Chapter 7
Liver Disease in α1-Antitrypsin Deficiency

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Introduction

Liver disease was first described in α1-antitrypsin deficiency (ATD) by Sharp and colleagues who discovered it in a child with cirrhosis in 1969 [1]. Since that time ATD has been widely recognized as the most common genetic diagnosis for children who undergo liver transplantation. Eriksson and colleagues showed in 1986 that adults with ATD also had a predilection for cirrhosis and hepatocellular carcinoma (HCC) [2], and onset of liver disease later in life is now known to be more common than previously recognized. The liver damage is predominantly characterized by fibrosis and ultimately cirrhosis with relatively limited inflammation. Nevertheless, there is wide variability in the incidence and severity of liver disease among individuals with the classical form of ATD, as shown by analysis of a cohort of Swedish individuals identified in a nationwide screening study by Sveger [3]. Only ~8% of the cohort had clinically significant liver disease over the first three decades of life. A greater proportion of this population will probably be impacted as they reach older ages, but we do know that a significant number of affected homozygotes completely escape clinical symptoms of liver disease throughout their lifetime [2].

Using a variety of model systems, we have come to learn that the liver is damaged in ATD by a gain-of-toxic function mechanism that is dependent on the accumulation of misfolded α1-antitrypsin Z (ATZ) within the early compartments of the secretory pathway [4]. Several theories for how this proteotoxicity leads to excessive collagen deposition and hyperproliferation in the liver have been proposed. Putative genetic and environmental modifiers are thought to play a role in managing the "proteotoxicity" and to determine susceptibility to and severity of liver disease among homozygotes [5]. Furthermore, our studies have implicated two types of

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mechanisms that are putative targets of genetic or environmental modifiers: cellular pathways for degradation of misfolded ATZ and signaling mechanisms that permit cellular adaptation to the presence of misfolded ATZ [5]. Indeed, several novel concepts for therapy that capitalize on the degradation pathways are currently being investigated.

In this chapter we will review what is known about the clinical characteristics of this liver disease, pathogenesis of fibrosis and carcinoma in the liver, and current and novel therapeutic strategies.

Clinical Characteristics of ATD-Associated Liver Disease

Liver disease may have onset in infancy, childhood, adolescence, or middle-aged adults, and it is likely that these represent four distinct forms of the disease. Jaundice is the first sign of the disease in infants. Usually this is when the physiologic jaundice of the newborn is prolonged to 4–6 weeks of age. These infants often have elevated serum conjugated bilirubin and transaminase levels, and in some cases hepatomegaly may be evident. ATD is an important entity to consider in the differential diagnosis of neonatal cholestasis, as the infantile presentation of ATD can closely mimic extrahepatic biliary atresia in terms of overall clinical appearance and laboratory evaluation. A study conducted by the Biliary Atresia Research Consortium (BARC) found that pathologists blinded to clinical information regarding infant liver biopsies under review for the assessment of cholestatic liver disease were not consistently able to distinguish between cases due to biliary atresia from those due to ATD [6]. Furthermore, neonates with classical ATD may not demonstrate biliary excretion on hepatoscintigraphy due to their significant cholestasis, making it difficult to differentiate the possibility of ATD from biliary atresia using this modality in such cases [7]. Occasionally, the infant with ATD is first recognized because of pruritus and hypercholesterolemia, making it important to distinguish ATD from inborn errors of bile secretion that cause infantile cholestasis. Severe manifestations reflective of portal hypertension such as ascites, splenomegaly with hypersplenism, gastrointestinal bleeding, and poor growth can rarely occur during infancy [8].

The typical presentation for this disease in childhood, in adolescence, or during adulthood is one or more of the complications of portal hypertension. In many cases it is enlargement of the liver and/or spleen together with leukopenia and thrombocytopenia indicative of hypersplenism. Some people will present with ascites, gastrointestinal bleeding from esophageal varices, or hepatic encephalopathy. Occasionally the diagnosis is made when symptoms of abdominal pain are attributed to gallstones, and imaging studies are suggestive of hepatic cirrhosis. A number of affected individuals will be diagnosed incidentally when a nodular appearance to the liver is noticed during abdominal surgery for another diagnosis.

There is an increased incidence of HCC in persons with ATD. This was first shown by Eriksson and colleagues by an autopsy study in Sweden [2]. Statistical

analysis indicated that the incidence of hepatic carcinoma in ATD was higher than expected from the general population and also significantly higher than what would be expected in association with cirrhosis alone. This study also showed that both cirrhosis and HCC could be present incidentally in individuals that had never been diagnosed with liver disease and had died of other causes. In most cases HCC is discovered at the time of diagnosis of ATD-associated liver disease or soon after that diagnosis has been made. It is almost always in the adult form of the disease although one case of hepatic carcinoma was diagnosed in a 12-year-old [9]. The incidence of liver cancer in ATD is still probably not fully appreciated clinically. In a review of liver transplants performed at the University of Pittsburgh Medical Center (UPMC) from 1991 to 2012 in which ATD was at least one of the pathology-confirmed diagnoses from review of the explant, we found that 9 out of 98 adult patients (9.2 %) had explants positive for HCC, and another eight patients (8.2 %) had explants that demonstrated evidence of dysplasia (unpublished data). However, as noted below, not all of these patients were homozygotes, and several had other causes of liver disease, including three with a history of alcohol abuse, one had a history of alcohol abuse and autoimmune hepatitis, and one had been diagnosed with hemochromatosis (unpublished data). In one series of 164 cases of primary liver carcinoma, 13 cases had tumors surrounded by intrahepatocytic globular inclusions immunostained with a monoclonal antibody to ATZ [10]. This was taken to mean that 7.9 % of primary liver cancers were associated with ATD, but these cases were not independently confirmed by AT phenotype determination.

Over the past two decades, there has been an increase in the number of adults who undergo liver transplantation with a diagnosis of ATD. Indeed, over the last 10 years, 85–90 % of all liver transplant procedures done in the USA for ATD are for adults, typically at around 50–65 years of age (United Network of Organ Sharing, personal communication). The apperent change from childwood to adult predominance is partially explained by a backlog of childhood cases that underwent liver transplantation prior to that time, but it may also be partially explained by improved recognition of ATD as a cause of chronic liver disease [11].

The most important study of incidence and severity of liver disease in ATD comes from a nationwide screening study carried out by Sveger in the early 1970s [3]. In this study, the investigators screened 200,000 newborns from which they identified 127 individuals who were homozygotes for ATZ. From this group of homozygotes, 14 cases exhibited prolonged obstructive jaundice, and 9 out of the 14 developed clinical manifestations of severe liver disease. Notably, slightly more than half of the remaining infants identified in this cohort were found to have elevated blood transaminase levels but otherwise exhibited no other signs of liver disease. In the four decades following the initiation of this study, this cohort has been followed relatively comprehensively, and the results of subsequent follow-up analyses have revealed that only about 8 % of the population has developed clinically significant liver disease [12]. Because this cohort has not reached the peak age for adult-onset liver disease, 50–65 years of age, and because liver biopsies have not been a part of the study, it is likely that the true incidence of liver disease in an unbiased background is greater than 8 %. However, the study shows that there is wide

variability in the hepatic phenotype of ATD. Furthermore, it provides powerful evidence that genetic and/or environmental modifiers are critical in determining whether a homozygote is susceptible to or protected from clinical liver disease.

The natural history of ATD-associated liver disease is also quite variable. The majority of infants diagnosed with ATD because of prolonged neonatal jaundice will experience slow resolution of jaundice and elevated serum transaminases over the first 6–12 months of life. Even when splenomegaly or other signs and symptoms of portal hypertension are present, the disease may progress very slowly. In one report of 17 patients with cirrhosis and portal hypertension, it took 4 years before liver transplantation was required in nine patients, and seven of the patients were living relatively healthy lives for up to 23 years after diagnosis [13]. In other patients the liver disease progresses more rapidly, but it is almost always within the category of chronic and not acute liver failure.

