

1. Hamesch K, Mandorfer M, Pereira VM, et al. Liver fibrosis and metabolic alterations in adults with alpha1 antitrypsin deficiency caused by the Pi*ZZ mutation. Gastroenterology 2019;157:705–719.e18.
2. 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;69:1357–1364.
3. 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;71:1272–1274.
4. Friedrich-Rust M, Poynard T, Castera L. Critical comparison of elastography methods to assess chronic liver disease. Nat Rev Gastroenterol Hepatol 2016;13:402–411.
5. Castera L, Foucher J, Bernard PH, et al. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. Hepatology 2010;51:828–835.
6. Tapper EB, Lok ASF. Use of Liver Imaging and Biopsy in Clinical Practice. N Engl J Med 2017;377:2296–2297.
7. **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;66:1022–1030.
8. Fu DA, Campbell-Thompson M. Immunohistochemistry staining for human alpha-1 antitrypsin. Methods Mol Biol 2017;1639:139–143.
9. Kleiner DE, Brunt EM, Van Natta M, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology 2005;41:1313–1321.
10. Friedrich-Rust M, Ong MF, Martens S, et al. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. Gastroenterology 2008;134:960–974.

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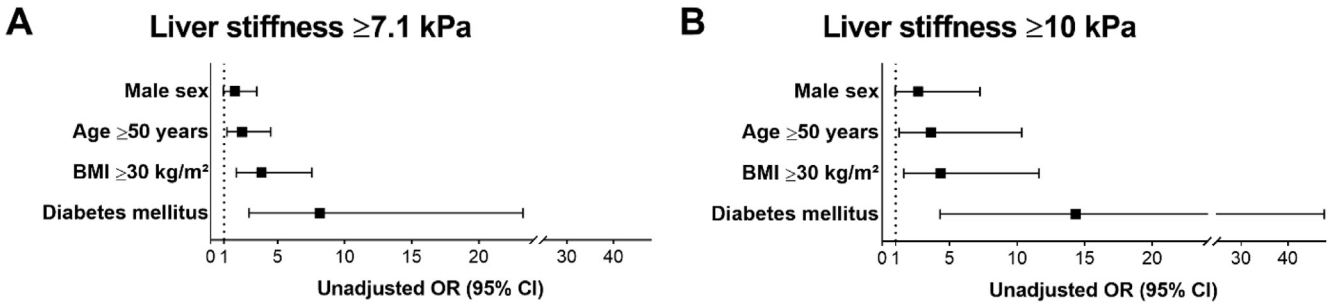
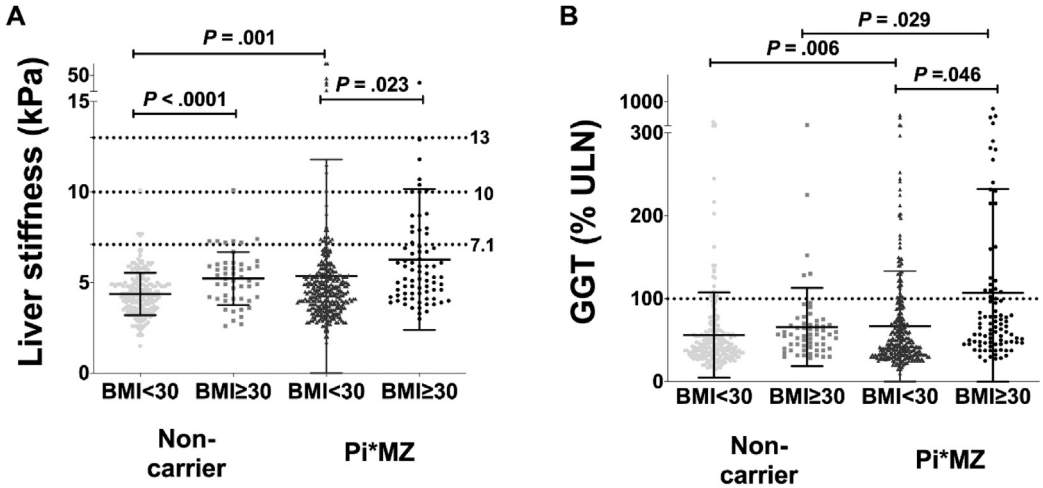
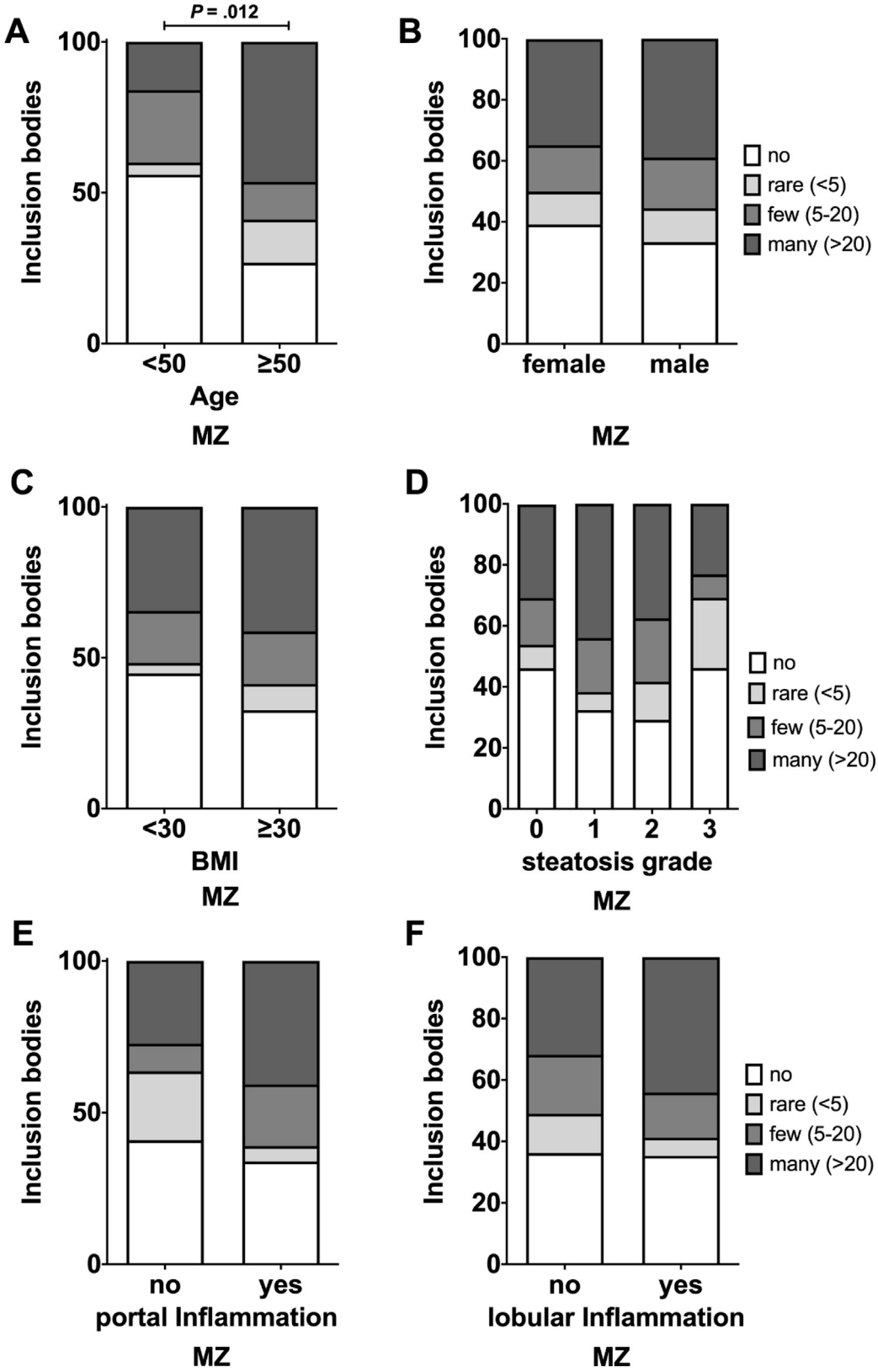


