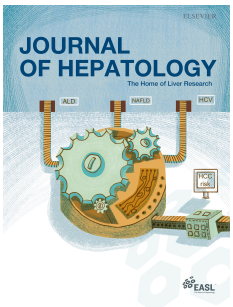


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# Alpha-1 antitrypsin deficiency: a re-surfacing adult liver disorder

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**Summary**

Alpha-1 antitrypsin deficiency (AATD) arises from mutations in the *SERPINA1* gene encoding alpha-1 antitrypsin (AAT) that lead to AAT retention in the endoplasmic reticulum of hepatocytes, causing proteotoxic liver injury and loss-of-function lung disease. The homozygous Pi*Z mutation (Pi*ZZ genotype) is responsible for the majority of severe AATD cases and can precipitate both pediatric and adult liver diseases, while the heterozygous Pi*Z mutation (Pi*MZ genotype) is an established modifier of liver disease. We review genotype-related hepatic phenotypes/disease predispositions. We also describe the mechanisms and factors promoting the development of liver disease, as well as approaches to evaluate the extent of liver fibrosis. We discuss the emerging therapeutic approaches, diagnosis, and clinical management of this neglected disorder.

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

**Key points**

- Alpha-1 antitrypsin (AAT) is a secreted protein produced primarily in hepatocytes.
- Inherited variants of the *SERPINA1* gene encoding AAT may impair AAT secretion and give rise to alpha-1 antitrypsin deficiency (AATD).
- AATD presents with liver disease due to proteotoxicity of the mutant AAT and lung emphysema due to a loss-of-function mechanism.
- While >100 *SERPINA1* variants have been reported, the heterozygous and homozygous Pi*Z variants (Pi*MZ/Pi*ZZ) are the most clinically relevant genotypes.
- Weekly intravenous augmentation therapy with AAT has clinical efficacy in Pi*ZZ individuals with lung involvement, and its immunomodulatory effects might also be effective in other disorders.
- Pi*MZ predisposes individuals with cystic fibrosis and alcoholic and metabolic-associated fatty liver disease to the development of advanced liver disease.
- Pi*ZZ causes neonatal hepatitis and liver cirrhosis in adults.
- While no treatment is yet available for AATD-related liver disease, siRNA application has yielded encouraging results in early phase 2 clinical trials.

## Background

Alpha-1 antitrypsin deficiency (AATD) is one of the most common potentially life-threatening genetic disorders. It predisposes patients to lung and liver damage, and both organs constitute the most prevalent causes of AATD-related mortality.[1] AATD arises from mutation-based misfolding of the anti-protease alpha-1 antitrypsin (AAT), which is primarily expressed in hepatocytes and then secreted into the bloodstream to protect the lungs from proteolytic degradation by neutrophil elastase. The mutations lead to enhanced protein degradation and/or aggregation in the endoplasmic reticulum (ER) of hepatocytes, thereby causing proteotoxic liver stress and damage (Figure 1). Proteolytic lung damage results from decreased systemic AAT levels and the resulting insufficient inhibition of proteases (Figure 1).[2, 3] Clinically, AATD manifests as early-onset panlobular lung emphysema and/or chronic obstructive pulmonary disease whose development might be slowed down by AAT augmentation therapy.[1, 4] Lung affection represents the leading cause of death in severe AATD, and lung destruction progresses more rapidly in smokers.[5] Given the decreased secretion of AAT into the bloodstream in individuals with AATD, measurement of serum levels of AAT constitutes a cost-effective screening assay, and the diagnosis is further established by genetic analysis, which might be complemented with AAT protein phenotyping.[1, 6]

More than 100 variants of *SERPINA1*, the gene encoding AAT, have been described. They are grouped based on the migration of mutant AAT in the electrical field.[7, 8] For example, Pi*M indicates the medium (i.e., normal) velocity of the wild-type allele. Pi*Z (rs28929474) and Pi*S (rs17580) are the most clinically relevant variants and display very slow or slow movement, respectively (Table 1).[1, 9] The rare Pi*F isoform exhibits fast migration, while the Pi*Q0 alleles constitute a heterogeneous group of variants that yield no detectable protein in serum (Table 1).[10-12] Finally, some variants, such as Pi*$M_{malton}$ or Pi*$M_{procida}$, produce functionally deficient AAT with migration similar to the wild-type isoform (Table 1).[13]

Classic severe AATD (genotype Pi*ZZ) is caused by the homozygous Pi*Z variant affecting approximately 1:2000 individuals of European descent (Figure 2).[14-16] It is characterized by markedly decreased concentrations of serum AAT that confer a strong predisposition to lung disease (Table 2).[1] In contrast, the heterozygous genotype Pi*MZ can be found in 1:50 Caucasians and results in normal/slightly decreased AAT serum concentrations (Table 2, Figure 2). It predisposes individuals to lung emphysema when additional risk factors, such as smoking, are present.[1] The compound heterozygous genotype Pi*SZ arises from the simultaneous presence of both the Pi*Z and the Pi*S variants *in trans*. It affects 1:500

Caucasians, displays intermediate AAT serum concentrations, and moderately increases susceptibility to lung disease (Table 2, Figure 2).[17, 18] The Pi*SS genotype (i.e., homozygous Pi*S allele) is approximately as common as Pi*SZ but does not seem to constitute a clinically relevant risk factor for the development of liver disease.[18]

**Mechanisms of AATD-related liver injury**

The severe homozygous Pi*Z variant leads to an ~85% decrease in the amount of secreted protein and an accumulation of the Z-AAT protein at the production site (i.e., the ER).[19, 20] Seventy percent of the protein is degraded, and 15% of the protein forms insoluble polymers. While ER-associated degradation (ERAD) disposes monomeric Z-AAT,[21] autophagy is the primary degradation machinery for Z-AAT polymers (Figure 1).[22] Polymerized AAT is rather indistinct in routine hematoxylin and eosin sections but yields characteristic purple, roundish inclusions after periodic acid-Schiff-diastase (PAS-D) staining (Figure 3).[7] Immunohistochemistry with an anti-Pi*Z antibody constitutes the most sensitive method to visualize Pi*Z accumulation (Figure 3).

Several tools have been employed to delineate the consequences of Pi*Z-related liver injury. Among them, mice transgenic for the human Pi*Z variant (termed Pi*Z mice) develop chronic proteotoxic liver injury with characteristic PAS-D-positive globules but do not display a lung phenotype due to the unaffected synthesis of the wild-type murine AAT. Notably, mice with a quintuple deletion of endogenous AAT alleles (in contrast to humans, mice have multiple genes encoding AAT) have recently become available and may constitute the more relevant genetic model for lung disease but not for liver disease.[23] Regardless, Pi*Z mice have yielded several important insights into AATD liver disease. For example, enhancement of autophagic degradation, either via gene transfer of the master regulator TFEB or via autophagy-enhancing drugs (such as carbamazepine or rapamycin), diminished Z-AAT accumulation and ameliorated liver injury (Figure 1).[24-26] The mouse model was also instrumental in determining the extent of ER-related stress. Although no activation of the unfolded stress response was detected in cells expressing Z-AAT, an ER overload response was observed.[27] Among the ER stress-related pathways, NF-kB activation constitutes a protective response promoting protein degradation.[28] On the other hand, JNK phosphorylation and increased expression of the proapoptotic protein CHOP accelerated cell death and the development of liver injury by transcriptional upregulation of *SERPINA1*, resulting in an overload of proteotoxic Z-AAT.[29,

[30] JNK activation also activated forkhead box O3 (FOXO3) and upregulated microRNA-34b/c (miR-34b/c) expression. Notably, miR-34b/c reduced profibrotic signaling of platelet-derived growth factor (PDGF), and its deletion in Pi*Z mice resulted in the early development of liver fibrosis.[31] Moreover, mitochondrial injury is another well-established consequence of Z-AAT accumulation observed both in Pi*Z mice and in human liver specimens.[32] Finally, the mouse model was instrumental to investigate the role of cofactors in the modulation of liver disease. In that respect, overexpression of hepatitis B surface protein, as seen in individuals with chronic hepatitis B infection, was found to aggravate liver injury, fibrosis, and the frequency of hepatocellular carcinoma in Pi*Z mice,[33] while the mild iron accumulation seen in homeostatic iron regulator (*Hfe*) gene knockout animals did not have a significant effect.[34] Notably, administration of the nonsteroidal anti-inflammatory drug indomethacin increased liver damage via activation of the IL-6-STAT-3 pathway in Pi*Z mice.[35] Finally, Pi*Z mice are used to evaluate the efficacy of new treatment strategies. For example, the administration of antisense oligonucleotides or RNA interference effectively decreased hepatic Pi*Z accumulation and ameliorated/reversed the development of the associated disease.[36]

In addition to transgenic animals, several cellular models are available. Patient-derived induced pluripotent stem cells (iPSCs) have attracted significant interest, since they can be differentiated into hepatocyte-like cells and might reflect interindividual variability in the expression of liver disease.[37-39] Activation of the unfolded protein response and inflammatory networks were the primary alterations seen in the Pi*ZZ-derived cell lines.[40] Although limited by the availability of human tissues, the generation of liver organoids from Lgr5+ stem cells constitutes another attractive approach.[41] The first published data are encouraging and point toward a decreased production of hepatocyte-specific genes in Pi*ZZ organoids.[42] This is well in line with transgenic animal data showing downregulated hepatocyte nuclear factor-4 alpha-related signaling resulting in loss of liver zonation.[43] Finally, several cell lines overexpressing Pi*Z or other *SERPINA1* variants have been developed. Their advantage is that they are relatively easy to handle. However, they are not well suited to study processes that occur in terminally differentiated hepatocytes. On the other hand, they are useful for dissecting basic molecular mechanisms. For example, they were instrumental in the identification of a potentially novel degradative process based on the delivery of polymerized Z-AAT directly to the lysosome.[44] Moreover, they yielded important insights into the handling of mutated AAT by ERAD. This is facilitated by ER mannosidase I, which removes the protein from the calnexin-calreticulin refolding machinery.[45, 46] In subsequent steps, misfolded AAT is stabilized via

interaction with chaperones and lectins, such as OS9, ERLEC1, or GRP94, and translocated in the cytosol to be ultimately degraded by the proteasome.[21, 47] However, these data, as well as other data generated in cell lines, await validation in animal models or human samples. On the other hand, several human observations need to be further dissected in experimental models. These include an alteration in lipid metabolism with more pronounced liver steatosis and decreased levels of serum triglycerides, as well as identification of extracellular vesicles with profibrogenic cargo in sera from Pi*ZZ individuals.[15, 48]

