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Review

Liver disease with unknown etiology – have you ruled out alpha-1 antitrypsin deficiency?

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***Abstract*:** Although a less well-known consequence of alpha-1 antitrypsin deficiency (AATD) liver disease is the second leading cause of death among patients with the condition. The alpha-1 antitrypsin (AAT) protein is produced by hepatocytes within the liver, which retain pathological variants of AAT instead of secreting the proteinase inhibitor into the systemic circulation. This intracellular retention is caused by inefficient folding and polymerization of mutant AAT and the accumulation of these AAT aggregates leads to diverse manifestations of liver disease, which can present differently in both children and adults. The progression from hepatocyte apoptosis to liver inflammation, fibrosis and cirrhosis, and liver failure is still not fully understood, but in older patients, liver disease can surpass lung disease as the principal cause of death. Liver function tests (LFTs) can measure plasma levels of liver enzymes to assess liver function but require careful interpretation. Non-invasive tests are being developed that can detect early liver disease, but liver biopsy is still the gold standard for assessing liver fibrosis once abnormal LFTs have been detected in a patient. Currently, there is no licensed treatment for AATD-related liver disease (intravenous AAT therapy is not indicated for this purpose), but liver transplantation is associated with positive outcomes and may even slow emphysema progression. Therefore, new strategies are being developed to address treatment of AATD-related liver disease, such as accelerating degradation of mutant AAT and assisting hepatocytes in the folding and secretion of mutant AAT, but these approaches remain at early stages of development.

Keywords: alpha-1 antitrypsin, alpha-1 antitrypsin deficiency, cirrhosis, hepatocellular carcinoma, liver disease, liver transplant

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Introduction

Liver disease is a less well-known consequence of alpha-1 antitrypsin deficiency (AATD), but it is the second leading cause of death among both adults and children with AATD.[1] Liver disease is thought to affect around 10% of individuals with AATD and is also the cause of death in approximately 10% of individuals with AATD.[2–4] As is the case with lung disease, AATD is under-diagnosed in patients with liver disease.[5] In this chapter, the pathophysiology, presentation, and diagnosis/management of liver disease related to AATD will be discussed, as well as the current open research questions and future developments in the management of AATD-associated liver disease.

Pathophysiology and genetics

Hepatocytes are responsible for producing the majority of the body's alpha-1 antitrypsin (AAT) protein, which functions to protect the lungs by inhibiting a range of pro-inflammatory proteases.[6] The wild-type AAT protein, referred to as the M type, is normally synthesized in the hepatocyte and rapidly secreted by the endoplasmic reticulum (ER) in large quantities. In contrast, the Z

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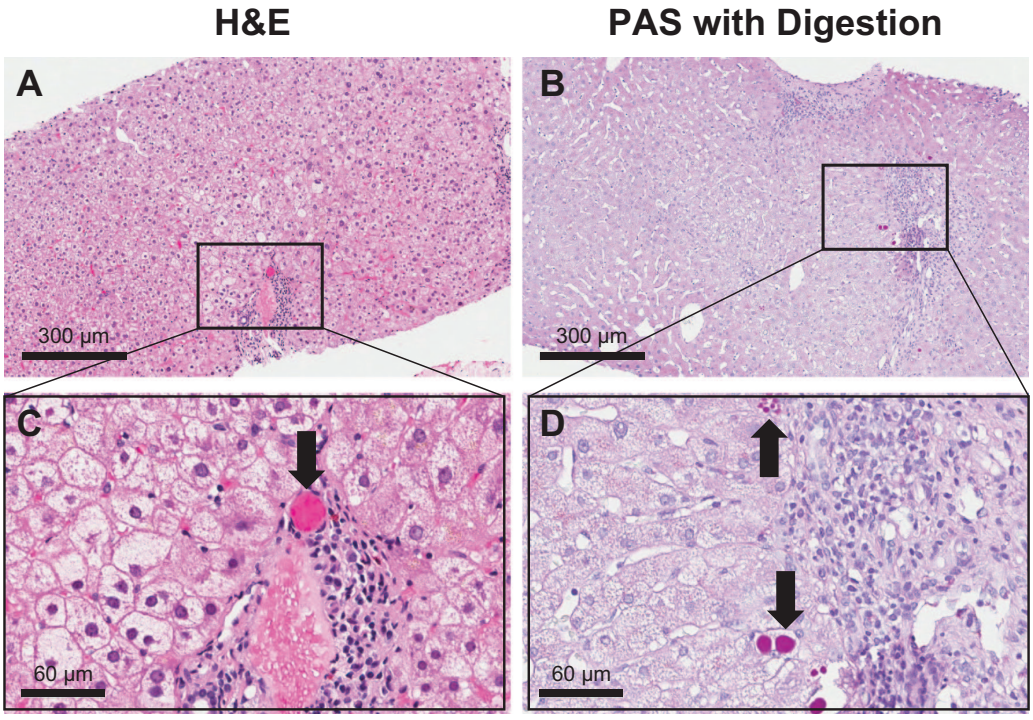