Supplementary Figure 1. Factors associated with liver stiffness measurements indicative of significant and advanced liver fibrosis in Pi*MZ subjects (Cohort 1). Unadjusted ORs with their corresponding 95% confidence intervals (CI) are shown for liver stiffness ≥7.1 kPa indicative of significant liver fibrosis (*A*) and liver stiffness ≥10 kPa indicative of advanced liver fibrosis (*B*).



Supplementary Figure 2. Liver stiffness and serum GGT in obese and nonobese Pi*MZ individuals compared with corresponding noncarriers (Cohort 1). (*A*) Scatter plot of liver stiffness measurements determined by transient elastography in obese and nonobese subgroups, stratified by BMI ≥30 kg/m^2, of Pi*MZ subjects and noncarriers. The *dotted lines* represent the following etiology-unspecific cutoff levels[1,10]: 7.1 kPa (suggestive of fibrosis stage ≥2), 10.0 kPa (suggestive of fibrosis stage ≥3), and 13.0 kPa (suggestive of fibrosis stage 4). (*B*) Scatter plots of serum GGT levels, normalized to the sex-specific ULN, marked with a *dotted line*. The shown multivariable *P* values were adjusted for age, sex, diabetes mellitus, and mean alcohol consumption.



Supplementary Figure 3. Comparison of inclusion bodies in Pi*MZ individuals with different clinical and histological features (Cohort 3). The abundance of inclusion bodies was scored, using IHC, as described[2]: no, no inclusions visible; rare, inclusions in up to 5 cells; few, inclusion bodies in 5–20 cells; and many, inclusions in more than 20 cells. Univariate *P* values are shown in all panels. (*A*) Amounts of inclusion bodies in Pi*MZ individuals with age $<$50 or age $\geq$50 years. (*B*) Amounts of inclusion bodies per field in female and male Pi*MZ individuals. (*C*) Amounts of inclusion bodies per field in Pi*MZ individuals with BMI $<$30 or $\geq$30 kg/m^2. (*D*) Amounts of inclusion bodies per field in Pi*MZ individuals distributed by using Kleiner's steatosis scoring system.[9] (*E*) Amounts of inclusion bodies per field in Pi*MZ individuals distributed by the presence of portal inflammation. (*F*) Amounts of inclusion bodies per field in Pi*MZ individuals distributed by the presence of portal inflammation.

Supplementary Table 1. Laboratory Parameters in Pi*MZ Individuals and Pi*ZZ Subjects Compared With Noncarriers (Cohort 1)

	Non-carriers *n*=284	Pi*MZ *n*=419	Pi*ZZ° *n*=309	*P* value *Pi*MZ vs noncarriers (univariable)*	*P* value *Pi*MZ vs noncarriers (multivariable)*	*P* value *Pi*MZ vs Pi*ZZ (univariable)*	*P* value *Pi*MZ vs Pi*ZZ (multivariable)*
Liver-related blood parameters							
ALT (% of ULN)	67±38	75±54	76±49	**.042**	.18	.78	.25
ALT ≥ULN (%)	11	16	18	.068	.25	.65	.20
AST (% of ULN)	63±23	67±37	76±39	.10	.27	**.002**	**.0003**
AST ≥ULN (%)	4	6	16	.29	.93	**<.0001**	**<.0001**
GGT (% of ULN)	59±52	75±84	114±131	**.005**	**.007**	**<.0001**	**.002**
GGT ≥ULN (%)[a]	10	18	33	**.002**	**.030**	**<.0001**	**.001**
ALP (% of ULN)	60±21	62±20	67±28	.13	**.004**	**.024**	.55
ALP ≥ULN (%)[b]	4	5	9	.51	**.012**	**.015**	.13
Bilirubin (% of ULN)	48±36	52±35	60±39	.18	.50	**.003**	.060
Bilirubin ≥ULN (%)	7	7	11	.81	.96	**.044**	.34
Platelets (G/L)	255±63	259±67	230±69	.51	.075	**<.0001**	**<.0001**
Platelets <150 G/L (%)	2	4	9	.080	.24	**.010**	.064
INR (units)[c]	0.97±0.10	1.01±0.17	1.04±0.18	**.001**	**.027**	.066	.18
Albumin (g/L)	4.7±0.5	4.6±0.4	4.4±0.5	.081	**.003**	**<.0001**	.29
Lipid metabolism							
Triglycerides (mg/dL)	126±71	123±90	103±56	.67	**.003**	**.002**	**.001**
Cholesterol (mg/dL)	217±47	208±49	209±42	**.031**	.24	.82	.19