**Pediatric liver disease in AATD**

Although this review is focused on liver disease in adults, it needs to be highlighted that AATD also causes pediatric liver injury, typically in the form of neonatal cholestasis.[49] The strongest evidence stems from the Swedish neonatal screening program that identified 120 newborns with Pi*ZZ out of 200,000 newborns, while a variety of other reports relied on data from tertiary centers.[50-52] In the population-based cohort, 12% of Pi*ZZ neonates displayed prolonged jaundice, and 8% of these neonates had severe liver disease.[50] Although biochemical abnormalities were seen in >50% of the Pi*ZZ neonates identified by newborn screening, the values often normalized during follow-up.[51] As a result, only 2-3% of Pi*ZZ children develop end-stage liver disease that may require liver transplantation, and at age 18, only 12% of them display elevated ALT or GGT.[49, 51, 53] Interestingly, increased CHOP, which upregulates *SERPINA1* transcription, was detected in diseased livers of Pi*ZZ children but not in adults, suggesting that CHOP plays an important role in hepatic disease by increasing the burden of proteotoxic Z-AAT, particularly in the pediatric population.[30] Notably, a clinically significant pediatric liver disease in less severe genotypes, such as Pi*MZ or Pi*SZ, is exceedingly rare and might be at least in part caused by additional comorbidities, such as cystic fibrosis.[49]

**Novel insights into adult AATD-associated liver disease**

The recent gain in knowledge about AATD-related adult liver disease largely comes from three independent sources. First, the United Kingdom Biobank (UKB) constitutes a population-based cohort study comprising approximately 500,000 individuals with available AATD genotyping and clinical data. The recruited volunteers gave informed consent for data linkage to medical reports, which allowed us to collect the available ICD codes. After excluding individuals with pathological alcohol consumption and viral

hepatitis, the cohort was left with >17,000 Pi*MZ, >800 Pi*SZ, and ~140 Pi*ZZ individuals.[18] Although the lack of a detailed assessment of liver fibrosis is a limitation of this cohort, the population-based recruitment of participants independently of their AATD genotype constitutes a major advantage, given that most individuals with AATD remain undiagnosed their whole life.[1] Second, the EASL AATD consortium recruited >400 Pi*MZ individuals, ~240 Pi*SZ individuals, and nearly 600 Pi*ZZ study participants from fourteen European countries, the US, and Australia. All individuals underwent standard serological testing and noninvasive assessment of liver fibrosis by transient elastography. While this cohort possesses only a limited number of liver biopsies,[15, 54, 55] it includes systematic biobanking of blood samples, thus making a valuable resource for ancillary studies. The third cohort from North America included 94 Pi*ZZ individuals evaluated by routine laboratory tests, noninvasive assessment of liver fibrosis in most participants, and liver biopsies.[56]

**Liver enzymes in AATD – as seen on a population-basis**

The UKB constitutes the largest resource for the assessment of routine liver function tests. Mean alanine aminotransferase (ALT) values were significantly higher in all analyzed AATD genotypes as compared to those in noncarriers, but differences between the genotypes were modest. On the other hand, mean aspartate aminotransferase (AST) concentrations were clearly the highest in Pi*ZZ individuals, but they were also elevated in Pi*MZ and Pi*SZ subjects (Table 2). Gamma-glutamyl transferase (GGT) concentrations were comparable in noncarriers and Pi*MZ, Pi*SS, and Pi*SZ individuals. Although Pi*ZZ participants presented more often with elevated serum GGT levels compared to noncarriers, the differences were only borderline significant (Table 2).[18] Notably, serum GGT elevation may reflect metabolic alterations or the predominant involvement of the periportal area in AATD liver disease.[15, 56] Compared to the UKB, elevated liver function tests were found to be more frequent in the participants of the EASL AATD consortium, likely because UKB was a cohort of healthy subjects.[18] Regardless, the available studies clearly demonstrate that only a minority of individuals with AATD display liver enzymes outside the normal range. Elevated serum liver enzyme concentrations are uncommon, even in Pi*ZZ subjects, the most severe common AATD genotype. Consequently, elevated liver enzymes in patients with AATD should always trigger a thorough diagnostic workup.

**ICD-10 diagnoses of liver fibrosis/cirrhosis and primary malignant liver neoplasm in AATD**

Data linkage to UKB participants' medical reports allowed the evaluation of the prevalence of liver-related ICD-10 codes. It demonstrated a strong predisposition of Pi*ZZ individuals for liver fibrosis/cirrhosis and primary malignant neoplasms of the liver.[18] The risk of liver fibrosis/cirrhosis in Pi*ZZ subjects was 20 times higher than that in noncarriers (Figure 2), which is well in line with the liver stiffness measurements from the EASL AATD consortium, as well as the increased risk of individuals with AATD requiring liver transplantation.[18, 57] In contrast, only very limited data on the susceptibility of Pi*ZZ individuals to liver tumors are available. While the UKB analysis indicated an odds ratio >40 times elevated, further studies are needed to clarify this liver cancer risk in Pi*ZZ individuals.[18] Pi*SZ individuals harbored a three times higher risk for liver fibrosis/cirrhosis, while the risk for liver cancer was seven times higher (Figure 2). Pi*MZ individuals displayed a slightly increased odds ratio of 1.7 for liver fibrosis and a similar risk for liver-related mortality (Figure 2), [18, 58] whereas their risk of liver cancer was not significantly increased. Notably, the risk of liver fibrosis/cirrhosis and/or a liver tumor in Pi*SZ carriers was comparable to that in individuals homozygous for the *PNPLA3* (rs738409) and *TM6SF2* (rs5854926) single nucleotide polymorphisms, while the risks in Pi*ZZ subjects clearly exceeded the risks reported for these well-established disease modifiers.[18] Given the high risk for the development of liver cancer, we advocate regular hepatic ultrasound follow-ups for both Pi*ZZ and Pi*SZ individuals with significant liver fibrosis. However, larger prospective studies are needed to justify this approach.

**Evaluation of liver fibrosis and pathology in individuals with AATD**

Although liver biopsy remains the gold standard for the evaluation of liver fibrosis, pathology data on subjects with AATD are limited.[59] Clark et al. examined 94 adult Pi*ZZ biopsies, and the data revealed a prevalence of 35.1% for clinically significant liver fibrosis (i.e., fibrosis grade $F \geq 2$) in Pi*ZZ individuals.[56] When established clinical routine PAS-D staining was used, characteristic roundish AAT inclusions were seen in 95% of Pi*ZZ participants (Figure 3). Notably, they became more abundant at advanced liver fibrosis stages.[55] Similar findings were made by the EASL AATD consortium, which also showed higher sensitivity of the immunohistochemistry staining with the Pi*Z-specific antibody compared to PAS-D (Figure 3). Only 40% of Pi*MZ individuals harbor Z-AAT inclusions by PAS-D vs. 63% by immunohistochemistry with an antibody specific for polymeric Z-AAT.[55, 60] These data demonstrate that

the presence/absence of inclusion bodies by staining cannot be used to diagnose/rule out AATD. While the average number of AAT inclusions was much lower in Pi*MZ livers than in Pi*ZZ livers, the abundance of AAT inclusions in subjects with the same genotype increased with fibrosis stage. This suggests that individuals with advanced liver fibrosis might have an impaired ability to degrade misfolded Z-AAT, which may lead to a vicious cycle of protein accumulation and liver injury.

Liver stiffness measurement (LSM) by transient elastography was the most precise method for the recognition of advanced liver fibrosis (i.e., fibrosis grade F ≥ 3).[56, 61] In contrast, there is no optimal tool for the detection of intermediate liver fibrosis, and multiple approaches (serum GGT, serum ALT, LSM by transient elastography, and AST-to-platelet ratio index [APRI], which is a serum-based fibrosis predictor) yielded only modest results.[56] Although there are multiple other methods to noninvasively investigate liver fibrosis,[1] their sensitivity and specificity remain to be validated against liver biopsies. As with other liver diseases, magnetic resonance elastography might provide an accurate assessment, but current evidence is very limited.[62]

**Phenotype of Pi*ZZ and Pi*SZ individuals**

Both the EASL AATD consortium and the biopsy study by Clark et al. demonstrated a high liver fibrosis burden in Pi*ZZ individuals and identified male sex, age ≥50 years, presence of metabolic syndrome, and increased liver enzyme levels as risk factors for clinically significant liver fibrosis (i.e., fibrosis stage ≥2 on an F0-F4 scale) (Figure 2).[15, 56] Notably, Pi*ZZ individuals might be susceptible to the development of liver steatosis, and an impaired ability to secrete lipids might be responsible for this defect.[15] On the other hand, no correlation between the extent of lung and liver fibrosis was observed.[15] Importantly, significant or even advanced liver fibrosis was sometimes seen even in individuals with relatively low LSM scores. These data need to be considered when counseling patients, and based on that evidence, we advocate a low threshold for performing liver biopsy, particularly in young individuals with repeatedly elevated liver enzyme levels.

Compared to Pi*ZZ subjects, Pi*SZ individuals seem to display a substantially lower liver fibrosis burden,[18] which is consistent with their moderate risk for AATD-related lung disease.[63] However, factors promoting the development of liver fibrosis are similar between Pi*ZZ and Pi*SZ individuals (i.e., male sex, age ≥50 years, obesity, and the presence of diabetes mellitus) (Figure 2).[18]

**Heterozygous carriage of the Pi*Z variant (Pi*MZ genotype) as a disease modifier**

Unlike the Pi*ZZ genotype, Pi*MZ status is a risk factor rather than a disease-causing agent, and "second hits" are necessary to induce disease.[55] In line with that theory, Pi*MZ subjects in the UKB displayed only a 1.7 times increase in liver-related mortality.[58] However, the simultaneous presence of other liver disorders or risk factors substantially increased their liver-related risks. This has been particularly clearly demonstrated in subjects with cystic fibrosis and alcoholic/nonalcoholic fatty liver disease (ALD/NAFLD), in whom heterozygous Pi*Z carriage markedly increased the risk of liver cirrhosis.[54, 64] A large genome-wide association study supported this finding by showing that the odds ratio of the Pi*Z variant to display ALD-/NAFLD-related cirrhosis surpasses the risk conferred by other established genetic modifiers of liver disease, such as *PNPLA3* p. Ile148Met (rs738409), *HSD17B13*:T (rs72613567), and *TM6SF2* p. Glu167Lys (rs5854926).[65] The fibrosis-promoting effect of Pi*Z in cystic fibrosis might be related to the predisposition to gallstone formation of the Pi*Z variant, because cystic fibrosis can cause cholestatic liver disease.[1] The other risk factors seem to be shared with other Pi*ZZ and Pi*SZ individuals (i.e., obesity and diabetes mellitus were the strongest modifiers, and age ≥50 years was a weak risk factor) (Figure 2).[55] These data are not surprising, since obesity is known to amplify the profibrogenic effect of genetic variants.[66, 67] In AATD, both obesity and diabetes may aggravate the occurrence of ER stress and are associated with increased oxidative stress and lipolysis.[68, 69] The greater risk of older individuals is also not surprising, since they are exposed to the inherited condition for a longer time. Moreover, aging may impair the efficiency of the degradation of misfolded proteins.[55, 70] While it was clearly shown to predispose individuals to ALD-/NAFLD-related fibrosis, the contribution of the Pi*MZ status to other liver disorders, such as hemochromatosis or viral hepatitis, remains unclear (Table 3).

