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REVIEW

Alpha-1 antitrypsin deficiency: A conformational disease associated with lung and liver manifestations

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Summary Alpha-1 antitrypsin (A1AT) is a serine antiprotease produced chiefly by the liver. A1AT deficiency is a genetic disorder characterized by serum levels of less than 11 μmol/L and is associated with liver and lung manifestations. The liver disease, which occurs in up to 15% of A1AT-deficient individuals, is a result of toxic gain-of-function mutations in the A1AT gene, which cause the A1AT protein to fold aberrantly and accumulate in the endoplasmic reticulum of hepatocytes. The lung disease is associated with loss-of-function, specifically decreased anti-protease protection on the airway epithelial surface. The so-called 'Z' mutation in A1AT deficiency encodes a glutamine-to-lysine substitution at position 342 in A1AT and is the most common A1AT allele associated with disease. Here we review the current understanding of the molecular pathogenesis of A1AT deficiency and the best clinical management protocols.

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Abbreviations

A1AT	alpha-1 antitrypsin
ATRA	all *trans*-retinoic acid
BAL	bronchoalveolar lavage
COPD	chronic obstructive pulmonary disease
EDEM	ER degradation-enhancing α-mannosidase-like protein
eIF2α	translation initiation factor e2-α
EOR	ER overload response
ER	endoplasmic reticulum
ERAD	ER-associated degradation
ERSE	ER stress response element
FENIB	familial encephalopathy with neuroserpin inclusion bodies
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
GOLD	Global initiative for Chronic Obstructive Lung Disease
HRCT	high-resolution computed tomography
Ire1	inositol-requiring kinase 1
NE	neutrophil elastase
PERK	PKR-like ER kinase

PR-3	proteinase-3
RNAi	RNA interference
siRNA	small interfering RNA
TMAO	trimethylamine oxide
UPR	unfolded protein response
UPRE	unfolded protein response element

Introduction

Alpha 1-Antitrypsin (A1AT) deficiency (OMIM +107400) is a lethal hereditary disorder characterized by low plasma levels of A1AT (Laurell and Eriksson 1963). The condition is associated with a substantially increased risk for the development of pulmonary emphysema by the third or fourth decade of life and is also associated with risks for development of hepatic disease, cutaneous panniculitis, arterial aneurysm, bronchiectasis, and renal disease. A1AT deficiency is a genetic disorder characterized by misfolding of the A1AT protein and it belongs to a class of genetic diseases associated with aberrant protein folding which are collectively known as conformational disorders.

A1AT

The A1AT gene is a 12.2-kilobase-pair gene composed of 7 exons and 6 introns, encoded by the protease inhibitor (Pi) locus located on chromosome 14q32.1 (Darlington et al 1982; Schroeder et al 1985). The gene is expressed in cells of several lineages, with expression being highest in hepatocytes (Rogers et al 1983). This is consistent with the fact that A1AT is an acute-phase reactant. Translation of the gene results in a 418-amino-acid protein that includes a signal peptide. The A1AT protein is glycosylated and posttranslationally modified in the endoplasmic reticulum (ER) and its carbohydrate side-chains are modified in the cis-Golgi apparatus before being packaged and released. The final product of the gene is a 52 kDa glycosylated protein. Serum A1AT is almost totally derived from hepatic production; however, A1AT is also actively transcribed and secreted by other cells, including mononuclear phagocytes, enterocytes, renal parenchymal cells and intestinal epithelium (Carlson et al 1988; Molmenti et al 1993).

A1AT is the archetype of the serine protease inhibitor or serpin superfamily, members of which have closely related structures and functions. A1AT has a structural conformation that allows it to tightly grasp and pseudo-irreversibly inhibit serine proteases including neutrophil elastase (NE), cathepsin G and proteinase 3 (PR-3) (Carrell 1986). A1AT functions by presenting its reactive centre residue on an exposed loop of the molecule such that it forms an ideal substrate for proteolytic enzymes. The exact fit between enzyme and inhibitor results in a tightly bound complex, which inhibits the enzyme and allows it to be eliminated from the circulation.