Quantitative measures are expressed as means and standard deviations or as relative frequencies (%).
INR, international normalized ratio.
[a]Pi*MZ vs noncarriers: adjusted OR = 2.6 [1.1–6.0], Pi*MZ vs Pi*ZZ: adjusted OR = 0.4 [0.2–0.9].
[b]Pi*MZ vs noncarriers: adjusted OR = 5.1 [1.1–23.0], Pi*MZ vs Pi*ZZ: adjusted OR = 0.2 [0.1–0.6].
[c]Only patients not taking anticoagulant medication were analyzed. All multivariable analyses were adjusted for age, sex, BMI, presence of diabetes mellitus, and mean alcohol consumption. *P* values <.05 are indicated in bold font.

Supplementary Table 2. Characteristics of Pi*MZ Individuals Compared With Noncarriers and Pi*ZZ Subjects, All ≥50 Years Old (Cohort 1)

	Noncarriers, Age ≥50 *n*=171	Pi*MZ, Age ≥50 *n*=181	Pi*ZZ, Age ≥50 *n*=186	*P* value *Pi*MZ vs noncarriers (univariable)*	*P* value *Pi*MZ vs Pi*ZZ (univariable)*
Characteristics					
Age (y)	61±7	60±8	60±8	.12	.73
Women (%)	50	59	50	.076	.080
BMI (kg/m^2)	26.3±4.8	26.5±4.6	25.0±4.4	.74	**.002**
Mean alcohol consumption (g/d)	8.0±10.2	4.7±7.7	4.6±7.6	**.001**	.92
AAT serum level (mg/dL)	140±25	87±18	29±16	**<.0001**	**<.0001**
Modifiable risk factors					
BMI ≥30 kg/m^2 (%)	21	26	12	.25	**.001**
Diabetes mellitus (%)	6	7	4	.74	.20
Relevant alcohol intake[a] (%)	15	9	7	.13	.43
Liver status					
ALT (% of ULN)	65±38	75±40	76±49	**.016**	.82
ALT ≥ ULN (%)[b]	7	16	16	**.008**	.96
AST (% of ULN)	62±20	70±28	80±34	**.002**	**.004**
AST ≥ ULN (%)[c]	2	9	20	**.007**	**.007**
GGT (% of ULN)	62±52	83±95	122±147	**.011**	**.004**
GGT ≥ ULN (%)[d]	11	20	32	**.013**	**.013**
ALP (% of ULN)	62±21	67±24	68±26	**.024**	.75
ALP ≥ ULN (%)	4	6	12	.46	.066
Bilirubin (% of ULN)	47±28	52±28	61±40	.11	**.020**
Bilirubin ≥ ULN (%)	8	7	12	.82	.12
Platelets (G/L)	252±61	253±66	228±74	.91	**.001**
Platelets <150 G/L (%)	3	7	12	.10	.15
INR (units)[e]	0.97±0.12	1.02±0.24	1.06±0.23	**.027**	.23
Albumin (g/L)	4.7±0.5	4.5±0.4	4.4±0.5	**.014**	**.025**
Liver stiffness (kPa)	4.5±1.3	6.2±8.6	7.3±6.8	**.013**	.16
Liver stiffness ≥7.1 kPa (%)[f]	6	14	32	**.015**	**<.0001**
Liver stiffness ≥10.0 kPa (%)[g]	1	7	15	**.002**	**.024**
CAP (dB/m)	252±55	263±63	263±58	.096	.98
CAP ≥280 dB/m (%)[h]	32	42	35	**.045**	.19
Lipid metabolism					
Triglycerides (mg/dL)	129±69	136±89	111±65	.43	**.005**
Cholesterol (mg/dL)	227±48	2220±50	219±42	.18	.92