Figure 1. Liver biopsy histology from a PI*ZZ alpha-1 antitrypsin deficiency (AATD) patient.
Hematoxylin and eosin (H&E) staining (A and C) and periodic acid-Schiff (PAS) with digestion staining (B and D) showing positive globules indicative of alpha-1 antitrypsin (AAT) polymerization/accumulation within hepatocytes (arrows).

variant folds inefficiently into its final conformation and polymerizes, resulting in 85% ER retention through protein quality control pathways.[7] Liver disease from AATD arises due to this polymerization behaviour associated with certain AAT protein variants, such as the Z and S variants, as well as the rare M_{Malton} variant.[8,9] With these AAT variants, the mutated AAT protein is not secreted from hepatocyte ER due to misfolding during synthesis and is intracellularly retained. The polymerized AAT is subjected to intracellular proteolysis rather than being secreted into the systemic circulation where it functions as an enzymatic inhibitor.[10] These misfolded mutant proteins aggregate in the ER of the hepatocytes and form large polymers/globules, which can be identified microscopically by periodic acid-Schiff staining with diastase digestion on liver biopsy tissue samples (Figure 1). This abnormal accumulation of mutated AAT in the liver has been shown to cause mitochondrial autophagy and caspase activation in hepatocytes,[11] which can further lead to hepatocellular apoptosis, liver inflammation, liver fibrosis and cirrhosis, as well as end-stage liver disease.[12] Emerging data suggest that circulating extracellular vesicles produced as a result of hepatic injury in AATD patients contain pro-fibrinogenic factors that may promote liver fibrosis. Whether this occurs *in situ* is yet to be determined.[13]

AATD is one of the three most frequently occurring genetic liver disorders, along with Wilson's disease and hemochromatosis.[14] Although there are many genotypes associated with AATD, liver disease is more strongly associated with the PI*ZZ and PI*SZ genotypes, and has some association with the PI*MZ genotype.[15–18] The PI*ZZ genotype has been known to be associated with liver disease for some time, but recent studies have revealed an association between the PI*SZ genotype and liver disease in both adults and children.[17] These cases are often less prevalent and less clinically severe than in patients with the PI*ZZ genotype.[17] The PI*MZ genotype has also recently been confirmed as a risk factor for the development of liver disease,[19,20] and an association has been noted with the rare M_{Malton} variant, which also causes AAT protein polymerization.[9] Nevertheless, further data are required on the prevalence of liver disease in AATD, the risk of liver disease associated with

different AATD genotypes, and the impact of aggravating factors (e.g. hepatotoxic drugs, alcohol).

Disease presentation

Much like the presentation of lung disease in AATD, the potential manifestations of liver injury are diverse and include chronic hepatitis, cirrhosis, cholestatic jaundice, fulminant hepatic failure, and hepatocellular carcinoma (HCC).[21] The typical presentation of liver disease also differs between adults and children with AATD.[12]

In children, the age of diagnosis varies significantly. AATD-related liver disease has been identified in children between the ages of 1 month and 15 years,[16,22] although the most well-known presentation in children is neonatal hepatitis syndrome, which is characterized by severe and prolonged jaundice.[21] However, multiple birth screening studies in Europe and North America suggest that most PI*ZZ children are healthy and therefore go undiagnosed in childhood.[23,24] A small number of PI*ZZ neonates develop cholestatic hepatitis (neonatal hepatitis syndrome), which manifests as elevated levels of conjugated serum bilirubin, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels.[25,26] The majority of neonates with cholestatic hepatitis improve spontaneously,[27,28] although some PI*ZZ neonates exhibit severe complications of liver disease and go on to develop cirrhosis, portal hypertension and liver failure at a variety of ages, from pre-school to early adulthood.[4,27,28] In addition, a subsection of PI*ZZ children who do not exhibit neonatal cholestasis go on to develop liver failure later in childhood. The exact factors influencing progression to liver failure are still unknown, although several new studies are shedding more light on this.