Liver disease has been reported in individuals with AT allotypes other than the homozygous ATZ allotype (Table 7.1). Compound heterozygotes for the S and Z

Table 7.1 Deficiency variants of α1-antitrypsin

			Clinical disease		
Variant	Defect	Site	Liver	Lung	Cellular defect
Z	Single base substitution M1 (Ala213)	Glu342-Lys	+	+	IC accumulation
S	Single base substitution	Glu264-Val	–	–	IC accumulation
$M_{Heerlen}$	Single base substitution	Pro369-Leu	–	+	IC accumulation
$M_{Procida}$	Single base substitution	Leu41-Pro	–	+	IC accumulation
M_{Malton}	Single base deletion	Phe52	+	+	IC accumulation
M_{Duarte}	Unknown	Unknown	+?	+	Unknown
$M_{Mineral\ Springs}$	Single base substitution	Gly57-Glu	–	+	No function; EC degradation?
S_{iiyama}	Single base substitution	Ser53-Phe	–	+	IC accumulation
P_{Duarte}	Two base substitution	Arg101-His Asp256-Val	+?	+	Unknown
P_{Lowell}	Single base substitution	Asp256-Val	–	+	IC degradation?
$W_{Bethesda}$	Single base substitution	Ala336-Thre	–	+	Accelerated catabolism?
$Z_{Wrexham}$	Single base substitution	Ser19-Leu	?	?	Unknown
F	Single base substitution	Arg223-Cys	–	–	Unknown
T	Single base substitution	Glu264-Val	–	–	Unknown
I	Single base substitution	Arg39-Cys	–	–	IC degradation
$M_{Palermo}$	Single base deletion	Phe51	–	–	Unknown
$MN_{ichinan}$	Single base deletion and single base substitution	Phe52 Gly148-Arg	–	–	Unknown
$Z_{Ausburg}$	Single base substitution	Glu342-Lys	–	–	Unknown
King's	Single base substitution	His334-Asp	+		IC accumulation
M_{pisa}	Single base substitution	Lys259-Ile	–	+?	IC accumulation
$E_{taurisano}$	Single base substitution	Lys368-Glu	–	+	IC accumulation
$Y_{orzinuovi}$	Single base substitution	Pro391-His	+?	–	IC accumulation

allele appear to have a risk of liver disease that is similar to ZZ homozygotes [12]. In a follow-up analysis of a subset of 26-year-old subjects from the Swedish screening/cohort study, 3 out of 32 individuals (9 %) with the SZ allotype had elevated liver transaminases compared with 4 out of 70 subjects with the ZZ allotype (6 %). A subsequent follow-up study of a subset of 30-year-old patients from the same cohort found that 4 of 39 (10 %) subjects with SZ had elevated alanine transaminase (ALT) compared with 4 of 89 (5 %) subjects with ZZ [14]. Patients with the SZ allotype were found to represent 4 % of pathology-confirmed adult cases of ATD in our study of the UPMC liver transplant database from 1991 to 2012 (unpublished data). Data from three liver transplant centers from 1987 to 2012 indicated that 50 ZZ and 23 SZ adults had undergone liver transplantation [15]. Interestingly, the patients with the SZ allotype were more likely than the ZZ patients to have another reason for liver disease. Liver disease in individuals with the SZ allotype is likely to be related to accumulation of misfolded protein in the intracellular secretory compartments because the S variant is known to be prone to intracellular accumulation, albeit to a milder extent than the Z variant [16]. A few cases of liver disease have been reported in individuals with the homozygous M_{malton} variant of AT [17, 18], probably attributable to the tendency for this variant to misfold and accumulate within cells in a way that is similar to the Z variant [19]. The variant S_{iiyama}, which also has a tendency to misfold and accumulate within cells, was reported in a patient with emphysema and hepatocyte inclusions, but the patient did not manifest clinical liver disease [20]. The W variant was reported in three consanguineous children homozygous for the allele, two of whom died during infancy of severe liver disease [21]. Fra et al. reported a novel variant, Yorzinuovi (Pro391His), which was associated with reduced serum AT levels and an increased tendency to form intracellular polymers with increased intracellular accumulation [22]. The patient was 46 years old and had a 10-year history of elevated serum transaminases, but there was no further characterization of the liver involvement and no indication as to whether the patient had been evaluated for other causes of liver disease. However, AT inclusions were not detected in the liver biopsy by immunohistochemistry [22]. Miranda et al. reported the novel King's variant that was discovered in a 6-week-old boy with prolonged jaundice [23]. Laboratory studies demonstrated a significant reduction in the patient's serum AT level and a liver biopsy that was characteristic of classic ATD with intrahepatocytic inclusions. The child was found to be compound heterozygous for the Z and King's variant. Biochemical studies showed that the King's variant forms polymers within the endoplasmic reticulum and accumulates within cells [23]. In reports of rare AT variants, it is important to note the difficulty in ascertaining whether the AT allele truly causes liver damage unless alcohol consumption, viral hepatitis, and autoimmune disease have been extensively characterized. Furthermore, based on what we know about the incidence of liver disease among ZZ homozygotes, we could only anticipate a subpopulation of individuals with each allotype to be susceptible to clinical effects on the liver. Nevertheless, one of the correlative concepts that have arisen from the association of AT alleles with liver damage is that all of these alleles have polymerogenic properties. This concept has led to theory that polymerization plays an important role in the pathobiology of hepatotoxicity.

A subject of some controversy has been whether MZ heterozygotes are at higher risk for developing liver disease. An obstacle to addressing this question has been the tendency in the literature to address this issue with retrospective studies that derive from single-center experiences and small case series and are therefore unfortunately biased in ascertainment. An early study of liver biopsies found a relatively higher prevalence of MZ heterozygosity in patients with cryptogenic cirrhosis and chronic active viral hepatitis [24]. A cross-sectional case–control study by Regev et al. did not find an overall association between MZ and the presence of chronic liver disease or cryptogenic cirrhosis, but they did find a significantly higher prevalence of MZ in patients with decompensated liver disease from all causes. Furthermore, they found that there appeared to be an association between the presence of MZ phenotype and the severity of liver disease in patients with hepatitis C and nonalcoholic fatty liver disease [25]. A retrospective study by Graziadei et al. at a liver transplant center found that 8.2 % of their patients who underwent liver transplantation were MZ heterozygotes, compared with 2–4 % of the general population based on their review of published studies [26]. A small study by Vecchio et al. that reviewed 80 consecutive cases of cryptogenic cirrhosis and chronic active hepatitis found no cases of MZ heterozygosity. Given the small number of cases, it is likely that this study was underpowered to detect cases of MZ [27]. Although it is still difficult to know from these studies whether MZ heterozygosity truly causes liver disease by itself, we have seen a number of cases of MZ individuals with severe liver disease and no other potential cause despite extensive evaluation. A number of adults with the MZ phenotype undergo liver transplantation each year. In our review of liver transplants at UPMC from 1991 to 2012, AT phenotypes were done on 89 patients with pathological features of ATD and 44 of these were MZ heterozygotes (unpublished data). Furthermore, according to our conceptual model for the pathogenesis of liver disease in ZZ homozygotes, it seems likely that some MZ heterozygotes who have a complement of particularly powerful negative genetic and/or environmental modifiers would be susceptible to liver damage from the proteinopathy.

There has also been extensive discussion in the literature about whether other causes of liver disease act in concert with ATD. One intriguing study investigated the possibility that ATD and cystic fibrosis (CF) occurs together and increases the risk of liver disease in each of the populations. This case–control study examined genotyping data for five candidate genes in patients with cystic fibrosis (CF) and severe liver disease with portal hypertension from more than 100 CF centers across North America and Japan and found as its primary conclusion that having even a single copy of the Z allele is a risk factor for incidence and severity of liver disease in CF. No ZZ homozygotes were identified in that study [28]. In adults with ATD who come to clinical attention for liver disease, there is often evidence for other causes of liver disease such as alcoholic liver disease, nonalcoholic steatohepatitis, viral hepatitis, autoimmune liver disease, or hemochromatosis that can confound their clinical picture. The degree to which any given hepatic comorbidity can interact with ATD to accelerate the development of cirrhosis and HCC is not fully understood. Furthermore the majority of studies on this issue are confusing because

patients with ATD include mostly heterozygotes grouped together with a small or even unknown number of homozygotes. In regard to ATD together with hemochromatosis, one study found that the MZ heterozygous phenotype was about ten times more common compared with the general population (20 vs. 2.2 %) [29]. However, other studies have not found an association between these two conditions [30, 31]. There are case reports of more rapid clinical progression when hepatitis C virus infection is encountered in the setting of at least one Z allele [32, 33]. However, studies by Simsek et al. and Elzouki et al. did not reveal a clear synergizing effect [34, 35]. There is still very limited data about liver disease in ATD in combination with nonalcoholic fatty liver disease. One survey of an uncontrolled registry suggested that obesity is common in ATD patients with active liver disease [36], and we have seen a number of middle-aged patients who are Z homozygotes with hepatic steatosis as a part of the liver histological picture.