Quantitative measures are expressed as mean ± standard deviation or as relative frequency (%). Multivariable were analyses adjusted for age, BMI, presence of diabetes mellitus, and mean alcohol consumption.
INR, international normalized ratio.
[a]Alcohol intake > 12 g/d women, > 24 g/d men (Individuals with alcohol consumption > 40 g/d for females or > 60 g/d for males had been excluded a priori).
[b]Pi*MZ vs noncarriers: Unadjusted OR=2.5 [1.2–5.2].
[c]Pi*MZ vs noncarriers: Unadjusted OR=4.2 [1.4–12.9], Pi*MZ vs Pi*ZZ: Unadjusted OR =0.4 [0.2–0.8].
[d]Pi*MZ vs noncarriers: Unadjusted OR=2.1 [1.2–4.0], Pi*MZ vs Pi*ZZ: Unadjusted OR=0.5 [0.3–0.9].
[e]Only patients not taking anticoagulant medication were considered.
[f]Pi*MZ vs noncarriers: Unadjusted OR =2.4 [1.2–5.1], Pi*MZ vs Pi*ZZ: Unadjusted OR =0.4 [0.2–0.6].
[g]Pi*MZ vs noncarriers: Unadjusted OR =13.2 [1.7–101.7], Pi*MZ vs Pi*ZZ: Unadjusted OR=0.5 [0.2–0.9].
[h]Pi*MZ vs noncarriers: Unadjusted OR =1.6 [1.0–2.5].

Supplementary Table 3. Logistic Regression Models for Liver Stiffness as Noninvasive Surrogate of Liver Fibrosis in Pi*MZ Subjects vs Noncarriers (Cohort 1)

	Liver stiffness ≥7.1 kPa		Liver stiffness ≥10 kPa	
	Odds ratio (OR) [95% CI]	*P* value	Odds ratio (OR) [95% CI]	*P* value
Unadjusted	4.2 [1.8–9.8]	.001	9.0 [1.1–70.7]	.037
Adjusted for age and sex	5.3 [2.2–12.7]	.0002	10.4 [1.3–84.0]	.028
Adjusted for BMI, DM, and mean alcohol consumption	3.8 [1.6–9.0]	.003	8.1 [1.006–65.331]	.049
Adjusted for CAP	4.2 [1.8–9.6]	.001	8.9 [1.1–70.2]	.038
Adjusted for AAT serum level	5.9 [1.8–18.7]	.003	38.8 [3.0–503.1]	.005
Adjusted for age, sex, and CAP	5.0 [2.1–11.9]	.0003	9.6 [1.2–77.3]	.033
Adjusted for age, sex, BMI, DM, and mean alcohol consumption	4.8 [2.0–11.8]	.001	9.3 [1.1–77.2]	.040
Adjusted for all above	6.2 [1.7–23.2]	.007	64.1 [3.3–1256.6]	.006

"Unadjusted" represents the comparison between Pi*MZ individuals and noncarriers without accounting for the mentioned cofactors. The cutoff for liver stiffness measured by transient elastography (TE) was selected based on etiology-unspecific recommendations, ie, ≥7.1 kPa suggesting the presence of significant fibrosis (fibrosis stage ≥2) and ≥10 kPa suggesting the presence of advanced fibrosis (fibrosis stage ≥3).
CI, confidence interval; CAP, controlled attenuation parameter; DM, diabetes mellitus. *P* values <.05 are indicated in bold font.