Scarring of the liver, that is, hepatic cirrhosis, usually progresses slowly in patients with AATD and is more commonly seen in adult patients over 50 years of age. The prognosis for these patients is typically poor,[29] and liver disease surpasses lung disease as the principal cause of death in older patients, particularly in patients who have never smoked.[1] The progression of liver disease into HCC is not well understood, with suggested possible mechanisms including saturation of autophagy, and altered regulation of genes that promote cellular proliferation and tumorigenesis.[30] In heterozygous individuals (i.e. those with the PI*MZ genotype), development of HCC appears to be dependent on the presence of pre-existing viral hepatitis infection,[31] and in homozygous patients, HCC has been found to develop independently of viral infection and/or pre-existing liver damage.[32]

Liver disease diagnosis in AATD

Analysis of a patient's serum AAT protein phenotype and *SERPINA1* genotype are considered the gold standard tests for diagnosing AATD.[1] Protein phenotype gel analysis (isoelectric focusing electrophoresis) requires technical expertise and is therefore best performed in a reference laboratory with experience in this technique.[33] Diagnosis of liver disease *via* liver biopsy is not necessarily required for AATD diagnosis, but can be useful in assessing the degree and extent of liver injury, as well as fibrosis and cirrhosis.

PI*ZZ patients should theoretically not produce AAT protein levels in the near-normal range. However, experience from the authors suggest that PI*SZ patients with active liver disease and inflammation occasionally show serum AAT levels within the normal range, and systemic inflammation has been shown to mask the presence of the PI*MZ variant.[34] Therefore, testing serum AAT levels should be interpreted carefully and should not be used alone for diagnosing AATD. Serum AAT levels are often higher in neonates and then rapidly decrease over the first few months of life, which may not be reflected in the reference ranges of many laboratories. If a patient has recently received a plasma transfusion, measuring serum AATD levels or phenotype testing should be avoided, as results will reflect the donor's plasma levels rather than the patient's own plasma levels. Similarly, this is true for patients with emphysema who regularly receive AAT protein replacement therapy.

Monitoring and management of liver disease in AATD

There are several methods used to detect liver disease in AATD. One easy and simple method is to measure plasma levels of liver enzymes using standard blood tests (liver function tests; LFTs). PI*ZZ and PI*SZ patients have been shown to have increased ALT and AST levels compared to control patients, although plasma levels may still be within the normal range.[35,36] However, levels of liver

enzymes should be interpreted carefully as enzyme levels can be influenced by the use of contraceptive medication, alcohol use and increased body weight with liver steatosis, and therefore should not solely be relied on for diagnosing liver disease.[35,36] These factors also add to problems in defining the 'normal' reference ranges for these LFTs. The presence of elevated liver enzymes may not accurately reflect ongoing liver injury with AATD and therefore will require further investigation.

Liver biopsy is the gold standard for assessing liver fibrosis and could be used to detect AATD-associated liver disease after the detection of abnormal LFTs, but due to the invasiveness, procedural pain, complications and diagnostic accuracy concerns, non-invasive imaging-based methods for assessing liver disease are preferred. Elastography methods such as magnetic resonance elastography (MRE), acoustic radiation force impulse (ARFI) quantification and two-dimensional shear wave elastography (2D-SWE) have the potential to be suitable imaging tools for the assessment of AATD-related liver fibrosis as they can detect early liver fibrosis before the development of cirrhosis, but require further investigation with larger patient populations.[37–39] FibroScan is another non-invasive method that has proved to be accurate in diagnosing liver fibrosis through liver stiffness measurements.[19,40] FibroScan can give an estimation of liver fibrosis staging from blood fibrosis test results without the need for liver biopsy,[40] and has been validated in AATD patients, with the proportion of invalid scans in children decreasing with age.[41,42] Blood tests may also be performed for markers of liver injury, for example, reduced thrombopoietin, or more specific parameters may be assessed using HepaScore and FibroTest. If blood test and elastography results are discordant, liver biopsy may be considered.[39]

AATD patients with advanced liver disease (cirrhosis or failure) may ultimately require liver transplantation, which is associated with positive outcomes in terms of survival in both children and adult patients.[15,22] Liver transplantation may also slow the progression of emphysema, owing to the return to normal levels of AAT protein post-transplant.[15,43] However, in some PI*ZZ and PI*SZ patients, levels of forced expiratory volume in 1 s continue to decline unexpectedly post-liver transplant.[15]

Currently, there is no licensed pharmacological treatment for AATD-related liver disease and liver transplantation is the only way of resolving advanced liver cirrhosis. Therefore, as with liver cirrhosis of any etiology, avoidance of non-steroidal anti-inflammatory drugs (NSAIDs) is typically advised.[44,45] Animal model studies of AATD suggest that NSAIDs can be toxic to a PI*ZZ liver uniquely, even in the absence of cirrhosis. The mechanism for NSAID toxicity in PI*ZZ individuals is thought to be due to the prostaglandin production blockade induced by NSAIDs, which can increase AAT synthesis, and therefore, increase misfolded protein accumulation.[46] This pattern of injury by NSAIDs in a PI*ZZ liver has only been reported in animal models and has not yet been shown in humans. Therefore, in case of fever or pain in PI*ZZ patients, many authorities suggest overall NSAID avoidance in favour of moderate doses of acetaminophen.