In children there have also been studies exploring the possibility that heterozygosity for the Z allele is a risk factor for liver disease of other causes. In one retrospective study at a single pediatric liver disease center, AT heterozygosity was more common in children with other chronic liver diseases [37]. Specifically, when they compared their population to an American reference database, the Z variant represented 2.2 % of AT alleles in their pediatric liver disease population, compared with 0.56 % in the reference database. This difference remained true when their population was subdivided between biliary atresia and non-biliary atresia patients. They also found that in the subset of their patients with biliary atresia who were referred for liver transplantation, possession of non-M alleles was associated with a shorter mean age to transplantation compared to MM homozygotes (235 vs. 779 days, $p = 0.036$).

The diagnosis of ATD should be considered in any individual being evaluated for symptoms and signs of liver disease. In infants this includes jaundice or pruritus. In older children hepatomegaly or splenomegaly may be the only signs. Elevated serum transaminases in an otherwise asymptomatic individual should also lead to evaluation for ATD. Certainly any signs of portal hypertension should trigger diagnostic studies for ATD. The diagnosis of ATD is established by the determination of serum AT protein phenotype by isoelectric focusing (IEF), an electrophoretic technique that permits the identification of AT variants by separation along a pH gradient. Serum AT concentrations also play an important role in the diagnostic evaluation. They may be used for screening, with levels below normal (85–215 mg/dL) being suggestive of the presence of at least one abnormal AT allele. It is ideal to determine both the AT phenotype and serum concentration as these assays are complementary in their functions. A technical limitation of phenotypic analysis by IEF is that it cannot establish the presence of a null AT allele combined with a normal or mutant allele. Serum AT levels are crucial in these cases for raising suspicion of the presence of a null allele [38]. On the other hand, serum AT levels alone are inadequate. They may appear to be borderline normal in Z heterozygotes, and AT levels can in some cases be falsely reassuring since AT, as an acute phase reactant, may rise in the setting of an inflammatory response. AT levels may also be affected by blood transfusions [36]. PCR-based targeted mutation analysis is available for the most

common alleles (M, Z, and S), which may be helpful when a patient's phenotype analysis is ambiguous, but it is limited in that it will not detect less common alleles. In rare situations in which routine testing is inconclusive and a rare or novel variant is suspected, next generation sequence analysis may be of value [39].

The characteristic histologic finding on liver biopsy is periodic acid Schiff (PAS)-positive, diastase-resistant globules and/or granules within hepatocytes, often in the periportal regions, accompanied by fibrosis and mild inflammation. The inclusions are round to oval, eosinophilic, and 1–40 μm in diameter. There may also be evidence of hepatocellular necrosis and bile duct epithelial cell destruction [40]. The degree of inflammation may vary depending on the presence of comorbid conditions such as steatohepatitis or viral hepatitis. Although suggestive, the presence of PAS-D globules is not absolutely pathognomonic for the diagnosis of ATD, as these structures have been reported to occasionally occur in individuals without ATD [41]. Inclusions may also be scant or absent in some ATD cases in the first few weeks of life [42]. Immunohistochemical stains for AT are useful for confirming the nature of the inclusions. Liver histology may also reflect the presence of dysplasia or carcinoma.

Mechanism of Deficiency

The classical form of ATD is characterized by a point mutation that substitutes lysine for glutamate 342 in the mutant protein, ATZ [4]. Studies in several different model systems using pulse-chase radiolabeling have shown that there is reduced secretion and intracellular accumulation of ATZ when compared to the wild-type AT [43–47]. Immunostaining and biochemical analyses show that mutant ATZ accumulates in early compartments of the secretory pathway, including most notably the rough endoplasmic reticulum (ER), and perhaps other as yet undefined compartments that do not stain with classical markers of the rough ER [46]. It is important to note that in these model systems the secretion of ATZ is reduced, whereas it is completely absent for a variety of null variants when they are expressed in similar model systems [48–50]. This reflects what is seen in humans with serum levels of ATZ at 10–15 % of normal while those of the null variant are undetectable. Together, these observations make it possible to conclude that these model systems faithfully recapitulate the human disease and definitively prove that the primary abnormality is defective biogenesis. Furthermore, site-directed mutagenesis studies have shown that the single amino acid substitution of lysine for glutamate 342 is sufficient to cause the cellular defect in which the mutant ATZ is unable to efficiently translocate through the intracellular secretory pathway [43].

It is still not entirely clear how the amino acid substitution leads to diminished secretion and intracellular accumulation of ATZ. Lomas and colleagues have argued that the primary defect is polymerization [51]. These authors demonstrated polymers and insoluble aggregates in liver biopsy specimens and plasma from patients with ATD and provided evidence for a loop–sheet insertion mechanism of

polymerization. In this conceptualization of polymerization, the characteristic lysine substitution is at the hinge of the mobile reactive-site loop, and because it has more bulk than the glutamate that is ordinarily at that site, it prevents the reactive-site loop from relaxing into a known gap in the A sheet that is part of the flexible conformational changes of the AT molecule. This permits the reactive-site loop on another ATZ molecule to insert into the gap and begin the oligomerization and ultimately polymerization process. In an important study, Sidhar et al. showed that the secretory defect that characterizes ATZ was partially corrected by introducing a second mutation that suppressed polymerization [52]. More recent studies by Huntington and colleagues have suggested a different mechanism for polymerogenic and aggregation-prone properties of ATZ that involves at least two different domain-swapping phenomena [53–55].

Nevertheless, several lines of evidence suggest that misfolding is the primary defect responsible for impaired secretion/intracellular accumulation of ATZ, and in this conceptualization polymerization/aggregation is a result of rather than the primary defect itself. First, only 18 % of the intracellular pool of ATZ is in polymers at steady state in a mammalian cell line model that faithfully recapitulates the intracellular accumulation/fate of ATZ [49]. Second, a naturally occurring variant of ATZ which has the same E342K mutation as ATZ and a carboxyl-terminal truncation accumulates in the ER even though it does not form polymers suggesting that misfolding is sufficient to lead to intracellular accumulation of ATZ [48]. Third, the results of Sidhar et al. [52] do not exclude the possibility that diminished secretion of ATZ is partially corrected by the second engineered mutation because this second mutation also prevents the primary misfolding defect. Furthermore, in a very interesting study, a small molecule that prevents polymerization in vitro does not correct the secretory defect of ATZ in vivo but rather leads to enhanced degradation [56]. It is hard to know for certain about the applicability of this last study because there was no analysis of whether polymerization was prevented by the small molecule in vivo. Taken together, we believe that misfolding is the primary defect and that polymerization and aggregation are time-dependent effects of the accumulation of ATZ because of misfolding. This conceptualization is also consistent with the domain-swapping mechanism of polymerization described by Huntington in which polymerization is viewed as a "kinetic" result, or delayed folding, of monomeric ATZ and explains how some ATZ gets secreted.

Pathogenesis of Liver Injury/Fibrosis

In most cases, ATD liver disease is slowly progressive and the dominant pathology is characterized by fibrosis/cirrhosis and carcinogenesis [57]. The mechanism of liver injury is now known to involve gain-of-function processes wherein the accumulation of the mutant ATZ in the ER of hepatocytes triggers a series of events that eventually results in proteotoxic damage. The most compelling evidence for the gain-of-toxic function mechanism comes from mouse models of ATD in which

transgenic expression of the mutant human ATZ gene is sufficient to reproduce the features of liver disease known to occur in humans [58, 59]. The PiZ mouse, generated with a human genomic fragment encompassing all of the exons and introns of the human ATZ gene [59] and which is the most extensively characterized model, has intrahepatocytic globules reflecting accumulation of misfolded ATZ in the ER, slowly progressing fibrosis, low-grade inflammation, dysplasia, and increased prevalence of hepatic carcinoma [60, 61]. Because there are normal levels of AT and presumably other anti-elastases in these animals, as directed by endogenous murine genes, the liver injury in these transgenic mice cannot be attributed to a loss-of-function mechanism such as excess proteolytic activity.