Supplementary Table 4. Characteristics of Pi*ZZ Subjects and Pi*MZ Individuals, Both With LSM Suggesting Significant Liver Fibrosis (Cohort 1)

	Pi*ZZ, LSM ≥7.1 kPa n=78	Pi*MZ, LSM ≥7.1 kPa n=42	P value (univariable)
Characteristics			
Age (years)	55±14	51±15	.12
Women (%)	32	43	.24
BMI (kg/m^2)	26.1±5.9	28.3±4.6	**.046**
Mean alcohol consumption (g/d)	3.8±6.2	3.4±7.0	.78
Modifiable risk factors			
BMI ≥30 kg/m^2 (%)[a]	16	42	**.002**
Diabetes mellitus (%)[b]	3	17	**.016**
Relevant alcohol intake[c] (%)	6	3	.42
Liver status			
ALT (% of ULN)	100±75	90±56	.47
ALT ≥ULN (%)	36	20	.069
AST (% of ULN)	97±62	75±38	**.047**
AST ≥ULN (%)[d]	33	13	**.025**
GGT (% of ULN)	195±201	112±128	**.022**
GGT ≥ULN (%)[e]	55	33	**.029**
ALP (% of ULN)	74±35	71±34	.69
ALP ≥ULN (%)	18	5	.065
Bilirubin (% of ULN)	70±44	59±34	.19
Bilirubin ≥ULN (%)	17	10	.33
Platelet count (G/L)	192±64	239±68	**.001**
Platelets <150 G/L (%)	25	13	.12
INR (units)[f]	1.13±0.31	1.04±0.09	.16
Albumin (g/L)	4.3±0.5	4.6±0.4	**.012**
Liver stiffness (kPa)	13.2±11.6	14.7±16.0	.54
CAP (dB/m)	274±63	272±61	.88
CAP 280 dB/m (%)	48	53	.67
Lipid metabolism			
Triglycerides (mg/dL)	114±60	140±90	.075
Cholesterol (mg/dL)	213±50	206±61	.52

Quantitative measures are expressed as means and standard deviations or as relative frequencies (%).
INR, international normalized ratio.
[a]Unadjusted OR=0.3 [0.1–0.6].
[b]Unadjusted OR=0.2 [0.0–0.8].
[c]Alcohol intake >12 g/d women, >24 g/d men (Individuals with alcohol consumption >40 g/d for females or >60 g/d for males had been excluded a priori).
[d]Unadjusted OR=0.3 [0.1–0.9].
[e]Unadjusted OR=0.4 [0.2–0.9].
[f]Only patients not taking anticoagulant medication were considered. *P* values <.05 are indicated in bold font.

Supplementary Table 5. Characteristics of Pi*MZ Subjects With and Without LSM Suggesting Significant Liver Fibrosis (Cohort 1)

	Pi*MZ, LSM <7.1 kPa *n*=377	Pi*MZ, LSM ≥7.1 kPa *n*=42	*P* value (univariable)
Characteristics			
Age (years)[a]	44±16	51±15	**.013**
Women (%)	58	43	.069
BMI (kg/m^2)[b]	25.7±4.6	28.3±4.6	**.001**
Mean alcohol consumption (g/d)	4.5±6.7	3.4±7.0	.36
AAT serum level (mg/dL)	87±20	90±19	.49
Modifiable risk factors			
BMI ≥30 kg/m^2 (%)[c]	16	42	**<.0001**
Diabetes mellitus (%)[d]	3	17	**<.0001**
Relevant alcohol intake[e] (%)	7	3	.30
Liver status			
Liver stiffness (kPa)	4.5±1.1	14.7±16.0	**<.0001**
CAP (dB/m)[f]	242±60	272±61	**.004**
CAP ≥248 dB/m (%)[g]	46	72	**.003**
CAP ≥280 dB/m (%)[h]	27	53	**.001**
ALT (% of ULN)	73±54	90±56	.065
ALT ≥ULN (%)	16	20	.49
AST (% of ULN)	66±36	75±38	.13
AST ≥ULN (%)[h]	5	13	**.044**
GGT (% of ULN)	71±78	112±128	**.004**
GGT ≥ULN (%)[i]	16	33	**.009**
ALP (% of ULN)	61±18	71±34	**.004**
ALP ≥ULN (%)	4	5	. 72
Bilirubin (% of ULN)	51±35	59±34	.14
Bilirubin ≥ULN (%)	6	10	.36
Platelet count (G/L)	261±64	239±88	.059
Platelets <150 G/L (%)[j]	3	13	**.006**
INR (units)[k]	1.01±0.18	1.04±0.09	.30
Albumin (g/L)	4.6±0.4	4.6±0.4	.41
Lipid metabolism			
Triglycerides (mg/dL)	121±90	140±89	.21
Cholesterol (mg/dL)	209±48	206±61	.77