Genetics of A1AT deficiency

Since Laurell and Eriksson (1963) first made the association between low levels of A1AT protein and emphysema we have learned a considerable amount about the genetic mechanisms underlying this under-diagnosed disease. A1AT deficiency is a classic monogenic disorder. The A1AT gene is highly pleomorphic, with approximately 100 alleles identified to date. Variants are inherited in an autosomal co-dominant fashion, i.e. the products of both alleles are expressed, and the protein phenotype is classified according to the 'Pi' system, as defined by plasma isoelectric focusing.

A1AT genotypes that confer an increased risk for developing pulmonary emphysema and/or liver disease are those in which deficiency or null alleles are combined in homozygous or heterozygous states, and encode A1AT plasma levels below a protective threshold of 11 μmol/L (Crystal 1998). On the basis of plasma levels and function of A1AT, variants are categorized as follows: (a) Normal: commonly M types which account for 95% of alleles in caucasian individuals and are characterized by normal plasma levels (more than 20 μmol/L); (b) Deficient: ZA1AT is a common deficiency variant, with plasma levels of homozygotes in the range of 5–6 μmol/L. The 'S' variant is also common and PiSS individuals have A1AT plasma levels of 8–11 μmol/L; (c) Null: these are variants associated with no detectable circulating A1AT in the plasma and are not associated with liver disease, e.g. QO_{lisbon}, a Thr68Ile exon II mutant; or (d) Dysfunctional: the unique Pittsburgh mutation (Met358Arg) which converts A1AT into an inhibitor of thrombin rather than elastase (Owen et al 1983). Null and dysfunctional mutations are rare. The majority of patients with A1AT deficiency are usually either homozygous or heterozygous PiZ or PiS.

The distribution of A1AT variants probably reflects the genetic origins of the disorder. The highest incidence of A1AT deficiency is in Europe, with up to 6% of people of European descent carrying at least one copy of the S gene and 3–4% carrying at least one copy of the Z variant (Hutchison 1990; de Serres 2002). The highest prevalence of the Z allele is in northern and western European countries with a mean

gene frequency of 0.014 or 14 per 1000, which using Hardy–Weinberg principles would yield an estimated ZZ homozygote prevalence of 1 in 5000 (de Serres 2002). The highest frequency of PiS is found in southern Europe, particularly in the Iberian Peninsula, suggesting that the mutation is likely to have arisen in that region. The mean gene frequency of PiS in southern Europe is 0.056 or 56 per 1000, yielding an estimated SS homozygote prevalence of 1 in 320 (Hutchison 1998). It must also be remembered that these gene frequencies vary widely as A1AT deficiency is so under-recognized.

Molecular basis of A1AT deficiency

Specific mutations of the A1AT gene that occur include base substitutions, in-frame deletions, frameshift mutations and exon deletions. The medically interesting variants associated with deficiency, are the S and Z genes commonly found in Europeans and the uncommon Null (non-production gene). Both S and ZA1AT result from single amino acid substitutions. In the S variant there is a substitution of a valine residue for glutamate at position 264 (Val264Glu) (Curiel et al 1989). The Z mutation (Glu342Lys) results from the substitution of a positively charged lysine for a negatively charged glutamine at the base of the reactive centre loop. This mutation distorts the relationship between the loop and the β-pleated 'A' sheet that forms the major feature of the molecule. The consequent perturbation in structure allows the loop of one molecule to interlock with the 'A' sheet of another to form fibril-like polymers (Lomas et al 1992). The formation of these loop–sheet polymers is temperature- and concentration-dependent and is likely to occur in the ER of hepatocytes. Chains of polymers become interwoven to form insoluble inclusions that are the pathological hallmark of A1AT liver disease (Fig. 1). Recent evidence has indicated the possibility of polymer formation outside the hepatocyte (Elliott et al 1998; Janciauskiene et al 2002; Mulgrew et al 2004).