**Therapeutic options for AATD-related liver disease**

For the treatment of AATD-related lung disease, intravenous augmentation therapy with plasma-purified AAT was approved by the Food and Drug Association in 1987 as the first disease-specific therapy for AATD.[1] The RAPID trial, the first randomized, placebo-controlled trial of AAT augmentation therapy, later showed a significant reduction in the annual rate of lung density loss as a surrogate for lung emphysema

by AAT augmentation. However, it did not show a significant effect on quality of life, FEV1 values, or exacerbations of chronic obstructive pulmonary disease.[71, 72]

While AAT augmentation therapy constitutes a disease-specific treatment of AATD-related lung disease, liver transplantation represents the only available relief for severe AATD-related liver disease.[1, 71, 72] Although data are limited, the existing evidence demonstrates excellent survival and rapid normalization of serum AAT concentrations in both adults and children following liver transplantation.[73-75] Notably, since individuals with AATD who have liver cirrhosis may rapidly decompensate,[76] evaluation for liver transplantation should be considered in the early stages. In line with the genotype-associated susceptibilities described above, only <10% of Pi*ZZ individuals undergoing liver transplantation had an additional liver disorder, compared to 40% and 90% of transplanted Pi*SZ and Pi*MZ subjects, respectively.[74] Although the implantation of a liver without AATD is thought to "cure" the disease, a decline in lung function was seen in some liver transplanted individuals.[74] Nevertheless, the risk of lung-related death seems to be lower in carefully selected individuals.[74]

Several approaches addressing AATD-related liver disease have yielded promising results in preclinical studies, and some of them have already been translated into clinical trials (Figure 1).[77] In an important proof-of-concept study, Burrows et al. demonstrated the ability of chemical chaperones to increase the secretion of mutant AAT.[78] Similarly, small peptides or intrabodies have been shown to block AAT polymerization.[79-81] Recently, Lomas et al. performed an extensive high-throughput screen and identified a small molecule that corrected AAT misfolding and increased secretion, both in vitro and in vivo.[82] These efforts are supported by recent advances in our understanding of the Z-AAT polymerization process.[83]

For clinical studies, small-interfering RNAs (siRNAs) are currently leading the way. They inhibit the production of mutated proteins, thereby alleviating proteotoxic stress. The employed siRNA is conjugated with N-acetylgalactosamine residues that mediate its hepatocyte-specific uptake via the asialoglycoprotein receptor.[84] Recently, the first data documenting the potential efficacy of this approach has become available. In the ARO-AAT2002 open-label trial (NCT03946449), four Pi*ZZ patients underwent three subcutaneous injections with ARO-AAT with a biopsy after 24 weeks, while five patients received five injections and were biopsied at week 48. siRNA treatment resulted in marked reductions in both serum and hepatic Z-AAT levels.[85] Moreover, subjects presented with decreased serum ALT and GGT

concentrations.[85] Most importantly, six out of the nine individuals displayed an improvement in liver fibrosis, including two individuals who were deemed cirrhotic at baseline. Two additional siRNA trials are either currently recruiting or are expected to be recruiting in the near future (NCT03945292, NCT04174118) and will include even Pi*ZZ individuals with preexisting liver cirrhosis.

Another pathway for decreasing the hepatic burden of AAT is autophagy, which degrades protein polymers that are too large to be processed via the proteasome.[86] In human studies, autophagy-inducing drugs, such as carbamazepine, are being used. A randomized and placebo-controlled trial assessing the safety and efficacy of a 52-week treatment with carbamazepine in PiMZ and Pi*ZZ individuals with liver cirrhosis is currently in phase 2 (NCT01379469), and the first results are expected by the end of this year.

An alternative approach relying on a folding corrector named VX-864 has been recently investigated in a phase 2 clinical trial in Pi*ZZ individuals (NCT04474197). While it significantly increased serum AAT concentrations, further development of the compound was discontinued since its efficacy was deemed insufficient to confer a clinical benefit. While the way ahead is still long, this strategy should be pursued further since it may potentially alleviate both lung and liver disease. Other orally administered folding correctors, namely ZF874, have been recently investigated in a phase 1, double-blinded, randomized, placebo-controlled clinical trial with healthy volunteers and Pi*MZ individuals (NCT04443192).[87, 88]

As the number of treatment strategies is growing, personalized approaches need to be developed for individuals with different genotypes and stages of liver disease. Moreover, genetic assessment should be expanded to include sequencing of modifier genes to identify individuals at risk for severe liver disease that will benefit the most from early therapeutic interventions.

While the current siRNA approach seems to be the most straightforward option for Pi*ZZ individuals with a high load of intrahepatic Z-AAT and advanced liver fibrosis, its long-term lung safety needs to be evaluated. As a result, concomitant AAT augmentation therapy may need to be considered to address lung disease. Additionally, Pi*Z-specific silencing represents an attractive option for Pi*MZ/Pi*SZ subjects with liver disease. Alternatively, folding correctors or substances stimulating protein degradation that do not diminish AAT serum levels may prove beneficial for these cases and for individuals with lower fibrosis stages.

**Therapeutic effects of AAT**

AAT possesses versatile immunomodulatory and cytoprotective functions. For example, AAT overexpression was able to extend the lifespan in *Drosophila*.[1, 89] In line with that, AAT supplementation was beneficial in experimental models of graft-versus-host disease and might even be helpful in humans with this condition (Table 4). It has been successfully investigated in experimental lung, kidney, and islet transplantation models (Table 4), while its efficacy in liver grafts warrants systematic investigation. Moreover, AAT supplementation exerts a protective function in several models of acute liver injury and in experimental alcoholic liver disease.[90, 91] Finally, AAT inhibits TMPRSS2 protease, which enables the entry of SARS-CoV-2 into cells, and the therapeutic effect of AAT for this condition is currently being assessed in several clinical trials (Table 4).[92, 93]

## CONCLUSIONS

Recent studies in preclinical models and patients have greatly improved our understanding of AATD-related liver disease. Depending on the genotype, AATD may either cause liver disease or be a disease modifier. Despite the gain in knowledge, the vast majority of individuals with AATD, even those with a severe Pi*ZZ genotype, remain undiagnosed and, in the case of liver disease, are mislabeled as having alcoholic liver disease or idiopathic liver cirrhosis. Moreover, several important questions remain unanswered (Table 5). Given that multiple therapeutic products are already or soon will be under clinical investigation, we need to improve testing and diagnosis for AATD. We also need genetic screening to systematically identify individuals with AATD who will develop liver disease, particularly when additional environmental risk factors are present. A minimization of coexistent risk factors is warranted for individuals with AATD genotypes conferring an increased risk of liver fibrosis (i.e., Pi*MZ, Pi*SZ, and Pi*ZZ). Inclusion in clinical trials should be attempted for subjects with significant liver fibrosis to stop/attenuate the development of liver scarring. Finally, longitudinal studies are needed to better evaluate the rate of progression and to more precisely calculate liver cancer risk. A systematic comparison between pediatric and adult cases, as well as long-term, standardized follow-up of affected children, is also warranted (Table 5).

## ABBREVIATIONS

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

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*Further remarks:* We attest that we did not use any copyright protected material in our manuscript. No writing assistance was provided. Figures were created with BioRender.com.

## CONFLICT OF INTEREST

*Declaration of competing interests:* All authors declare no support from any organization other than the abovementioned organizations for the submitted work, no financial relationships with any organizations that might have an interest in the submitted work in the previous 5 years, and no other associations or activities that could appear to have influenced the submitted work. Hence, all authors declare themselves independent of funders concerning this manuscript.

## AUTHORS' CONTRIBUTIONS

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## REFERENCES

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[1] Strnad P, McElvaney NG, Lomas DA. Alpha1-Antitrypsin Deficiency. N Engl J Med 2020;382:1443-1455.

[2] Janciauskiene S, Wrenger S, Immenschuh S, Olejnicka B, Greulich T, Welte T, et al. The Multifaceted Effects of Alpha1-Antitrypsin on Neutrophil Functions. Front Pharmacol 2018;9:341.

[3] Janciauskiene SM, Bals R, Koczulla R, Vogelmeier C, Kohnlein T, Welte T. The discovery of alpha1-antitrypsin and its role in health and disease. Respir Med 2011;105:1129-1139.

[4] Greene CM, Marciniak SJ, Teckman J, Ferrarotti I, Brantly ML, Lomas DA, et al. alpha1-Antitrypsin deficiency. Nat Rev Dis Primers 2016;2:16051.

[5] Tanash HA, Ekström M, Rönmark E, Lindberg A, Piitulainen E. Survival in individuals with severe alpha 1-antitrypsin deficiency (PiZZ) in comparison to a general population with known smoking habits. Eur Respir J 2017;50.

[6] Strange C, Moseley MA, Jones Y, Schwarz L, Xie L, Brantly ML. Genetic testing of minors for alpha1-antitrypsin deficiency. Arch Pediatr Adolesc Med 2006;160:531-534.

[7] Strnad P, Nuraldeen R, Guldiken N, Hartmann D, Mahajan V, Denk H, et al. Broad spectrum of hepatocyte inclusions in humans, animals, and experimental models. Compr Physiol 2013;3:1393-1436.

[8] Ferrarotti I, Carroll TP, Ottaviani S, Fra AM, O'Brien G, Molloy K, et al. Identification and characterisation of eight novel SERPINA1 Null mutations. Orphanet J Rare Dis 2014;9:172.

[9] Fagerhol MK, Laurell CB. The Pi system-inherited variants of serum alpha 1-antitrypsin. Prog Med Genet 1970;7:96-111.

[10] Lee JH, Brantly M. Molecular mechanisms of alpha1-antitrypsin null alleles. Respir Med 2000;94 Suppl C:S7-11.