Quantitative measures are expressed as means and standard deviations or as relative frequencies (%).
INR, international normalized ratio.
[a]Unadjusted OR=1.026 [1.005–1.047].
[b]Unadjusted OR=1.113 [1.044–1.186].
[c]Unadjusted OR=3.8 [1.9–7.5].
[d]Unadjusted OR=8.1 [2.9–23.2].
[e]Alcohol intake >12 g/d women, >24 g/d men (Individuals with alcohol consumption >40 g/d for females or >60 g/d for males had been excluded a priori).
[f]Unadjusted OR=1.009 [1.003–1.015].
[g]Unadjusted OR=3.0 [1.5–6.5].
[h]Unadjusted OR=2.84 [0.99–8.17].
[i]Unadjusted OR=2.6 [1.2–5.3].
[j]Unadjusted OR=4.2 [1.4–12.6].
[k]Only patients not taking anticoagulant medication were considered. *P* values <.05 are indicated in bold font.

Supplementary Table 6. Characteristics of Pi*MZ Individuals, Stratified by Elevated Serum GGT (Cohort 1)

	Pi*MZ, GGT <ULN *n*=328	Pi*MZ, GGT ≥ULN *n*=72	*P* value *(univariable)*	*P* value *(multivariable)*
Characteristics				
Age (years)	44±16	47±14	.11	
Women (%)	58	51	.33	
BMI (kg/m^2)	25.7±4.6	27.0±4.4	**.036**	
Mean alcohol consumption (g/d)	4.1±5.7	5.9±10.3	.053	
AAT serum level (mg/dL)	87±19	88±25	.91	.71
Modifiable risk factors				
BMI ≥30 kg/m^2 (%)	21	34	**.016**	
Diabetes mellitus (%)	3	7	.12	
Relevant alcohol intake[a] (%)	6	10	.22	
Liver status				
ALT (% of ULN)	64±28	121±98	**<.0001**	**<.0001**
ALT ≥ ULN (%)[b]	9	47	**<.0001**	**<.0001**
AST (% of ULN)	62±21	86±69	**<.0001**	**<.0001**
AST ≥ ULN (%)[c]	3	18	**<.0001**	**<.0001**
GGT (% of ULN)	47±20	203±134	**<.0001**	**<.0001**
ALP (% of ULN)	59±17	75±29	**<.0001**	**<.0001**
ALP ≥ ULN (%)[d]	3	15	**<.0001**	**<.0001**
Bilirubin (% of ULN)	51±35	75±29	.58	.54
Bilirubin ≥ ULN (%)	7	7	.82	.78
Platelets (G/L)	260±68	252±64	.32	.50
Platelets <150 G/L (%)	4	6	.45	.50
INR (units)[e]	1.01±0.18	0.99±0.12	.44	.36
Albumin (g/L)	4.6±0.4	4.6±0.5	.68	.49
Liver stiffness (kPa)[f]	4.8±2.2	8.5±13.2	**<.0001**	**<.0001**
Liver stiffness ≥7.1 kPa (%)[g]	8	18	**.009**	.060
Liver stiffness ≥10.0 kPa (%)[h]	2	13	**<.0001**	**.001**
CAP (dB/m)[i]	240±59	263±61	**.005**	.11
CAP ≥248 dB/m (%)[j]	44	69	**.0002**	**.005**
CAP ≥280 dB/m (%)[k]	25	47	**.0002**	**.008**
Lipid metabolism				
Triglycerides (mg/dL)[l]	114±76	162±130	**<.0001**	**.0004**
Cholesterol (mg/dL)	206±48	219±53	.057	.069