A1AT as a conformational disease

A1AT deficiency is classed among a group of disorders referred to as 'conformational diseases' (Carrell and Lomas 2002). Conformational diseases are caused by mutations altering the folding pathway or the final conformation of a protein. Many such diseases are caused by mutations in secretory proteins and range from metabolic diseases such as diabetes to neurological conditions such as Alzheimer disease. Other conformational diseases include cystic fibrosis and hereditary haemochromatosis, which are also associated with intracellular accumulation of misfolded proteins and ER stress (Knorre et al 2002; Kudo et al 2002; Lawless et al 2007). A subclass of conformational disease includes the serpinopathies and is associated with abnormal β-strand linkages in serine proteinases. ZA1AT deficiency is the paradigm for these diseases, which include thrombosis, angio-oedema and emphysema due to loss-of-function of antithrombin, C1 inhibitor and alpha-1 antichymotrypsin, respectively, and the recently characterized gain-of-function dementia 'familial encephalopathy with neuroserpin inclusion bodies' (FENIB) (Miranda et al 2004).

ER stress occurs as a result of an imbalance between the ER protein folding load and the ability to process the load, and is characterized by a number of intracellular responses (Fig. 2). These are distinct but not exclusive, and include the ER overload response (EOR), the unfolded protein response (UPR) and apoptosis (Fig. 2).

EOR

The EOR pathway culminates in activation of the transcription factor NFκB. Latent NFκB resides in the cytosol, complexed to its inhibitor IκB. Activation by a

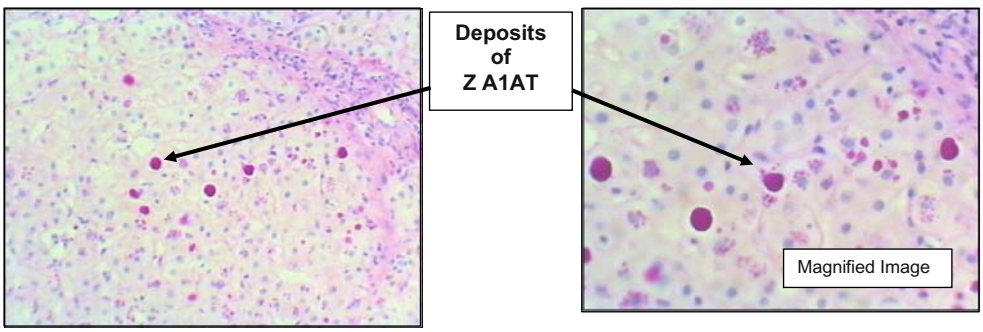


Fig. 1 Diastase-resistant periodic acid–Schiff-stained liver section from a ZA1AT-deficient individual showing A1AT deposits

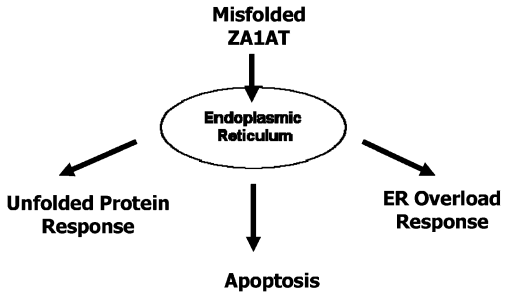


Fig. 2 Stress responses induced by accumulation of misfolded proteins in the ER

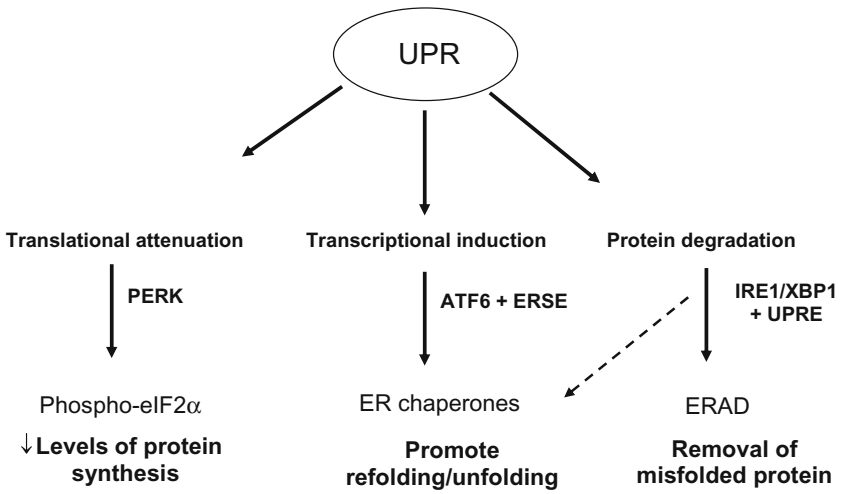
variety of stimuli, including accumulation of misfolded proteins in the ER, leads to phosphorylation and degradation of IκB and nuclear translocation of NFκB. This culminates in expression of NFκB-regulated genes. Expression of ZA1AT has been shown to activate NFκB and induce expression of interleukin-6 and interleukin-8 (Lawless et al 2004).