There is still relatively limited information about how proteinopathy leads to liver injury. Although structural and functional alterations in mitochondria and caspase-3 activation have been observed in liver tissue from the PiZ mouse model and from ATD patients [62, 63], mitochondrial dysfunction probably has mostly a cytostatic effect because apoptosis, necrosis, and inflammation are not major pathological characteristics of the liver in ATD.

The mechanisms responsible for the hepatic fibrotic response to proteinopathy are also not well understood. Genomic analysis of a mouse model with hepatocyte-specific inducible expression of mutant ATZ (Z mice), ideal for elucidating signal transduction pathways that are activated by the proteinopathy, has identified a relatively rich network of downstream targets of the TGFβ pathway, including the upregulation of connective tissue growth factor [64], and this pathway is known to play a central role in fibrosis [65–67]. We have also demonstrated that activation of the NFκB signaling pathway is an important and specific effect of cellular ATZ accumulation [50], and the NFκB pathway has been implicated in tissue fibrosis [68]. However, our studies have indicated a select repertoire of downstream targets for NFκB signaling in the setting of ATZ accumulation [64], suggesting that it is a unique response designed to affect hepatocellular proliferation (see below).

Several recent studies have suggested that fibrosis results from proteinopathies in several different tissues. Lung fibrosis has been described in several rare diseases characterized by proteinopathy in respiratory epithelial cells, including surfactant protein C deficiency and Hermansky–Pudlak syndrome [69, 70]. Similarly myocardial fibrosis has been described for desminopathy that affects cardiomyocytes [71] and skeletal muscle fibrosis in inclusion-body myositis [72]. Interestingly, by enhancing the degradation of misfolded proteins, autophagy has been shown to mitigate cardiac fibrosis from desminopathy [71] and skeletal muscle fibrosis from inclusion-body myositis [73] just as it does for hepatic fibrosis in the PiZ model of ATD (see below).

Although polymerization may not be the primary cause of defective secretion and intracellular accumulation, there is no question that polymers and aggregates form in the ER as a result of ATZ accumulation, and there are several lines of evidence that the ability to form polymers correlates with susceptibility to liver disease. First, all of the AT alleles that have been associated with liver disease appear to have the capacity to form polymers. Second, polymerogenic variants of another serpin that is expressed in neurons, neuroserpin, cause a neuronal degenerative disease [74].

Third, liver damage occurs in another genetic disease, inherited hypofibrinogenemia, associated with the accumulation of fibrinogen polymers and aggregates in the endoplasmic reticulum of liver cells [75]. Interestingly, liver disease has not been described for polymerogenic variants of other serpins, like C1 inhibitor [76], but this may be explained by the fact that these serpins are expressed at much lower concentrations and/or in cell types that are able to tolerate the variant serpins at these lower concentrations.

It is very important to point out that there is absolutely no in vitro or in vivo data that ATZ polymers themselves are directly toxic to liver cells. It is still possible that other forms of ATZ that accumulate in liver cells, such as monomeric misfolded ATZ or soluble ATZ oligomers or even a combination of misfolded monomers and polymers, are responsible for the cytotoxic effects. This means that polymerization could be a process that is associated with the generation of a toxic intermediate, and, if that is the case, it would represent a very reliable marker for some toxic misfolding event. It is even possible that polymerization is itself a mechanism for protecting cells from toxic misfolded ATZ and always occurs when toxic misfolding is present but in some cases is insufficient to provide the necessary protection. In studies of another proteinopathy, Alzheimer's disease, there is evidence suggesting that soluble oligomers of amyloid-β peptide are toxic to neurons [77], whereas aggregates, and particularly denser aggregates, are protective [78]. Further studies of this issue will be very important for determining novel treatment strategies in the future.

In an effort to further understand the mechanism by which proteinopathy leads to liver injury in ATD, the Perlmutter laboratory has investigated the mechanisms by which misfolded ATZ undergoes intracellular degradation. We hypothesized [5, 45] that differences in efficiency of the intracellular ATZ degradation could explain the differences in onset and severity of liver disease among Z homozygotes that were identified by the screening/cohort studies of Sveger in Sweden [3, 12]; in other words, genetic and environmental modifiers of the hepatic phenotype of ATD target intracellular degradative mechanisms (Fig. 7.1). The hypothesis was initially validated by studies showing that ATZ was degraded more slowly in ATD individuals with liver disease than in ATD individuals without apparent liver disease, using skin fibroblast line models engineered for the expression of ATZ using retroviral-mediated gene transfer [45]. Recently, studies by Tafaleng et al. have provided further validation of this hypothesis by showing slower degradation of ATZ in iPS-derived hepatocytes (iHeps) from ATD individuals with liver disease as compared to iHeps from ATD individuals without liver disease [46]. Interestingly, the rates of ATZ degradation in iHeps were almost identical to those in skin fibroblasts published 20 years ago with a half time of disappearance of ~4 h in those affected by liver disease compared to ~2 h in those without apparent liver disease. Furthermore, Tafaleng et al. found that large intracellular globular inclusions were only seen in the iHeps of the liver disease patients. In addition to showing that degradation may be relatively impaired in ATD individuals with liver disease, these results suggest the iHeps may be used to make premorbid predictions of liver disease susceptibility.

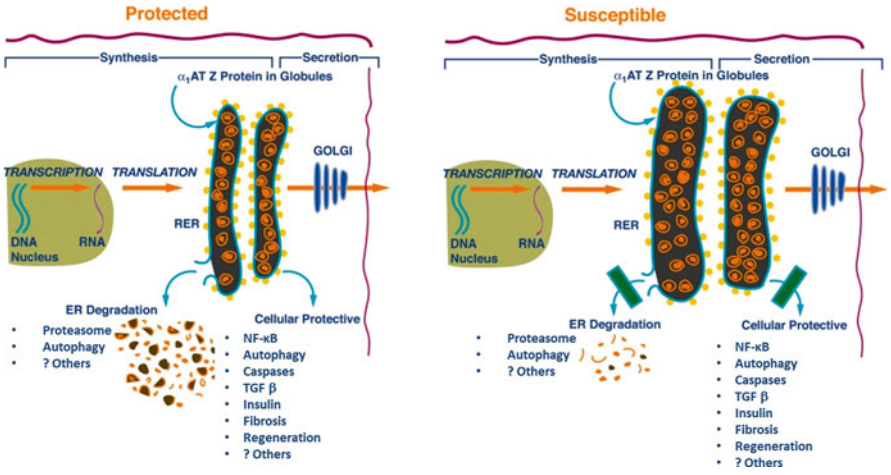


Fig. 7.1 Cellular factors that determine whether an AT-deficient individual is protected or susceptible to liver disease. In the susceptible host, there is greater accumulation of insoluble ATZ in the ER because of subtle alternations in putative proteostasis network regulatory mechanisms. Here these proteostasis regulatory mechanisms are envisioned as either ER degradation pathways or cellular protective responses. Reproduced from reference [57] with permission

Investigations of the mechanisms of ATZ degradation have shown that the proteasomal and autophagic pathways play critical roles. Early studies using yeast and mammalian cell lines showed that the proteasomal pathway participates in intracellular disposal of ATZ by a process that is now known as ER-associated degradation (ERAD) in which the substrate is extracted retrograde from the ER to the cytoplasm [79–81]. In fact, ATZ was one of the first identified substrates of the ERAD pathway [82].