[11] Talamo RC, Langley CE, Reed CE, Makino S. 1 -Antitrypsin deficiency: a variant with no detectable 1 -antitrypsin. Science 1973;181:70-71.

[12] Sinden NJ, Koura F, Stockley RA. The significance of the F variant of alpha-1-antitrypsin and unique case report of a PiFF homozygote. BMC Pulm Med 2014;14:132.

[13] Lomas DA, Irving JA, Gooptu B. Serinopathies. In: Strnad P, Brantly ML, Bals R, eds α1-Antitrypsin deficiency ERS monograph no 85 Lausanne, Swit- zerland: European Respiratory Society, 2019.

[14] Teckman JH. Liver disease in alpha-1 antitrypsin deficiency: current understanding and future therapy. Copd 2013;10 Suppl 1:35-43.

[15] Hamesch K, Mandorfer M, Pereira VM, Moeller LS, Pons M, Dolman GE, et al. Liver Fibrosis and Metabolic Alterations in Adults With alpha-1-antitrypsin Deficiency Caused by the Pi*ZZ Mutation. Gastroenterology 2019;157:705-719.e718.

[16] Blanco I, Bueno P, Diego I, Pérez-Holanda S, Casas-Maldonado F, Esquinas C, et al. Alpha-1 antitrypsin Pi*Z gene frequency and Pi*ZZ genotype numbers worldwide: an update. International journal of chronic obstructive pulmonary disease 2017;12:561-569.

[17] Blanco I, Bueno P, Diego I, Perez-Holanda S, Lara B, Casas-Maldonado F, et al. Alpha-1 antitrypsin Pi*SZ genotype: estimated prevalence and number of SZ subjects worldwide. Int J Chron Obstruct Pulmon Dis 2017;12:1683-1694.
[18] **Fromme M, Schneider CV**, Pereira V, Hamesch K, Pons M, Reichert MC, et al. Hepatobiliary phenotypes of adults with alpha-1 antitrypsin deficiency. Gut 2021.
[19] Lomas DA, Evans DL, Finch JT, Carrell RW. The mechanism of Z alpha 1-antitrypsin accumulation in the liver. Nature 1992;357:605-607.
[20] Teckman JH, Blomenkamp KS. Pathophysiology of Alpha-1 Antitrypsin Deficiency Liver Disease. Methods Mol Biol 2017;1639:1-8.
[21] Christianson JC, Shaler TA, Tyler RE, Kopito RR. OS-9 and GRP94 deliver mutant alpha1-antitrypsin to the Hrd1-SEL1L ubiquitin ligase complex for ERAD. Nat Cell Biol 2008;10:272-282.
[22] Teckman JH, Perlmutter DH. Retention of mutant alpha(1)-antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. Am J Physiol Gastrointest Liver Physiol 2000;279:G961-974.
[23] Borel F, Sun H, Zieger M, Cox A, Cardozo B, Li W, et al. Editing out five Serpina1 paralogs to create a mouse model of genetic emphysema. Proc Natl Acad Sci U S A 2018;115:2788-2793.
[24] Pastore N, Ballabio A, Brunetti-Pierri N. Autophagy master regulator TFEB induces clearance of toxic SERPINA1/α-1-antitrypsin polymers. Autophagy 2013;9:1094-1096.
[25] Hidvegi T, Ewing M, Hale P, Dippold C, Beckett C, Kemp C, et al. An autophagy-enhancing drug promotes degradation of mutant alpha1-antitrypsin Z and reduces hepatic fibrosis. Science 2010;329:229-232.
[26] Kaushal S, Annamali M, Blomenkamp K, Rudnick D, Halloran D, Brunt EM, et al. Rapamycin reduces intrahepatic alpha-1-antitrypsin mutant Z protein polymers and liver injury in a mouse model. Exp Biol Med (Maywood) 2010;235:700-709.
[27] Lawless MW, Greene CM, Mulgrew A, Taggart CC, O'Neill SJ, McElvaney NG. Activation of endoplasmic reticulum-specific stress responses associated with the conformational disease Z alpha 1-antitrypsin deficiency. J Immunol 2004;172:5722-5726.
[28] Mukherjee A, Hidvegi T, Araya P, Ewing M, Stolz DB, Perlmutter DH. NFκB mitigates the pathological effects of misfolded α1-antitrypsin by activating autophagy and an integrated program of proteostasis mechanisms. Cell Death Differ 2019;26:455-469.
[29] **Pastore N, Attanasio S**, Granese B, Castello R, Teckman J, Wilson AA, et al. Activation of the c-Jun N-terminal kinase pathway aggravates proteotoxicity of hepatic mutant Z alpha1-antitrypsin. Hepatology 2017;65:1865-1874.
[30] Attanasio S, Ferriero R, Gernoux G, De Cegli R, Carissimo A, Nusco E, et al. CHOP and c-JUN up-regulate the mutant Z α(1)-antitrypsin, exacerbating its aggregation and liver proteotoxicity. J Biol Chem 2020;295:13213-13223.
[31] Piccolo P, Ferriero R, Barbato A, Attanasio S, Monti M, Perna C, et al. Up-regulation of miR-34b/c by JNK and FOXO3 protects from liver fibrosis. Proc Natl Acad Sci U S A 2021;118.
[32] Perlmutter DH. Liver injury in alpha1-antitrypsin deficiency: an aggregated protein induces mitochondrial injury. J Clin Invest 2002;110:1579-1583.
[33] Kuscuoglu D, Bewersdorf L, Wenzel K, Gross A, Kobazi Ensari G, Luo Y, et al. Dual proteotoxic stress accelerates liver injury via activation of p62-Nrf2. J Pathol 2021;254:80-91.
[34] **Guldiken N, Hamesch K,** Schuller SM, Aly M, Lindhauer C, Schneider CV, et al. Mild Iron Overload as Seen in Individuals Homozygous for the Alpha-1 Antitrypsin Pi*Z Variant Does Not Promote Liver Fibrogenesis in HFE Knockout Mice. Cells 2019;8.
[35] Rudnick DA, Shikapwashya O, Blomenkamp K, Teckman JH. Indomethacin increases liver damage in a murine model of liver injury from alpha-1-antitrypsin deficiency. Hepatology 2006;44:976-982.
[36] Guo S, Booten SL, Aghajan M, Hung G, Zhao C, Blomenkamp K, et al. Antisense oligonucleotide treatment ameliorates alpha-1 antitrypsin-related liver disease in mice. J Clin Invest 2014;124:251-261.
[37] **Yusa K, Rashid ST**, Strick-Marchand H, Varela I, Liu PQ, Paschon DE, et al. Targeted gene correction of α1-antitrypsin deficiency in induced pluripotent stem cells. Nature 2011;478:391-394.
[38] Hannan NR, Segeritz CP, Touboul T, Vallier L. Production of hepatocyte-like cells from human pluripotent stem cells. Nat Protoc 2013;8:430-437.
[39] Tafaleng EN, Chakraborty S, Han B, Hale P, Wu W, Soto-Gutierrez A, et al. Induced pluripotent stem cells model personalized variations in liver disease resulting from α1-antitrypsin deficiency. Hepatology 2015;62:147-157.

[40] **Segeritz CP, Rashid ST**, de Brito MC, Serra MP, Ordonez A, Morell CM, et al. hiPSC hepatocyte model demonstrates the role of unfolded protein response and inflammatory networks in α(1)-antitrypsin deficiency. J Hepatol 2018;69:851-860.
[41] Huch M, Gehart H, van Boxtel R, Hamer K, Blokzijl F, Verstegen MM, et al. Long-term culture of genome-stable bipotent stem cells from adult human liver. Cell 2015;160:299-312.
[42] **Gómez-Mariano G, Matamala N**, Martínez S, Justo I, Marcacuzco A, Jimenez C, et al. Liver organoids reproduce alpha-1 antitrypsin deficiency-related liver disease. Hepatol Int 2020;14:127-137.
[43] Piccolo P, Annunziata P, Soria LR, Attanasio S, Barbato A, Castello R, et al. Down-regulation of hepatocyte nuclear factor-4α and defective zonation in livers expressing mutant Z α1-antitrypsin. Hepatology 2017;66:124-135.
[44] **Fregno I, Fasana E**, Bergmann TJ, Raimondi A, Loi M, Soldà T, et al. ER-to-lysosome-associated degradation of proteasome-resistant ATZ polymers occurs via receptor-mediated vesicular transport. Embo j 2018;37.
[45] Wu Y, Swulius MT, Moremen KW, Sifers RN. Elucidation of the molecular logic by which misfolded alpha 1-antitrypsin is preferentially selected for degradation. Proc Natl Acad Sci U S A 2003;100:8229-8234.
[46] Sun AH, Collette JR, Sifers RN. The cytoplasmic tail of human mannosidase Man1b1 contributes to catalysis-independent quality control of misfolded alpha1-antitrypsin. Proceedings of the National Academy of Sciences 2020;117:24825-24836.
[47] Greenblatt EJ, Olzmann JA, Kopito RR. Derlin-1 is a rhomboid pseudoprotease required for the dislocation of mutant α-1 antitrypsin from the endoplasmic reticulum. Nat Struct Mol Biol 2011;18:1147-1152.
[48] Khodayari N, Oshins R, Holliday LS, Clark V, Xiao Q, Marek G, et al. Alpha-1 antitrypsin deficient individuals have circulating extracellular vesicles with profibrogenic cargo. Cell Commun Signal 2020;18:140.
[49] Ruiz M, Lacaille F, Berthiller J, Joly P, Dumortier J, Aumar M, et al. Liver disease related to alpha1-antitrypsin deficiency in French children: The DEFI-ALPHA cohort. Liver Int 2019;39:1136-1146.
[50] Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med 1976;294:1316-1321.
[51] Sveger T, Eriksson S. The liver in adolescents with alpha 1-antitrypsin deficiency. Hepatology 1995;22:514-517.
[52] 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.
[53] Fromme M, Oliverius M, Strnad P. DEFI-ALFA: The French key to the alpha1 mystery? Liver Int 2019;39:1019-1021.
[54] **Strnad P, Buch S, Hamesch K**, Fischer J, Rosendahl J, Schmelz R, et al. Heterozygous carriage of the alpha1-antitrypsin Pi*Z variant increases the risk to develop liver cirrhosis. Gut 2019;68:1099-1107.
[55] **Schneider CV, Hamesch K,** Gross A, Mandorfer M, Moeller LS, Pereira V, et al. Liver Phenotypes of European Adults Heterozygous or Homozygous for Pi*Z Variant of AAT (Pi*MZ vs Pi*ZZ genotype) and Non-carriers. Gastroenterology 2020.
[56] Clark VC, Marek G, Liu C, Collinsworth A, Shuster J, Kurtz T, et al. Clinical and histologic features of adults with alpha-1 antitrypsin deficiency in a non-cirrhotic cohort. J Hepatol 2018;69:1357-1364.
[57] Adam R, Karam V, Delvart V, O'Grady J, Mirza D, Klempnauer J, et al. Evolution of indications and results of liver transplantation in Europe. A report from the European Liver Transplant Registry (ELTR). J Hepatol 2012;57:675-688.
[58] Schneider CV, Hamesch K, Strnad P. Response to: The Pi*MZ Allele in Alpha-1 Antitrypsin Increases Liver-Related Outcomes in a Population-Based Study. Gastroenterology 2021.
[59] Tapper EB, Lok AS. Use of Liver Imaging and Biopsy in Clinical Practice. N Engl J Med 2017;377:756-768.
[60] Fu DA, Campbell-Thompson M. Immunohistochemistry Staining for Human Alpha-1 Antitrypsin. Methods Mol Biol 2017;1639:139-143.
[61] Kumpers J, Fromme M, Schneider CV, Trautwein C, Denk H, Hamesch K, et al. Assessment of liver phenotype in adults with severe alpha-1 antitrypsin deficiency (Pi*ZZ genotype). J Hepatol 2019;71:1272-1274.
[62] Kim RG, Nguyen P, Bettencourt R, Dulai PS, Haufe W, Hooker J, et al. Magnetic resonance elastography identifies fibrosis in adults with alpha-1 antitrypsin deficiency liver disease: a prospective study. Aliment Pharmacol Ther 2016;44:287-299.