UPR

The UPR is a tripartite protective system including (i) the translational attenuation of global protein synthesis (Ron 2002); (ii) transcriptional induction of UPR target genes (Mori 2000) and (iii) ER-associated degradation (ERAD) in the proteasome (Kopito 1997) (Fig. 3).

Translational attenuation occurs as an immediate response that reduces the load of host protein synthesis in the ER and prevents further accumulation of unfolded proteins (Harding et al 2002). The type I transmembrane protein PKR-like ER kinase (PERK) phosphorylates translation initiation factor 2 (eIF2) on its alpha subunit (eIF2α) at serine-51, thus inhibiting the initiation of global translation and paradoxically promoting the translation of ATF4 mRNA, a bZIP transcription factor (Harding et al 2000). Targets of ATF4 include CHOP, GADD34, and ATF3 (Jiang et al 2004; Ma et al 2002). Transcriptional induction of UPR target genes involves the ER transmembrane protein inositol-requiring kinase 1 (IRE1) which can regulate chaperone induction, ERAD, and expansion of the ER in response to ER stress (Schroder and Kaufman 2005). IRE1 is an endoribonuclease that targets the basic leucine zipper (bZIP) transcription factor XBP-1 causing it to translocate into the nucleus and bind to ER stress response elements (ERSE) and/or unfolded protein response elements (UPRE), activating the transcription of ER chaperone genes, ER quality control genes, and folding enzymes (Yoshida et al 2001). The function of these gene products is to enhance correct folding of misfolded proteins and restore ER homeostasis. In a later phase of the UPR, components of ERAD are activated. This is the process whereby misfolded ER proteins are detected, prevented from progressing along the secretory pathway, and degraded by the ubiquitin–proteasome system (Kopito 1997; Travers et al 2000). The IRE1-XBP-1 pathway stimulates ERAD, increasing the capacity of ER-stressed cells to degrade irreversibly misfolded proteins. EDEM (ER degradation-enhancing α-mannosidase-like protein) is a type II transmembrane protein localized to the ER and is a key component of the ERAD machinery (Yoshida et al 2003). Rather than being separately dispensable, the UPR and ERAD are delicately coordinated, complementary pathways that eliminate unfolded protein accumulation and prevent its toxic effects. The degradation of misfolded A1AT has been shown to be mediated by EDEM, a postulated Man8B-binding protein that can accelerate degradation of terminally misfolded proteins by promoting their

Fig. 3 Signals activated by the unfolded protein response



release from calnexin in an *N*-glycan dependent manner (Hosokawa et al 2003; Oda et al 2003).

Apoptosis

Apoptosis is important for normal development and tissue homeostasis; however, alterations in the rate of apoptosis in certain tissues can cause disease. Prolonged ER stress leads to cell death, and is linked to the pathogenesis of a number of neurodegenerative conformational disorders, polycystic kidney disease and ischaemia. ER accumulation of ZA1AT is known to induce mitochondrial damage and caspase activation and is likely to play a role in ZA1AT-induced liver cell injury. Recent studies have reported evidence of cleavage and activation of the ER-specific caspase, caspase-4, *in vivo* in ZAAT-deficient patients (Hidvegi et al 2005), but a number of studies have failed to detect terminally apoptotic cells *in vivo*, probably owing to robust survival mechanisms in hepatocytes (Perlmutter 2002; Teckman et al 2004). The mechanism by which ZA1AT ER accumulation can activate the apoptotic process has recently been delineated (Miller et al 2007). siRNA studies demonstrated that caspase-4, although activated, is not essential for ZA1AT-induced apoptosis. P-I-3 kinase and Bad do play a role and the bile acid tauroursodeoxycholic acid can target this pathway to promote cell survival in ZA1AT-expessing cells. Further studies comparing the effects of ZA1AT on apoptosis in liver and lung cells will no doubt provide new insights into the mechanisms and outcomes involved.