Later, autophagy was discovered to be a second major pathway for disposal of misfolded ATZ [83]. Autophagy is an intracellular catabolic pathway by which cells digest subcellular structures and cytoplasm to generate amino acids as a survival mechanism. It is characterized by double-membrane vacuoles called autophagosomes which fuse with lysosomes for degradation of the internal constituents. Autophagy was firstly implicated in ATD when a marked increased autophagosomes were observed in human fibroblast cell lines engineered for the expression of mutant ATZ [83]. Increased autophagosomes were also observed in the liver of PiZ transgenic mice and in liver biopsy specimens from patients with ATD [83]. Definitive evidence for the role of autophagy in ATZ disposal was provided by genetic studies in which ATZ disposal was delayed in autophagy-deficient (Atg5-null) murine embryonic fibroblast cell lines [84] and Atg6-null yeast strains [85, 86]. Furthermore, by mating a transgenic mouse with liver-specific inducible expression of ATZ to the GFP–LC3 mouse that is characterized by GFP+ autophagosomes, we showed that accumulation of ATZ in liver cells is sufficient to activate the autophagic response [84]. The role of autophagy in intracellular ATZ degradation has been further validated recently by studies demonstrating that autophagy

enhancer drugs promote intracellular ATZ disposal and attenuate hepatic fibrosis in the PiZ mouse model of ATD in vivo [60, 87, 88]. One of the concepts originating from studies of ATZ disposal in autophagy-deficient yeast strains is that autophagy becomes particularly important at higher levels of ATZ expression [85]. These results taken together with the structural constraints of the proteasome have led to the supposition that the proteasomal pathway degrades soluble monomeric forms of ATZ, whereas autophagy is needed for soluble and insoluble polymers. However, it is also possible that autophagy also plays a role in the disposal of soluble monomeric ATZ that accumulates at levels of expression that exceed the capacity of the proteasome. Another important result from the studies by Kruse et al. in autophagy-deficient yeast also showed that a misfolded fibrinogen variant associated with liver disease in a rare inherited form of hypofibrinogenemia is degraded by autophagy in a manner almost identical to that of misfolded ATZ [86].

Several lines of evidence have suggested the existence of pathways for intracellular disposal of ATZ other than the proteasome and autophagy. Indeed, a sortilin-mediated pathway from Golgi to lysosome was recently shown to contribute to the degradation of ATZ in yeast and mammalian cell line models [85, 89]. Another non-proteasomal, non-autophagic pathway for ATZ disposal was recently identified in a powerful *C. elegans* model of ATD and found to be present in a mammalian cell line model as well [47]. Interestingly this pathway is suppressed by insulin signaling, and when upregulated by knocking down components of the insulin signaling pathway, it can completely mitigate ATZ proteotoxicity.

Studies designed to identify genetic and environmental modifiers of the liver disease phenotype in human populations have only recently been reported. In one interesting study, a single nucleotide polymorphism (SNP) in the *MAN1B1* gene was found to be overrepresented statistically in a series of infants with end-stage liver disease [90, 91]. This SNP was shown to decrease the levels of expression of the *MAN1B1* gene product, ER mannosidase I, and a recent study has provided evidence that such a polymorphism would theoretically impair ERAD/proteasomal degradation of misfolded proteins [92]. These results would appear to validate our hypothesis that intracellular degradation pathways would be targeted by modifiers of the liver disease phenotype. However, this SNP was not identified as a statistically significant modifier in another population [93] and will therefore likely require further population-based evaluation.

An SNP in the upstream flanking region of the AT gene has also been implicated in susceptibility to liver disease [94], but the nature of variant contradicted the rationale for how it might affect liver disease susceptibility. Furthermore, this study could lead to a different conclusion with legitimate alternate ways of classifying one of the populations used in the analysis.

Our hypothesis for variation in liver disease susceptibility also identifies signaling pathways that could increase or decrease ATZ proteotoxicity as potential targets for disease modifiers (Fig. 7.1). In order to begin to elucidate such pathways, we developed cell line and mouse models with inducible expression of ATZ as ideal systems. From these systems we now know that accumulation of ATZ specifically and selectively activates the autophagic response [84], NFκB signaling pathway and

cleavage of murine caspase-12 [50]. Gene expression profile analysis in the liver of the Z mouse with hepatocyte-specific inducible expression of ATZ implicated the TGFβ signaling pathway [64]. These signaling pathways could also act on ATZ proteotoxicity through a degradative mechanism. For instance, one of the genes most affected by the induction of ATZ accumulation in gene expression profile analysis was the regulator of G signaling RGS16 which likely acts by activating hepatic autophagy [64]. Insulin signaling could also affect ATZ proteotoxicity through an intracellular disposal mechanism [47]. Further studies of these types of proteostasis mechanisms could lead to the identification of additional modifiers in the future.

Pathogenesis of Hepatocellular Carcinoma in ATD

Although increased susceptibility to hepatic cancer was shown years ago [2], there have been only a few studies of potential mechanisms for carcinogenesis. Theorizing that hepatocellular hyperproliferation was likely to be involved, Rudnick et al. investigated the proliferation of liver cells by BrdU labeling in the PiZ mouse model of ATD [95]. In these studies hepatocellular proliferation was increased ~7-fold in the PiZ mouse compared to a wild-type control, and this degree of proliferation appeared to reflect the slowly progressing chronic nature of ATD liver disease. Most importantly, by using double immunohistochemical staining, it was shown that dividing hepatocytes were almost exclusively the ones that lacked intracellular ATZ inclusions, called the globule-devoid hepatocytes. Furthermore, it was shown that hyperproliferation of globule-devoid hepatocytes was driven by the number of adjacent

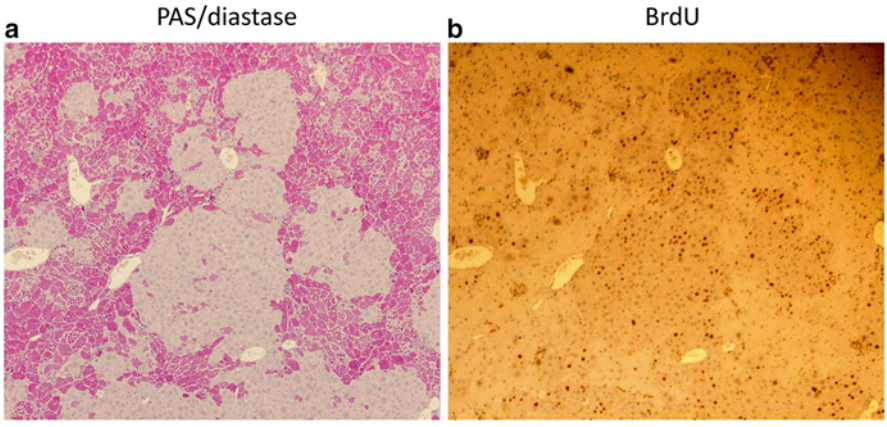


Fig. 7.2 Hepatocellular proliferation in the PiZ mouse model of AT deficiency. A liver section, stained with (**a**) PAS/diastase and (**b**) BrdU (brown staining of nuclei), shows proliferation only in globule-devoid hepatocytes or in inflammatory microabscesses. The tendency toward nodular areas of globule-devoid hepatocytes can be appreciated. Courtesy of Tunda Hidvegi, George Michalopoulos. Reproduced from Ref. [4] with permission

globule-containing hepatocytes. This last conclusion was based on the observation that the number of globule-containing hepatocytes was markedly increased in male PiZ mice or in testosterone-treated female PiZ mice, and this correlated directly with the degree of hyperproliferation of globule-devoid hepatocytes (Fig. 7.2).

Taken together these observations led to a theory that hepatocyte hyperproliferation was elicited by cross talk between globule-containing and globule-devoid hepatocytes ([96] and Fig. 7.3). The globule-devoid hepatocytes were viewed as younger cells capable of responding to trans-acting regenerative signals derived from the globule-containing hepatocytes. The globule-containing hepatocytes were viewed as having greater ATZ accumulation and proteotoxicity and unable to respond to the existing regenerative signals because of the proteotoxic effect on cell proliferation. Thus, the globule-containing hepatocytes are sick but not dead and stimulate the regeneration of the globule-devoid hepatocytes which have a selective proliferative advantage. Interestingly, the replicative defect in the globule-containing hepatocytes was shown to be relative because these cells could proliferate, as well as globule-devoid hepatocytes when regenerative stimuli were particularly powerful in PiZ mice that survived experimental partial hepatectomy [96]. The nature of the differences between the globule-containing and globule-devoid cells is not well elucidated. A study by Linblad et al. has suggested that the globule-devoid hepatocytes have lesser accumulation of ATZ [63], and this would be consistent with younger cells that have had less time to accumulate ATZ. It is also possible that globule-devoid hepatocytes are derived from globule-containing hepatocytes as they increase capacity for the degradation of ATZ. Several observations militate against this latter possibility. The number of globule-containing hepatocytes decrease with age [95], and Ding et al. [97] showed that transplanted hepatocytes have a selective proliferative advantage that also depends on the number of adjacent globule-containing hepatocytes in that it was much more evident in male PiZ mice that had significantly more globule-containing hepatocytes than female PiZ mice. This is associated with enhanced apoptosis of the host hepatocytes, hepatic repopulation with donor hepatocytes, and resolution of the liver fibrosis that occurs in untreated PiZ mice [97].