Regarding alcohol consumption, data are currently lacking in PI*ZZ patients without any evidence of liver damage. However, the PI*MZ genotype was found to increase the risk of developing cirrhosis in alcohol misusers, although the risk for the PI*MS genotype is less clear.[20,47,48] According to guidelines from the American Association for the Study of Liver Diseases (AASLD) for adults with hepatitis C infection, there is no known safe level of alcohol use, and therefore, all patients should be advised to abstain from alcohol;[49] this recommendation becomes more critical in hepatitis C-positive patients with AATD. However, it is currently unclear if a similar strategy should be recommended for patients with AATD and without hepatitis C infection.

Intravenous AAT augmentation therapy is not recommended for the treatment of AATD-related liver disease as clinical experience suggests that liver disease is unaffected by this therapy.[50] Augmentation therapy is also not recommended for AATD treatment in patients that have undergone liver transplantation, as a successful liver transplant should lead to normal circulating levels of the AAT protein in the patient post-transplant.[50]

Future developments

New strategies are required for the development of pharmacological interventions for the treatment of AATD-related liver disease and many

new approaches are currently being examined. Extensive studies have recently been published using *in vitro* analyses of the molecular structure of the PI*ZZ protein, and more than 10 different compounds have been shown to block liver injury in the PI*ZZ mouse model of AATD-related liver disease, although none are yet approved for use in humans.[51–54] Several gene repair technologies,[55–58] and the use of chemical chaperones,[59,60] are also being investigated to improve proper folding of mutant AAT proteins.

Several applications of RNA interference (RNAi) technology are also being examined to prevent mutant PI*ZZ protein synthesis and thereby prevent toxic hepatic accumulation and liver injury. In the PI*ZZ mouse model, these methods have been shown to eliminate liver injury, reverse liver disease, and prevent liver disease in young mice, therefore representing a promising therapy for AATD-related liver disease.[61] Phase I/II and phase II/III human trials using silencing RNA (siRNA) technology to inhibit PI*ZZ protein synthesis as a therapy are now underway in Europe and the United States, respectively.[62,63] The use of *in silico* or 'cell-free' systems has also been examined for designing therapeutic strategies for the disruption of mutant AAT protein polymerisation, which is likely to be an event distal to the protein retention signal.[54,64] However, the predicted effect of many compounds has not yet been reported in follow-up cell culture models, and the process of chemically creating medicinal molecules for trials in animal models has proved challenging. Another type of therapy, currently undergoing phase II trials, is being led by Vertex Pharmaceuticals. This therapy utilises small molecular chaperones to help reduce PI*ZZ AAT polymerization, which in theory could benefit AATD lung and liver disease simultaneously.[65]

Methods to accelerate intracellular degradation of mutant AAT proteins as a potential treatment for the liver have also been investigated. Several successful experiments in both cell culture and mouse models have demonstrated that up-regulating the autophagy degradation pathway can reduce the burden of the mutant AAT protein in the liver and reduce the associated liver injury.[21,27,66] Autophagy is an intracellular degradation pathway that is primarily important for balancing sources of energy during critical points of development and in response to nutrient stress. Autophagy is also important for removing misfolded/aggregated proteins and damaged organelles and is known to be activated in response to the accumulation of misfolded AAT proteins in the liver.[66] Enhancing autophagy using compounds such as sirolimus, carbamazepine and ursodeoxycholic acid, as well as genetic approaches to induce the expression of key autophagy regulators, have all been shown to decrease mutant AAT protein accumulation within cells and reduce liver cell injury.[51,52,67,68] However, excessively high doses of all of these agents were required to show any affect. A human trial is currently underway for the use of low doses of carbamazepine in PI*ZZ patients with liver cirrhosis.[69]

Conclusions

The manifestations of liver disease related to AATD are extremely diverse, and the etiology and exact prevalence are still not fully understood. As liver disease can manifest at any age, patients who develop chronic liver disease without any clear etiology, such as alcohol abuse or viral hepatitis, should be tested for AATD.[50] There is currently no specific treatment for AATD-related liver disease, and so effective pharmacological therapies to reduce the need for liver transplantation and disease progression are urgently needed. New technologies such as gene therapy and RNAi may hold promise for the treatment of liver disease caused by AATD, but further research and human trials are still required.

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Author contributions

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Conflict of interest

DP reports no conflicts of interest. JT reports grants from NIH, Alpha-1 Foundation, Vertex, Dicerna, Camp4, Arrowhead, KorroBio, and Gilead; and consulting fees from Alpha-1 Foundation, Vertex, Arrowhead, Dicerna, RestoreBio, Takeda, Retrophin, GLG, BioMarin, and Boehringer.

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