[63] Lara B, Miravitlles M. Spanish Registry of Patients With Alpha-1 Antitrypsin Deficiency; Comparison of the Characteristics of PISZ and PIZZ Individuals. Copd 2015;12 Suppl 1:27-31.
[64] Bartlett JR, Friedman KJ, Ling SC, Pace RG, Bell SC, Bourke B, et al. Genetic modifiers of liver disease in cystic fibrosis. Jama 2009;302:1076-1083.
[65] Abul-Husn NS, Cheng X, Li AH, Xin Y, Schurmann C, Stevis P, et al. A Protein-Truncating HSD17B13 Variant and Protection from Chronic Liver Disease. N Engl J Med 2018;378:1096-1106.
[66] Stender S, Kozlitina J, Nordestgaard BG, Tybjærg-Hansen A, Hobbs HH, Cohen JC. Adiposity amplifies the genetic risk of fatty liver disease conferred by multiple loci. Nat Genet 2017;49:842-847.
[67] Williams R, Aspinall R, Bellis M, Camps-Walsh G, Cramp M, Dhawan A, et al. Addressing liver disease in the UK: a blueprint for attaining excellence in health care and reducing premature mortality from lifestyle issues of excess consumption of alcohol, obesity, and viral hepatitis. Lancet 2014;384:1953-1997.
[68] Petersen MC, Shulman GI. Mechanisms of Insulin Action and Insulin Resistance. Physiol Rev 2018;98:2133-2223.
[69] Lebeaupin C, Vallée D, Hazari Y, Hetz C, Chevet E, Bailly-Maitre B. Endoplasmic reticulum stress signalling and the pathogenesis of non-alcoholic fatty liver disease. J Hepatol 2018;69:927-947.
[70] Leidal AM, Levine B, Debnath J. Autophagy and the cell biology of age-related disease. Nat Cell Biol 2018;20:1338-1348.
[71] Chapman KR, Burdon JG, Piitulainen E, Sandhaus RA, Seersholm N, Stocks JM, et al. Intravenous augmentation treatment and lung density in severe α1 antitrypsin deficiency (RAPID): a randomised, double-blind, placebo-controlled trial. Lancet 2015;386:360-368.
[72] McElvaney NG, Burdon J, Holmes M, Glanville A, Wark PA, Thompson PJ, et al. Long-term efficacy and safety of α1 proteinase inhibitor treatment for emphysema caused by severe α1 antitrypsin deficiency: an open-label extension trial (RAPID-OLE). Lancet Respir Med 2017;5:51-60.
[73] Guillaud O, Jacquemin E, Couchonnal E, Vanlemmens C, Francoz C, Chouik Y, et al. Long term results of liver transplantation for alpha-1 antitrypsin deficiency. Dig Liver Dis 2021;53:606-611.
[74] Carey EJ, Iyer VN, Nelson DR, Nguyen JH, Krowka MJ. Outcomes for recipients of liver transplantation for alpha-1-antitrypsin deficiency-related cirrhosis. Liver Transpl 2013;19:1370-1376.
[75] Townsend SA, Edgar RG, Ellis PR, Kantas D, Newsome PN, Turner AM. Systematic review: the natural history of alpha-1 antitrypsin deficiency, and associated liver disease. Aliment Pharmacol Ther 2018;47:877-885.
[76] Schaefer B, Mandorfer M, Viveiros A, Finkenstedt A, Ferenci P, Schneeberger S, et al. Heterozygosity for the alpha-1-antitrypsin Z allele in cirrhosis is associated with more advanced disease. Liver Transpl 2018;24:744-751.
[77] Turner AM, Stolk J, Bals R, Lickliter JD, Hamilton J, Christianson DR, et al. Hepatic-targeted RNA interference provides robust and persistent knockdown of alpha-1 antitrypsin levels in ZZ patients. J Hepatol 2018;69:378-384.
[78] Burrows JA, Willis LK, Perlmutter DH. Chemical chaperones mediate increased secretion of mutant alpha 1-antitrypsin (alpha 1-AT) Z: A potential pharmacological strategy for prevention of liver injury and emphysema in alpha 1-AT deficiency. Proc Natl Acad Sci U S A 2000;97:1796-1801.
[79] Mahadeva R, Dafforn TR, Carrell RW, Lomas DA. 6-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerization. Implications for the prevention of Z alpha(1)-antitrypsin-related cirrhosis. J Biol Chem 2002;277:6771-6774.
[80] Chang YP, Mahadeva R, Chang WS, Shukla A, Dafforn TR, Chu YH. Identification of a 4-mer peptide inhibitor that effectively blocks the polymerization of pathogenic Z alpha1-antitrypsin. Am J Respir Cell Mol Biol 2006;35:540-548.
[81] Ordóñez A, Pérez J, Tan L, Dickens JA, Motamedi-Shad N, Irving JA, et al. A single-chain variable fragment intrabody prevents intracellular polymerization of Z α1-antitrypsin while allowing its antiproteinase activity. Faseb j 2015;29:2667-2678.
[82] Lomas DA, Irving JA, Arico-Muendel C, Belyanskaya S, Brewster A, Brown M, et al. Development of a small molecule that corrects misfolding and increases secretion of Z α(1) -antitrypsin. EMBO Mol Med 2021;13:e13167.
[83] **Faull SV, Elliston ELK, Gooptu B**, Jagger AM, Aldobiyan I, Redzej A, et al. The structural basis for Z α(1)-antitrypsin polymerization in the liver. Sci Adv 2020;6.
[84] Remih K, Amzou S, Strnad P. Alpha1-antitrypsin deficiency: new therapies on the horizon. Current Opinion in Pharmacology, submitted 2021.
[85] Strnad P, Mandorfer M, Choudhury G, Griffiths W, Turner AM, Trautwein C, et al. ARO-AAT reduces serum and intra-hepatic Z-AAT protein in PiZZ alpha-1 antitrypsin deficient patients with liver

disease leading to improvements in clinically relevant liver biomarkers. AASLD The Liver Meeting Digital Experience 2020.
[86] Perlmutter DH. Autophagic disposal of the aggregation-prone protein that causes liver inflammation and carcinogenesis in alpha-1-antitrypsin deficiency. Cell Death Differ 2009;16:39-45.
[87] Zhou A, Stein PE, Huntington JA, Sivasothy P, Lomas DA, Carrell RW. How small peptides block and reverse serpin polymerisation. J Mol Biol 2004;342:931-941.
[88] **Yamasaki M, Sendall TJ**, Pearce MC, Whisstock JC, Huntington JA. Molecular basis of α1-antitrypsin deficiency revealed by the structure of a domain-swapped trimer. EMBO Rep 2011;12:1011-1017.
[89] Yuan Y, DiCiaccio B, Li Y, Elshikha AS, Titov D, Brenner B, et al. Anti-inflammaging effects of human alpha-1 antitrypsin. Aging Cell 2018;17.
[90] Jedicke N, Struever N, Aggrawal N, Welte T, Manns MP, Malek NP, et al. α-1-antitrypsin inhibits acute liver failure in mice. Hepatology 2014;59:2299-2308.
[91] Grander C, Schaefer B, Schwärzler J, Grabherr F, de Graaf DM, Enrich B, et al. Alpha-1 antitrypsin governs alcohol-related liver disease in mice and humans. Gut 2020.
[92] Wettstein L, Weil T, Conzelmann C, Müller JA, Groß R, Hirschenberger M, et al. Alpha-1 antitrypsin inhibits TMPRSS2 protease activity and SARS-CoV-2 infection. Nat Commun 2021;12:1726.
[93] Ritzmann F, Chitirala P, Krüger N, Hoffmann M, Zuo W, Lammert F, et al. Therapeutic Application of Alpha-1 Antitrypsin in COVID-19. Am J Respir Crit Care Med 2021;204:224-227.
[94] American Thoracic Society/European Respiratory Society statement: standards for the diagnosis and management of individuals with alpha-1 antitrypsin deficiency. Am J Respir Crit Care Med 2003;168:818-900.
[95] Luukkonen PK, Salomaa V, Åberg F. The Pi∗MZ Allele in Alpha-1 Antitrypsin Increases Liver-Related Outcomes in a Population-Based Study. Gastroenterology 2021;160:1874-1875.
[96] Hakim A, Moll M, Qiao D, Liu J, Lasky-Su JA, Silverman EK, et al. Heterozygosity of the Alpha 1-Antitrypsin Pi*Z Allele and Risk of Liver Disease. Hepatol Commun 2021;5:1348-1361.
[97] Regev A, Guaqueta C, Molina EG, Conrad A, Mishra V, Brantly ML, et al. Does the heterozygous state of alpha-1 antitrypsin deficiency have a role in chronic liver diseases? Interim results of a large case-control study. J Pediatr Gastroenterol Nutr 2006;43 Suppl 1:S30-35.
[98] Cacciottolo TM, Gelson WT, Maguire G, Davies SE, Griffiths WJ. Pi*Z heterozygous alpha-1 antitrypsin states accelerate parenchymal but not biliary cirrhosis. Eur J Gastroenterol Hepatol 2014;26:412-417.
[99] Goltz D, Hittetiya K, Vossing LM, Kirfel J, Spengler U, Fischer HP. Alpha(1)-antitrypsin PiMZ heterozygosity has an independent aggravating effect on liver fibrosis in alcoholic liver disease. Virchows Arch 2014;465:539-546.
[100] Propst T, Propst A, Dietze O, Judmaier G, Braunsteiner H, Vogel W. High prevalence of viral infection in adults with homozygous and heterozygous alpha 1-antitrypsin deficiency and chronic liver disease. Ann Intern Med 1992;117:641-645.
[101] Hashemi M, Alavian SM, Ghavami S, de Serres FJ, Salehi M, Doroudi T, et al. High prevalence of alpha 1 antitrypsin phenotypes in viral hepatitis B infected patients in Iran. Hepatol Res 2005;33:292-297.
[102] Eigenbrodt ML, McCashland TM, Dy RM, Clark J, Galati J. Heterozygous alpha 1-antitrypsin phenotypes in patients with end stage liver disease. Am J Gastroenterol 1997;92:602-607.
[103] Serfaty L, Chazouillères O, Poujol-Robert A, Morand-Joubert L, Dubois C, Chrétien Y, et al. Risk factors for cirrhosis in patients with chronic hepatitis C virus infection: results of a case-control study. Hepatology 1997;26:776-779.
[104] Rabinovitz M, Gavaler JS, Kelly RH, Van Thiel DH. Association between heterozygous alpha 1-antitrypsin deficiency and genetic hemochromatosis. Hepatology 1992;16:145-148.
[105] Kaserbacher R, Propst T, Propst A, Graziadei I, Judmaier G, Vogel W. Association between heterozygous alpha 1-antitrypsin deficiency and genetic hemochromatosis. Hepatology 1993;18:707-708.
[106] Elzouki AN, Hultcrantz R, Stål P, Befrits R, Eriksson S. Increased PiZ gene frequency for alpha 1 antitrypsin in patients with genetic haemochromatosis. Gut 1995;36:922-926.
[107] Fargion S, Bissoli F, Fracanzani AL, Suigo E, Sergi C, Taioli E, et al. No association between genetic hemochromatosis and alpha1-antitrypsin deficiency. Hepatology 1996;24:1161-1164.
[108] Schaefer B, Haschka D, Finkenstedt A, Petersen BS, Theurl I, Henninger B, et al. Impaired hepcidin expression in alpha-1-antitrypsin deficiency associated with iron overload and progressive liver disease. Hum Mol Genet 2015;24:6254-6263.