Quantitative measures are expressed as mean ± standard deviation or as relative frequency (%). Multivariable analyses were adjusted for age, BMI, presence of diabetes mellitus, mean alcohol consumption, and controlled attenuation parameter (CAP). INR, international normalized ratio.

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

[b]Adjusted OR=10.6 [5.5–20.6].

[c]Adjusted OR=7.2 [2.8–18.6].

[d]Adjusted OR=7.4 [2.5–21.5].

[e]Only patients not taking anticoagulant medication were considered.

[f]Adjusted OR=1.09 [1.01–1.17].

[g]Adjusted OR=1.8 [0.8–4.1].

[h]Adjusted OR=4.1 [1.3–13.3].

[i]Adjusted OR=1.004 [0.999–1.010].

[j]Adjusted OR=2.5 [1.3–4.7].

[k]Adjusted OR=2.2 [1.2–4.4].

[l]Adjusted OR=1.004 [1.001–1.007]. *P* values <.05 are indicated in bold font.

Supplementary Table 7. Logistic Regression Models for Elevated Serum GGT in Pi*MZ Subjects vs Noncarriers (Cohort 1)

	GGT ≥ULN	
	Odds ratio (OR) [95% CI]	*P* value
Unadjusted	2.3 [1.3–4.0]	.004
Adjusted for age and sex	2.6 [1.4–4.5]	.001
Adjusted for BMI, DM, and mean alcohol consumption	2.4 [1.3–4.2]	.003
Adjusted for CAP	2.3 [1.3–4.0]	.004
Adjusted for AAT serum level	2.5 [1.1–5.7]	.025
Adjusted for age, sex, and CAP	2.5 [1.4–4.4]	.002
Adjusted for age, sex, BMI, DM, and mean alcohol consumption	2.6 [1.4–4.7]	.002
Adjusted for all above	2.9 [1.2–6.8]	.018

"Unadjusted" represents the comparison between Pi*MZ individuals and noncarriers without accounting for the mentioned cofactors. The sex-specific ULN was used.
CI, confidence interval; CAP, controlled attenuation parameter; DM, diabetes mellitus. *P* values <.05 are indicated in bold font.

Supplementary Table 8. Characteristics of Pi*MZ Individuals in the Europe-wide Registry (Cohort 1) and Those Who Received Liver Biopsy (Cohort 2)

	Pi*MZ, registry (cohort 1) *n*=419	Pi*MZ, liver biopsy (cohort 3) *n*=84	*P* value *(univariable)*
Characteristics			
Age (years)	45±16	55±16	**<.0001**
Women (%)	56	44	**<.0001**
BMI (kg/m^2)	26.0±4.6	31.4 ± 6.5	**<.0001**
Mean alcohol consumption (g/d)	4.4±.6.8	5.1 ± 10.3	.45
Modifiable risk factors			
BMI ≥30 kg/m^2 (%)	18	61	**<.0001**
Diabetes mellitus (%)	4	31	**<.0001**
Relevant alcohol intake[a] (%)	7	5	.14

Quantitative measures are expressed as mean ± standard deviation (except liver stiffness: median and interquartile range) or as relative frequency (%).
[a]Alcohol intake >12 g/d women, >24 g/d men (individuals with alcohol consumption >40 g/d for females or >60 g/d for males had been excluded a priori). *P* values <.05 are indicated in bold font.