It is interesting to note that HCC develops with aging in male PiZ mice [61], and males with ATD were also disproportionately affected by hepatic cancer in the autopsy studies of Eriksson et al. [2]. Moreover, in cases of HCC associated with ATD, a staining pattern in which the carcinoma is negative for inclusions but surrounded by adjacent liver cells that are positive for inclusions (Fig. 7.4) is consistent with the theory proposed by Rudnick and Perlmutter [96].

We believe that the NFκB signaling pathway may be particularly important in the mechanism of hepatic carcinogenesis. This pathway is powerfully activated when ATZ accumulates in the liver [50]. However, genomic analysis of liver after controlled induction of ATZ accumulation identified a relatively limited repertoire of downstream targets of this pathway [64]. One of those targets, early growth response Egr1, was markedly downregulated [64], and it is known to be a transcriptional activator of hepatocyte regenerative activity [98]. This could mean that NFκB activation leads to decreased Egr1 expression and relatively decreased proliferation of globule-containing hepatocytes in ATD.

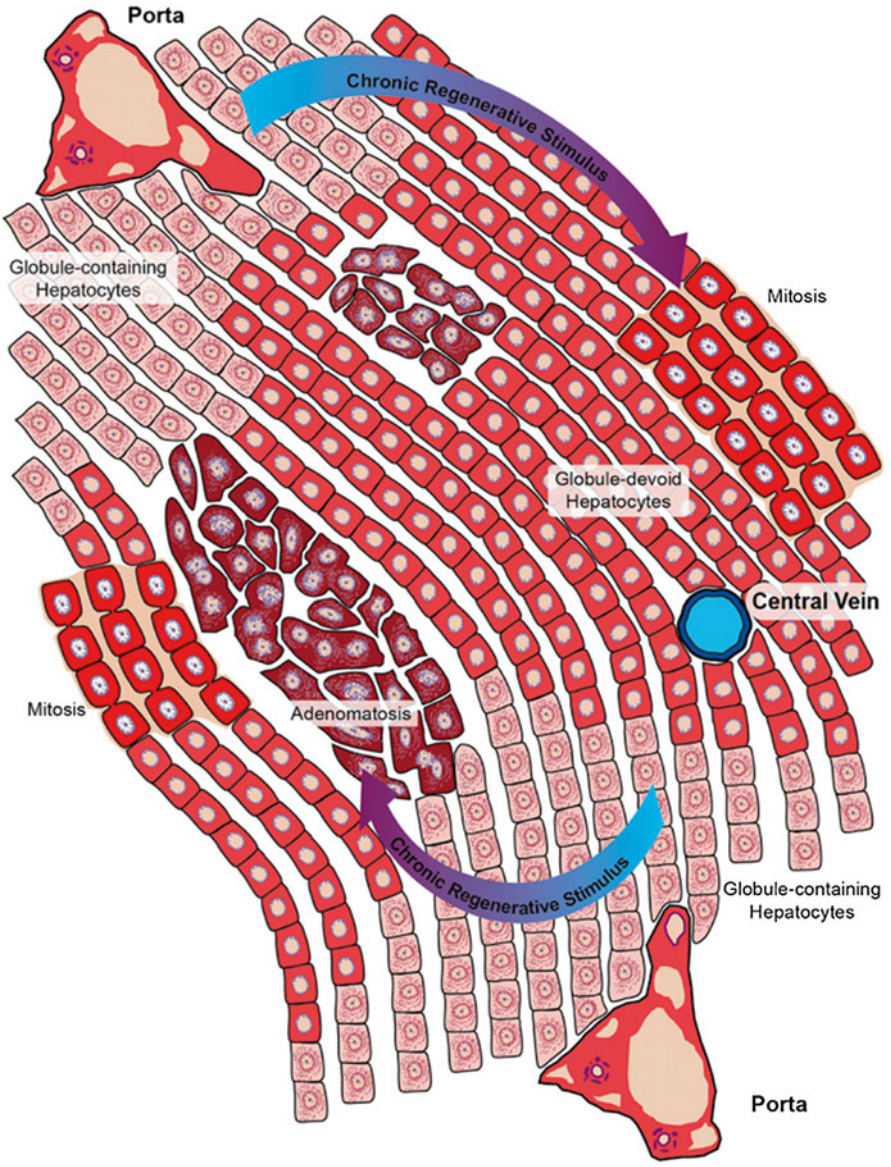


Fig. 7.3 Hypothetical model for hepatocarcinogenesis in ATD. Globule-containing hepatocytes (*pale pink*) tend to be periportal. They are "sick but not dead" and generate chronic regenerative signals which can only be received effectively in "trans" by globule-devoid hepatocytes (*deep pink*). The globule-devoid hepatocytes tend to be in the centrilobular regions. When regenerative signals are received by globule-devoid hepatocytes by this cross talk, it drives mitosis and ultimately carcinogenesis (*dark red*) in the globule-devoid regions. Reproduced from Ref. [93] with permission

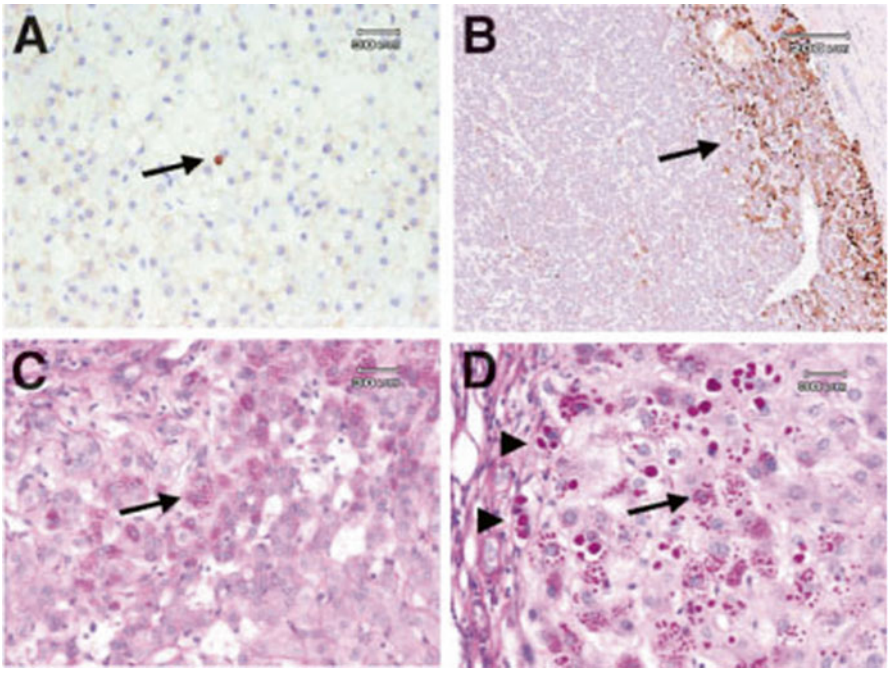


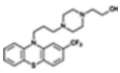
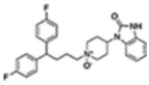
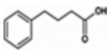
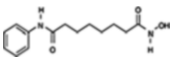
Fig. 7.4 Proliferation of hepatocytes in hepatocellular carcinoma associated with ATD. (**a**) The low proliferation rate of the tumor is demonstrated by the staining of only a single hepatocyte within the HCC (*arrow*) in this field (original magnification × 400); (**b**) immunohistochemistry for AT shows intracytoplasmic deposits in the normal hepatocytes at the periphery of the tumor; (**c**) and (**d**) PAS/diastase staining shows that there are deposits in the tumor cells, but they are fine granules (*arrow*) as compared to the globules of varying size in the surrounding normal hepatocytes (*arrowhead*) (**d**). Reproduced from Ref. [9] with permission

Therapeutic Strategies for Liver Disease of ATD

Orthotopic liver transplantation has been used for treatment of ATD-associated progressive liver dysfunction and liver failure with 5-year survival rates greater than 90 % for children and greater than 80 % for adults [99]. Interestingly, a number of patients with severe ATD liver disease experience low rates of disease progression and lead relatively normal lives for extended time period [13]. These individuals can therefore sometimes be observed and supported without transplantation, especially if living related donor transplantation is an option for them.