[109] Boëlle PY, Debray D, Guillot L, Corvol H. SERPINA1 Z allele is associated with cystic fibrosis liver disease. Genet Med 2019;21:2151-2155.
[110] Tawara I, Sun Y, Lewis EC, Toubai T, Evers R, Nieves E, et al. Alpha-1-antitrypsin monotherapy reduces graft-versus-host disease after experimental allogeneic bone marrow transplantation. Proc Natl Acad Sci U S A 2012;109:564-569.
[111] Marcondes AM, Karoopongse E, Lesnikova M, Margineantu D, Welte T, Dinarello CA, et al. α-1-Antitrypsin (AAT)-modified donor cells suppress GVHD but enhance the GVL effect: a role for mitochondrial bioenergetics. Blood 2014;124:2881-2891.
[112] Geiger S, Ozay EI, Geumann U, Hereth MK, Magnusson T, Shanthalingam S, et al. Alpha-1 Antitrypsin-Expressing Mesenchymal Stromal Cells Confer a Long-Term Survival Benefit in a Mouse Model of Lethal GvHD. Mol Ther 2019;27:1436-1451.
[113] Daemen MA, Heemskerk VH, van't Veer C, Denecker G, Wolfs TG, Vandenabeele P, et al. Functional protection by acute phase proteins alpha(1)-acid glycoprotein and alpha(1)-antitrypsin against ischemia/reperfusion injury by preventing apoptosis and inflammation. Circulation 2000;102:1420-1426.
[114] Lewis EC, Shapiro L, Bowers OJ, Dinarello CA. Alpha1-antitrypsin monotherapy prolongs islet allograft survival in mice. Proc Natl Acad Sci U S A 2005;102:12153-12158.
[115] Lewis EC, Mizrahi M, Toledano M, Defelice N, Wright JL, Churg A, et al. alpha1-Antitrypsin monotherapy induces immune tolerance during islet allograft transplantation in mice. Proc Natl Acad Sci U S A 2008;105:16236-16241.
[116] Koulmanda M, Bhasin M, Hoffman L, Fan Z, Qipo A, Shi H, et al. Curative and β cell regenerative effects of α1-antitrypsin treatment in autoimmune diabetic NOD mice. Proceedings of the National Academy of Sciences 2008;105:16242-16247.
[117] Koulmanda M, Bhasin M, Fan Z, Hanidziar D, Goel N, Putheti P, et al. Alpha 1-antitrypsin reduces inflammation and enhances mouse pancreatic islet transplant survival. Proceedings of the National Academy of Sciences 2012;109:15443-15448.
[118] Abecassis A, Schuster R, Shahaf G, Ozeri E, Green R, Ochayon DE, et al. α1-antitrypsin increases interleukin-1 receptor antagonist production during pancreatic islet graft transplantation. Cell Mol Immunol 2014;11:377-386.
[119] Jonigk D, Al-Omari M, Maegel L, Müller M, Izykowski N, Hong J, et al. Anti-inflammatory and immunomodulatory properties of α1-antitrypsin without inhibition of elastase. Proc Natl Acad Sci U S A 2013;110:15007-15012.
[120] Gao W, Zhao J, Kim H, Xu S, Chen M, Bai X, et al. α1-Antitrypsin inhibits ischemia reperfusion-induced lung injury by reducing inflammatory response and cell death. J Heart Lung Transplant 2014;33:309-315.
[121] Iskender I, Sakamoto J, Nakajima D, Lin H, Chen M, Kim H, et al. Human α1-antitrypsin improves early post-transplant lung function: Pre-clinical studies in a pig lung transplant model. J Heart Lung Transplant 2016;35:913-921.
[122] Lin H, Chen M, Tian F, Tikkanen J, Ding L, Andrew Cheung HY, et al. α(1)-Anti-trypsin improves function of porcine donor lungs during ex-vivo lung perfusion. J Heart Lung Transplant 2018;37:656-666.
[123] Götzfried J, Smirnova NF, Morrone C, Korkmaz B, Yildirim A, Eickelberg O, et al. Preservation with α(1)-antitrypsin improves primary graft function of murine lung transplants. J Heart Lung Transplant 2018;37:1021-1028.
[124] Magenau JM, Goldstein SC, Peltier D, Soiffer RJ, Braun T, Pawarode A, et al. α(1)-Antitrypsin infusion for treatment of steroid-resistant acute graft-versus-host disease. Blood 2018;131:1372-1379.
[125] Brener A, Lebenthal Y, Interator H, Horesh O, Leshem A, Weintrob N, et al. Long-term safety of α-1 antitrypsin therapy in children and adolescents with Type 1 diabetes. Immunotherapy 2018;10:1137-1148.

1 **TABLES**

| rs number | Deficiency alleles | *SERPINA1* variant | Characteristic genotypes and corresponding clinical features |
|---|---|---|---|
| rs28929474 | Z | p.Glu342Lys | Strong (Pi*ZZ)/mild (Pi*MZ): predisposition to liver disease and lung emphysema |
| rs17580 | S | p.Glu264Val | Pi*SZ: moderate predisposition to liver disease and lung emphysema<br>Pi*SS: only minimal risk |
| | F | p.Arg223Cys | Lung emphysema in compound heterozygotes |
| rs28931570 | I | p.Arg39Cys | No clear phenotype |
| rs121912713 | Pittsburgh | p.Met358Arg | Bleeding disorder via inhibition of thrombin and factor XI |
| | Trento | p.Glu75Val | Lung emphysema in coinheritance with Pi*Z |
| rs775982338 | $M_{malton}$ | p.Phe52del (M2 variant) | Liver disease and lung emphysema in homozygotes |
| rs28931568 | $M_{mineral\ springs}$ | p.Gly67Glu | Lung emphysema in homozygotes |
| rs28931569 | $M_{procida}$ | p.Leu41Pro | Lung emphysema in homozygotes |
| rs199422211 | $Q0_{bellingham}$ | p.Lys217* | Lung emphysema in homozygotes and compound heterozygotes |
| | $Q0_{bolton}$ | p.Δ1 bpPro362 | Lung emphysema in homozygotes and compound heterozygotes |
| rs267606950 | $Q0_{granitefalls}$ | p.Δ1 bpTyr160 | Lung emphysema in coinheritance with Pi*Z |
| rs1057519610 | $Q0_{hongkong}$ | p.Δ2 bpLeu318 | Lung emphysema in homozygotes and compound heterozygotes |

2 **Table 1: Overview of the most prominent alpha-1 antitrypsin deficiency alleles with their clinical**

3 **characteristics.**

4 All deficiency alleles are displayed with their rs number, mutation, and clinical features.[1, 12, 13]

5 Abbreviations: rs, reference SNP ID number; Pi, proteinase inhibitor.

6

| *SERPINA1* genotype | Range of serum AAT (mg/dL) | AST ≥ ULN (%) | ALT ≥ ULN (%) | GGT ≥ ULN (%) |
|---|---|---|---|---|
| **Pi*ZZ** | 20-45 | 15 | 11 | 22 |
| **Pi*SZ** | 75-120 | 5 | 9 | 18 |
| **Pi*MZ** | 90-210 | 5 | 7 | 17 |

7 **Table 2: Range of alpha-1 antitrypsin (AAT) serum levels and proportion of Pi*ZZ individuals, Pi*SZ**

8 **subjects, and Pi*MZ participants with elevated liver enzymes.**

The liver enzyme levels are based on data obtained from the UK Biobank in participants without an obvious liver comorbidity.[18] Range of AAT serum levels is in mg/dL.[94] Abbreviations: AAT, alpha-1 antitrypsin deficiency; AST, aspartate aminotransferase; ALT, alanine aminotransferase; GGT, gamma-glutamyl transferase; ULN, upper limit of normal (sex-specific).