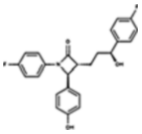
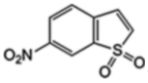
Several novel strategies for treatment of ATD liver disease that would obviate the need for organ transplantation and chronic immunosuppression are currently under investigation and at various stages of development. One of the relatively newer strategies involves autophagy enhancer drugs (Table 7.2). Autophagy was considered an excellent target because it is specifically activated when ATZ accumulates

Table 7.2 Therapeutic drug candidates

Name	Structure	Mechanism	Disease models tested	References
Carbamazepine (CBZ)		A well-known mood-stabilizing agent reported to induce autophagic disposal of insoluble ATZ and proteasomal degradation of soluble ATZ	Alpha-1 antitrypsin deficiency: mammalian cell lines; mouse model	Hidvegi et al. 2010 [57]
Fluphenazine (FLU)		Phenothiazine compound and identified as an autophagy inducer in high-throughput screening. Mechanism(s) of autophagic induction yet to be determined	Alpha-1 antitrypsin deficiency: high-throughput drug screen, *C. elegans* model; mammalian cell lines; mouse model	Gosai et al. 2010 [97] Li et al. 2014 [83]
Pimozide (PMZ)		Phenothiazine compound and identified as an autophagy inducer in high-throughput screening. Mechanism(s) of autophagic induction yet to be determined	Alpha-1 antitrypsin deficiency: high-throughput drug screen; *C. elegans* model	Gosai et al. 2010 [97]
4-Phenylbutyric acid (PBA)		Nonselective chemical chaperone that facilitates protein folding and reported to enhance secretion of ATZ	Alpha-1 antitrypsin deficiency: mammalian cell lines; mouse model	Burrows et al. 2000 [115] Teckman 2004 [116]
Suberoylanilide hydroxamic acid (SAHA)		Histone deacetylase 7 inhibitor, pharmacologically similar to PBA, and reported to enhance ATZ secretion and synthesis	Alpha-1 antitrypsin deficiency: mammalian cell lines	Bouchecareilh et al. 2012 [117]

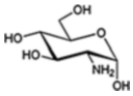
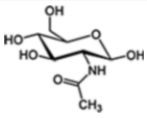
(continued)

Table 7.2 (continued)

Name	Structure	Mechanism	Disease models tested	References
Tat-beclin 1 peptide	YGRKKRRQRRR-GG-TNVFNATF EIWHDGEFGT	Novel autophagy-inducing peptide, which binds GAPR-1 releasing beclin-1 from Golgi, leading to increased beclin-1 in the cytosol which in turn induces autophagy	Infectious disease: mammalian cell lines; mouse model Huntington's disease: mammalian cell lines; mouse model	Shoji-Kawata et al. 2013 [108]
Ezetimibe		A cholesterol-reducing agent. Inactivates NPC1L1 by direct binding and reduces MTOR recruitment to lysosomes, thereby inhibiting mTORC1 activity, which leads to activation of autophagy	Alpha-1 antitrypsin deficiency: human primary hepatocytes	Yamamura et al. 2014 [110]
Spermidine		A naturally occurring polyamine; activates autophagy by altering cellular network of deacetylases and acetylases	Aging models: yeast model; mouse model; *C. elegans* model; mammalian cell lines	Eisenberg et al. 2009 [105] Morselli et al. 2011 [106]
Stattic		Chemical inhibitor of STAT3. It promotes PKR-dependent phosphorylation $eIF2^{\alpha}$ and consequent activation of autophagy	High-throughput drug screen; mammalian cell lines	Shen et al. 2012 [107]
Omega-6-polyunsaturated fatty acids		A class of PUFA that has been reported to activate autophagy. The molecular mechanism of autophagy induction not known	Aging model: *C. elegans* Mammalian cell lines	O' Rourke et al. 2013 [108]

(continued)

Table 7.2 (continued)

Name	Structure	Mechanism	Disease models tested	References
Glucosamine		An amino sugar which alleviates arthritic pain and improves skin quality. Induces autophagy in an mTOR-independent manner	Mammalian cell lines	Shintani et al. 2010 [109]
N-acetyl glucosamine		An amino sugar which reduces arthritic pain and improves skin conditions. Induces autophagy in an mTOR-independent manner	Mammalian cell lines	Shintani et al. 2010 [109]

in cells, and it also plays a critical role in intracellular disposal of ATZ. At the time when this approach was first investigated, several drugs which could enhance autophagic degradation of other misfolded proteins, such as mutant polyQ proteins that cause Huntington disease, were being described [100]. It had also become apparent that ATD liver disease could more frequently have its onset at 50–65 years of age coincident with the decline in autophagy function that is believed to trigger other age-dependent degenerative diseases associated with misfolded proteins. Hidvegi et al. first investigated the drug carbamazepine (CBZ), which is known for its widespread use in humans as an anticonvulsant and mood stabilizer, and found that it enhanced autophagic degradation of ATZ in mammalian cell line models of ATD [60]. Moreover, administration of this drug by oral gavage to the PiZ mouse model of ATD over a 3-week period significantly reduced hepatic ATZ load and hepatic fibrosis in vivo [60]. Because CBZ is already FDA-approved, it could be moved immediately into a Phase II/III clinical trial for the treatment of severe liver disease due to ATD.

The notion that autophagy enhancer drugs can mitigate proteotoxicity caused by intracellular accumulation of misfolded proteins has been bolstered by recent studies in *C. elegans* model system. High-throughput automated screening of a drug library (LOPAC) containing 1280 compounds in a *C. elegans* model system for ATD identified five hit compounds that decreased cellular ATZ load in a dose-dependent manner [101]. Four of these five compounds have autophagy enhancer activity and are already FDA approved for use in clinical practice. Interestingly, two of the hit compounds, fluphenazine and pimozide, belong to the phenothiazine drug family, which has structural similarity to tricyclic antidepressants, the structural family to which CBZ belongs. The phenothiazines were also previously shown to accelerate autophagic disposal of polyQ proteins [102, 103]. Fluphenazine has been

tested extensively in the *C. elegans* model of ATD, in mammalian cell line models, and also in the PiZ mouse. The results show that fluphenazine reduces hepatic ATZ load and hepatic fibrosis in vivo in all the model systems tested [87]. Altogether, the results from these studies show promising prospects for this type of high-throughput screening strategy and two novel strategies for chemical and computational-based drug discovery using the autophagy enhancer drug paradigm and the phenothiazine structure for discovering additional drugs for treating liver disease due to ATD [88, 104].