| | Evidence | Odds ratio | References |
|---|---|---|---|
| **Overall population** | ++ | Increased liver-related mortality (HR~1.7), higher odds for diabetic/obese individuals | Schneider et al., 2020[55]<br>Luukkonen et al., 2021[95]<br>Schneider et al., 2021[58]<br>Fromme et al., 2021[18]<br>Hakim et al., 2021[96] |
| **NAFLD-related cirrhosis** | ++ | OR~2-7 | Regev et al., 2006[97]<br>Cacciottolo et al., 2014[98]<br>Abul-Husn et al., 2018[65]<br>Strnad et al., 2019[54] |
| **ALD-related cirrhosis** | ++ | OR~2-6 | Goltz et al., 2014[99]<br>Cacciottolo et al., 2014[98]<br>Abul-Husn et al., 2018[65]<br>Strnad et al., 2019[54] |
| **Chronic hepatitis B-associated advanced liver fibrosis** | + | Smaller studies, OR~10 (one study) | Propst et al., 1992[100]<br>Hashemi et al., 2005[101]<br>Kuscuoglu et al., 2021[33] |
| **Chronic hepatitis C-associated advanced liver fibrosis** | +/- | Several studies, OR~4 in one of them | Propst et al., 1992[100]<br>Eigenbrodt et al., 1997[102]<br>Serfaty et al., 1997[103]<br>Regev et al., 2006[97] |
| **Hemochromatosis-associated advanced liver fibrosis** | +/- | Multiple studies, conflicting results | Rabinovitz et al., 1992[104]<br>Kaserbacher et al., 1993[105]<br>Elzouki et al., 1995[106]<br>Fargion et al., 1996[107]<br>Schaefer et al., 2015[108]<br>Guldiken et al., 2019[34] |
| **Cystic fibrosis-associated advanced liver disease** | ++ | OR~5 | Bartlett et al., 2009[64]<br>Boëlle et al., 2019[109] |
| **Liver transplantation** | + | Up to 10% of liver transplant recipients display Pi*MZ genotype | Carey et al., 2013[74]<br>Schaefer et al., 2018[76] |

**Table 3: Current evidence for the Pi*MZ genotype as a modifier of liver disease.**

Abbreviations: +/-, conflicting data; +, weak positive evidence; ++, robust positive evidence; AATD, alpha-1 antitrypsin deficiency; ALD, alcoholic liver disease; HCV, hepatitis C virus; HR, hazard ratio; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; ND, not determined; OR, odds ratio; Pi, protease inhibitor; Pi*MZ, AAT genotype with heterozygosity for the Pi*Z variant.

| | | |
|---|---|---|
| **A) Experimental** | | |
| Liver | Acute liver failure | • Jedicke et al., 2014[90] |
| | Alcoholic liver disease | • Grander et al., 2021[91] |
| | Graft-versus-host disease | • Tawara et al., 2012[110]<br>• Marcondes et al., 2014[111]<br>• Geiger et al., 2019[112] |
| | Kidney transplantation | • Daemen et al., 2000[113] |
| | Islet transplantation | • Lewis et al., 2005; 2008[114, 115]<br>• Koulmanda et al., 2008; 2012[116, 117]<br>• Abecassis et al., 2014[118] |
| Lung | Lung inflammation | • Jonigk et al., 2013[119] |
| | Lung transplantation | • Gao et al., 2014[120]<br>• Iskender et al., 2014[121]<br>• Lin et al., 2018[122]<br>• Götzfried et al., 2018[123] |
| | COVID-19 | • Wettstein et al., 2021[92] |
| **B) Clinical** | | |
| | Graft-versus-host disease | • Magenau et al., 2018[124] |
| | Type 1 diabetes | • Brener et al., 2018[125] |

**Table 4: Overview of the studies on alpha-1 antitrypsin supplementation.**

| | |
|---|---|
| **Pi*MZ** | • Is the modifier role liver disease etiology-specific?<br>• Is the contribution of AATD-related liver disease significant enough to warrant specific therapeutic intervention? |
| **Pi*ZZ** | • Are mechanisms underlying pediatric and adult liver disease identical?<br>• Are there any genetic and/or environmental modifiers? Can individuals at risk of severe liver disease be identified by specific tests?<br>• How to measure disease activity/identify rapid progressors?<br>• What are the most effective and clinically relevant end-points for evaluating the efficacy of investigational products? |

**Table 5. Key research questions for individuals with alpha-1 antitrypsin deficiency.**

Abbreviations: AATD, alpha-1 antitrypsin deficiency; Pi, protease inhibitor; Pi*MZ, AAT genotype with heterozygosity for the Pi*Z variant; Pi*ZZ, AAT genotype with homozygosity for the Pi*Z variant.

## FIGURE LEGENDS

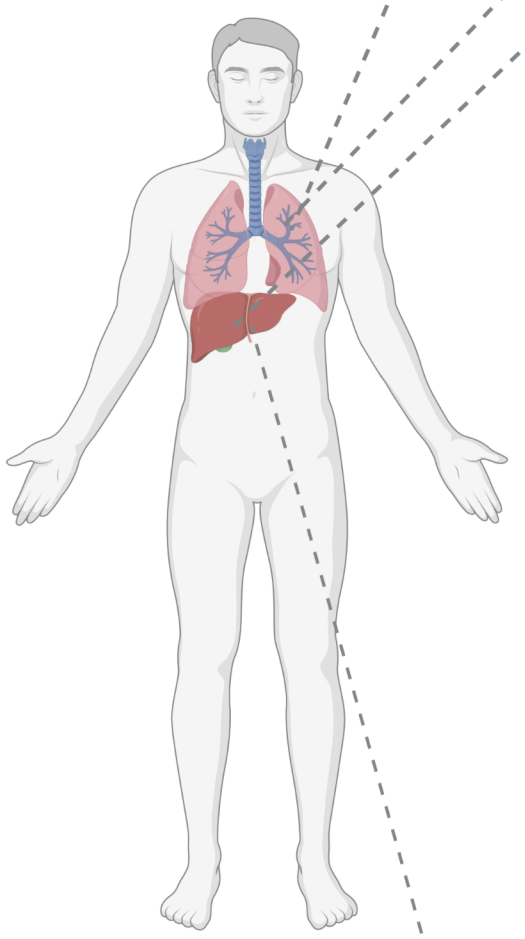
**Figure 1: Pathophysiology, clinical manifestations and treatment approaches for alpha-1 antitrypsin deficiency.** Alpha-1 antitrypsin deficiency (AATD) results from mutations in the gene encoding alpha-1 antitrypsin (AAT) named *SERPINA1* that lead to increased degradation and/or retention of AAT in the liver. The misfolding and polymerization of AAT confers proteotoxic stress that promotes the development of liver cirrhosis and liver tumors. The lack of AAT in the systemic circulation results in an insufficient inhibition of proteases (loss-of-function phenotype) that leads to proteolytic lung injury and panlobular emphysema. Histologically, the retention of AAT can be visualized as inclusion bodies in periodic acid-Schiff-diastase (PAS-D) staining. The characteristic homozygous Pi*Z mutation leads to an ~85% decrease in AAT secretion, and monomeric/polymeric Z-AAT is degraded by endoplasmic reticulum-associated degradation (ERAD) and autophagy. The resulting endoplasmic reticulum (ER) stress and/or environmental triggers stimulate AAT production, thereby causing a vicious cycle. Therapeutic approaches for AATD-related liver disease include small interfering RNA (siRNA), decreasing AAT production and secretion, and autophagy enhancers diminishing protein accumulation. Finally, polymerization inhibitors may facilitate both secretion and degradation.

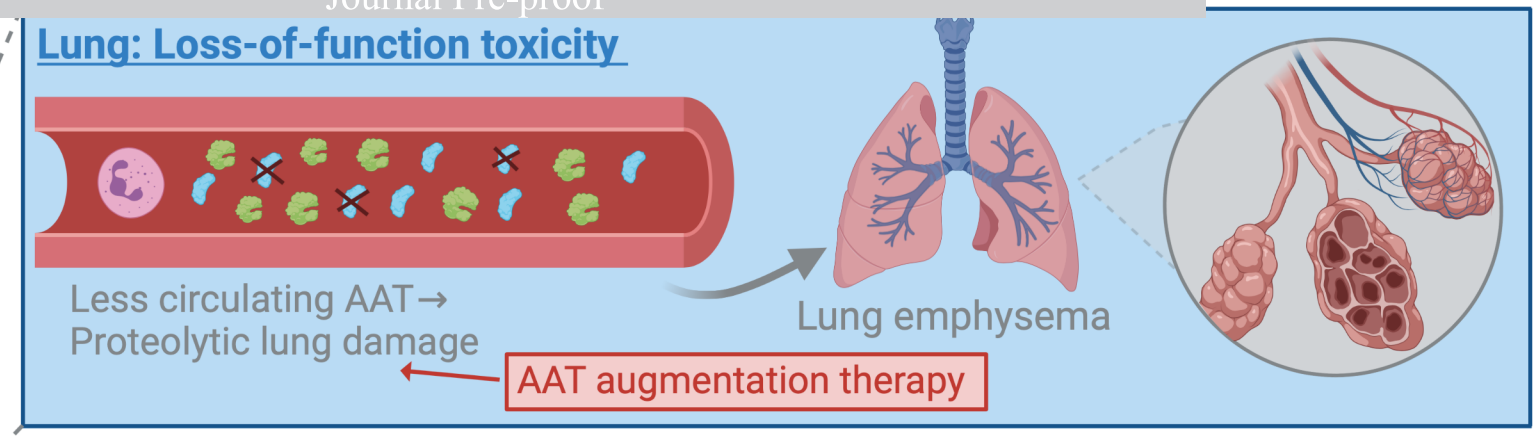
**Figure 2: Alpha-1 antitrypsin deficiency (AATD) genotypes and factors promoting the development of AATD-related liver disease.**

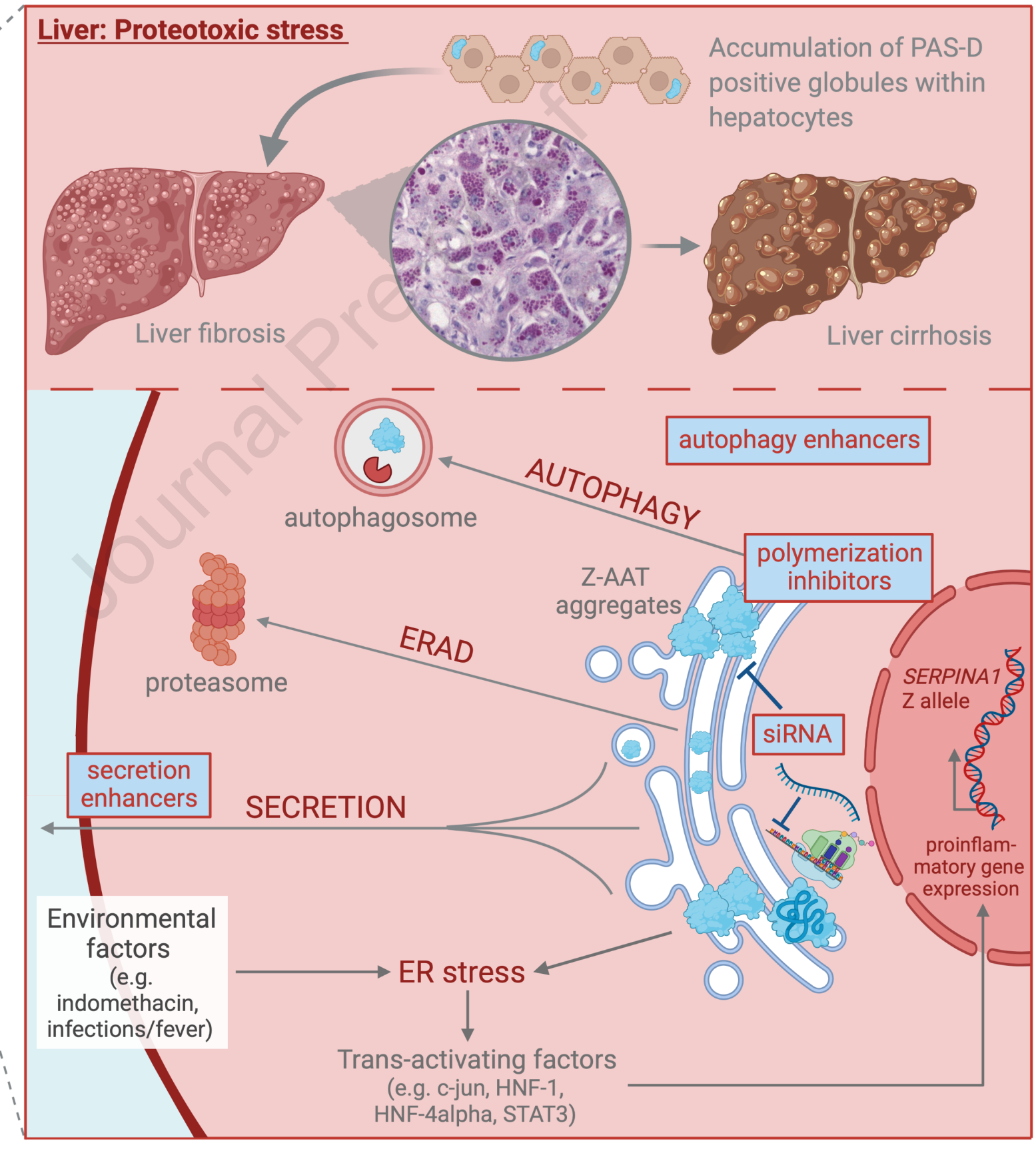
(A) The figure summarizes the frequency of the most relevant AATD genotypes in Caucasians, their odds of developing liver cirrhosis, and their share in adult liver transplantations performed in Europe. Pi*ZZ and Pi*MZ refer to a homozygous and heterozygous presence of the Pi*Z mutation, whereas Pi*SZ denotes compound heterozygosity of both the Pi*S and Pi*Z mutations. Notably, unlike Pi*ZZ, Pi*MZ contributes to rather than causes liver cirrhosis. (B) The established nongenetic risk factors associated with the occurrence of advanced liver disease in individuals with AATD are shown.

**Figure 3: Histological presentation of Pi*ZZ-associated liver disease.**

Liver sections from a 61-year-old man with the Pi*ZZ genotype and fibrosis stage 4 labeled with (A) hematoxylin and eosin (H&E), (B) periodic acid-Schiff-diastase (PAS-D) staining, and (C) immunohistochemistry (IHC) using an antibody specific for the Pi*Z variant of *SERPINA1*.[60]





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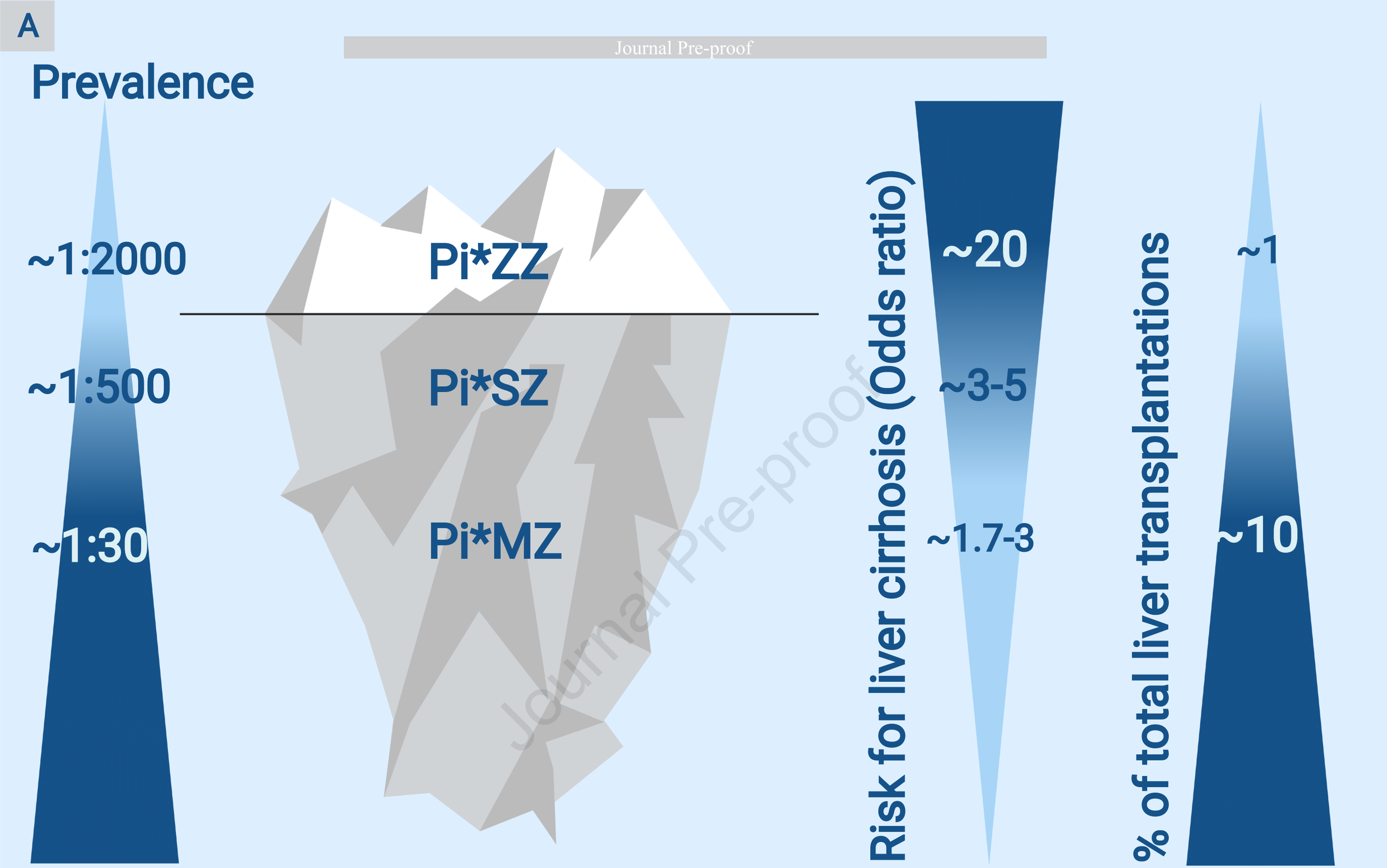
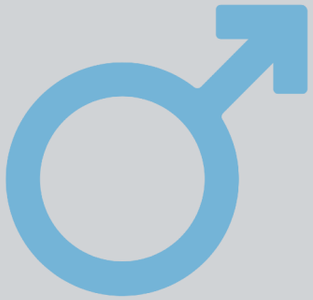



B

## Risk factors for the development of liver disease

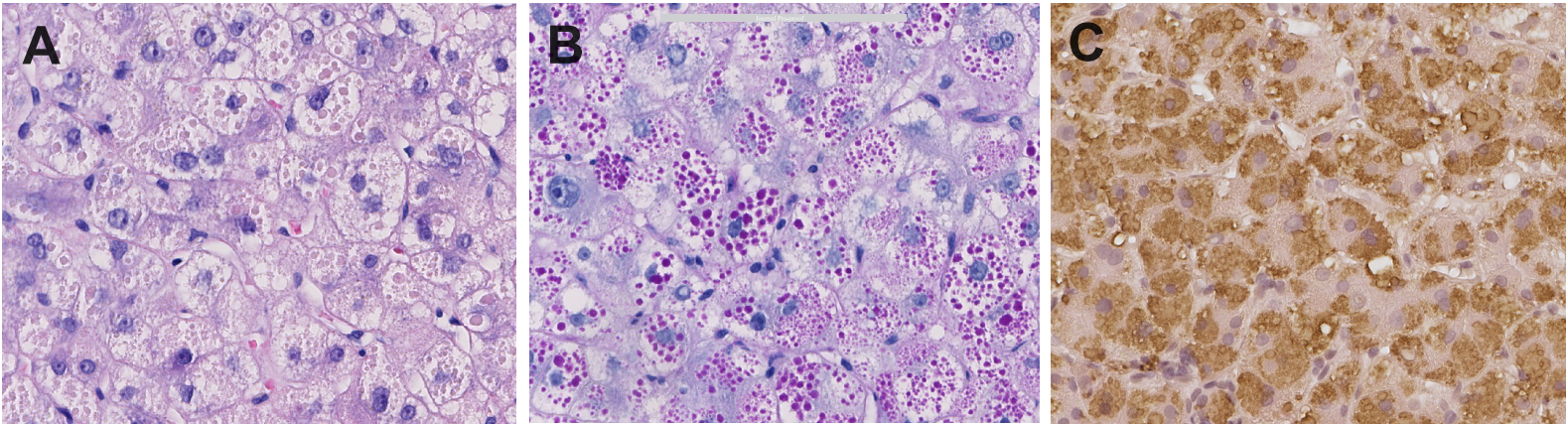
Diabetes mellitus

Obesity,
Metabolic syndrome



Male sex

Age ≥50 years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