Several other drugs that increase autophagy have recently been reported and could therefore be candidates for treating liver disease due to ATD. Eisenberg et al. showed that spermidine induces autophagy in several model systems including yeast, worms, and mammalian cell systems and increases lifespan in flies, worms, and mice [105]. Furthermore, Morselli et al. has reported that spermidine and resveratrol can synergistically induce autophagy in mammalian cell culture, mouse, and *C. elegans* model systems by altering the cellular network of deacetylases and acetylases [106]. Using a high-throughput screening strategy for autophagy enhancers, Shen et al. discovered that chemical inhibitors of transcription factor STAT3 such as JSI-124, Stattic, and WP1066 were potent inducers of autophagy [107]. Similarly, omega-6-polyunsaturated fatty acid has been reported to enhance autophagy in mammalian cell lines and *C. elegans* model systems [108]. In a *C. elegans* model system, omega-6-polyunsaturated fatty acids promoted longevity through this mechanism [108]. Glucosamine and *N*-acetylglucosamine have also been shown to enhance autophagy in mammalian cell lines [109]. A cholesterol-reducing drug, ezetimibe, has been shown to activate autophagy. Studies by Yamamura et al. showed that this drug enhances autophagy specifically in hepatocytes and intestinal epithelial cells [110], consistent with the inhibitory effect of this drug on cholesterol-binding protein Niemann–Pick-type C1-like 1 (NPC1L1) [111]. The authors reported that inhibition of NPC1L1 by ezetimibe reduced recruitment of mTOR to the lysosomes, leading to inhibition of mTORC1 activity and hence activation of autophagy. Importantly, the authors show that ezetimibe reduced cellular ATZ load in primary cultures of human hepatocytes engineered for ATZ expression. Further studies are required to establish effectiveness of this drug in reversing the proteotoxic effect of ATZ accumulation in animal model systems. A novel autophagy-inducing peptide Tat-beclin 1 enhances degradation of mutant huntingtin and several invasive viral and bacterial pathogens in vivo [112]. This peptide, or drugs based on its structure, has the potential for treatment of a broad range of human diseases caused by proteotoxicity and infectious pathogens.

A number of relatively new gene therapy strategies are being investigated. One of these involves new methods for silencing gene expression using vectors that are also capable of encoding wild-type AT to address both gain- and loss-of-function sequelae of ATD, respectively [113, 114]. In one approach, Li et al. utilized adeno-associated virus harboring short-hairpin RNA to knockdown endogenous ATZ expression together with a codon-optimized wild-type AT transgene cassette [113]. In another approach, Mueller et al. utilized an adeno-associated virus harboring microRNA to silence endogenous ATZ gene expression together with a microRNA-resistant wild-type AT gene [114]. In each case hepatic ATZ load was

reduced and levels of human AT increased in the serum of a transgenic mouse model of ATD. However, the effect on liver fibrosis by this strategy was not as compelling, and hence further studies are required to test whether more potent and widespread silencing would be more effective. Another study using antisense oligonucleotides by systemic administration to silence ATZ gene expression had a more impressive effect in reducing hepatic fibrosis in the PiZ mouse model system [115].

Another potential gene therapy approach being investigated is the transfer of genes that activate autophagy and therein reduce ATZ accumulation and proteotoxicity. Pastore et al. have championed this approach using TFEB, a master transcriptional activator of the autophagolysosomal system [116]. Using helper-dependent adenovirus for systemic delivery of TFEB and targeting of its expression to liver, this approach significantly reduced hepatic ATZ load and liver fibrosis in the PiZ mouse model. In vitro studies also validated that TFEB reduces cellular ATZ levels in an autophagy-dependent manner [116]. Although it will not address the loss-of-function mechanisms associated with ATD lung disease, gene therapy with TFEB or drugs that target TFEB activation constitute exciting potential therapeutic strategies for liver disease associated with ATD.

Ultimately genomic editing will be considered to definitively correct the genetic defect that causes ATD. The most recent development in this area, CRISPR/Cas-9-mediated genome editing, has been used in a mouse model in vivo to correct the genetic defect in dystrophin (Dmd) that causes Duchenne muscular dystrophy (DMD) [117]. Using this approach, the Dmd mutation was corrected in the germline of the mdx mouse model of DMD and produced genetically mosaic animals containing 2–100 % correction of the Dmd gene. The authors reported that only a subset of corrected cells in vivo could perform a complete phenotypic rescue. Hence, the extent of phenotypic rescue in the mosaic mice exceeded the efficiency of gene correction. One possibility for this is that the corrected muscle cells have a selective proliferative advantage in the presence of injured muscle cells. If the technological advancement eventually provides a way for genome editing of postnatal somatic cells, in the future this strategy could be used for correcting other diseases caused by single gene mutation events including ATD.

Several research groups are exploring a "structure-based" screening strategy that aim at generating peptides to prevent polymerization of the mutant ATZ with the hypothesis that this would facilitate secretion. A small-molecule compound designed against a lateral hydrophobic cavity in ATZ prevented its polymerization; however, further experiments in a cell line model revealed that this compound enhanced intracellular degradation only, with minimal effect on secretion [56]. These results provide evidence that it is the misfolding of ATZ, independent of its tendency to polymerize, which is primarily responsible for impaired secretion. Another small molecule based on a peptide that targets the reactive center loop of AT has been designed and introduced in cell line model systems with evidence for improved secretion of ATZ [118]. However, the efficacy of this peptide in an animal model system for either increasing secretion or reducing liver damage in vivo remains to be tested. It also remains possible that this type of peptide binding changes the conformation of the mutant protein in such a way that both misfolding and polymerization are reduced independently.

Chemical chaperones that can nonselectively facilitate folding of diverse misfolded proteins have also been investigated as a potential therapeutic option for ATD liver disease. Glycerol and 4-phenylbutyric acid (PBA) were found to mediate a robust enhancement in the secretion of ATZ in a mammalian cell line model, and its oral administration in PiZ mice increased blood levels of human AT reaching 20–50 % of the levels present in PiM mice and normal humans [119]. However, a pilot clinical trial involving 10 patients with ATD-associated liver disease failed to reveal any significant increase in serum levels of AT after 14 days of treatment with PBA [120]. It is not clear why the drug lacked effect but the large doses required are known to be quite challenging to tolerate and so it may be worthwhile to test in the future if newer, more tolerable formulations are developed. Recently, suberoylanilide hydroxamic acid (SAHA), another drug which has many pharmacological similarities to PBA, has been found to enhance secretion of ATZ in cell line models of ATD [121]. However, SAHA has not yet been tested in animal models. Moreover, detailed studies are needed to delineate whether this effect is due to increased synthesis of ATZ or due to its ability to reduce ATZ accumulation in cells or both. If increased secretion of ATZ is because of, or even associated with, increased synthesis, the treatment could produce more, rather than less, cellular proteotoxicity.

Hepatocyte transplantation therapy has also been investigated as a potential treatment for ATD. It has been tested in the past as a treatment for several metabolic liver diseases [122, 123]. Compared to orthotopic liver transplantation, it has the advantage of being a minimally invasive procedure with little known morbidity and is considerably less expensive than protein (AT) replacement therapy or liver transplantation. Importantly, recent studies have revealed that wild-type donor hepatocytes can repopulate almost the entire liver of the PiZ mouse model of ATD [97]. In the PiZ mouse model, the donor cells replaced both globule-containing and globule-devoid cells, indicating that both types of affected hepatocytes have impaired proliferative capacity compared to wild-type hepatocytes. Because the transplanted hepatocytes have a selective proliferative advantage over ATZ-containing endogenous hepatocytes and can substitute for the latter in a diseased liver, this option of therapy may be considered for ATD lung and liver disease.

Another exciting therapeutic strategy in which genomic editing is combined with hepatocyte transplantation has been tested in a transgenic mouse model of ATD. Studies by Yusa et al. have shown that the mutation in the AT gene could be corrected in human induced pluripotent stem (iPS) cells derived from an ATD patient using a combination of zinc-finger nucleases and transposon techniques [124]. Importantly, the corrected iPS cell lines could then be engrafted into the liver of the transgenic mouse model system, and, based on the observations of Ding et al. [97], the corrected cells should expand significantly because they will have a selective proliferative advantage. This strategy, if it proves successful in further preclinical models, has the potential to address both the loss- and gain-of-function mechanisms of organ damage and the advantage of personalized treatment options without any need for immunosuppression.

Summary

A subgroup of individuals with homozygous PIZZ ATD develop liver disease characterized by fibrosis/cirrhosis and HCC. Genetic and environmental modifiers are probably responsible for rendering the subgroup susceptible to liver disease. This liver disease is more common in adults than previously recognized, and this may mean that it is part of a collection of degenerative diseases that are impacted by the age-dependent decline in proteostasis mechanisms such as autophagy. The disease that affects infants, older children, and adolescents probably results from particularly powerful, rare combinations of modifiers. An impressive number and repertoire of therapeutic strategies have recently moved into various stages of development, including one autophagy enhancer drug that is already in an active phase II/III clinical trial. We believe these efforts will soon begin to improve the outcome for individuals with severe liver disease due to ATD.

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