A1AT deficiency-associated liver disease: clinical manifestations and pathology

A1AT deficiency associated with the PiZ and PiMmalton (ΔPhe52) (Fraizer et al 1989) mutations is most frequently associated with liver disease. In PiZZ individuals 10–15% develop clinically significant liver disease in their first 20 years of life (Sveger and Eriksson 1995) and are susceptible to liver damage as a result of the accumulation of ZA1AT polymers in the ER of hepatocytes. With the null mutation of A1AT there is no intracellular accumulation and therefore no hepatotoxicity or resulting liver damage.

The liver damage occurs through a gain-of-function mechanism, unlike the lung disease, which is due to loss-of-function. This gain-of function is also evident in ZA1AT transgenic mice where liver disease is apparent although normal levels of anti-elastases are still present (Perlmutter 2002). Sharp and colleagues (1969) first described cirrhosis in A1AT deficiency in 10 children from six families and later reported intrahepatocyte periodic acid–Schiff diastase-resistant inclusions, which occur owing to polymer formation of ZA1AT in the ER (Sharp et al 1971). In Sweden between 1972 and 1974, 200 000 neonates were screened for A1AT deficiency. 120 PiZZ, 2 PiZ−, 54 PiSZ and I PiS− children were found. Of these only 14 PiZZ children had prolonged jaundice, 9 of whom had severe liver disease. All infants appeared healthy at 6 months of age. Infants with a PiSZ phenotype had no signs of liver disease (Sveger 1976).

Hepatic disease associated with A1AT deficiency is most common in children. Of the 127 newborn PiZZ infants studied by Sveger (1976), all showed increased liver enzyme concentrations, 10% had prolonged neonatal jaundice and 1 in 10 of these developed cirrhosis and required liver transplantation. In early childhood the most common presentation of A1AT deficiency's effect on the liver is prolonged jaundice. The stools generally contain no yellow or green pigment, indicating cholestasis and mimicking biliary atresia. All patients have hepatomegaly and about 50% also have splenomegaly. Approximately 5% of the patients present with an increased bleeding tendency. This is due to vitamin K deficiency caused by the cholestasis-induced malabsorption. Less commonly children present later in childhood with hepatosplenomegaly or with cirrhosis (Kok et al 2007). Overall 10% of PiZZ neonates develop hepatitis and cholestasis. Cholestasis usually occurs in the first two months, though it may persist for up to eight months. Breastfeeding and vitamin E supplements are recommended for cholestatic children (Sokol et al 1985).

In Italy routine neonatal screening found that 5% of PiSZ children were affected by liver involvement with elevated liver enzymes in early childhood. By the ages of 5 and 10 years, none had liver disease (Kok 2007). Abnormal liver function is largely self-limiting, but it can sometimes persist into adolescence. Of the neonates screened in Sweden at the age of 16 years, elevated liver enzymes were found in 17% of PiZZ adolescents and in 8% of PiSZ adolescents. The adults with liver disease in infancy were clinically healthy (Sveger and Eriksson 1995). At the age of 26 years the PiZZ subjects were compared to PiMM individuals. The PiZZ subjects had normal lung function but 4–9% of them had mild liver abnormalities (Pittulainen et al 2005).

In adults, liver damage can manifest itself as chronic liver disease or hepatocellular carcinoma. Cirrhosis may develop and hepatocellular carcinoma will result in advanced cases; 5–10% of A1AT-deficient patients over the age of 50 years will develop cirrhosis. A study

examining 19 adult patients with A1AT deficiency and chronic liver disease revealed a late onset of symptomatic hepatic abnormalities. Thirteen patients (68%) were 60 years or older when the liver disease was discovered. The mean ages of the patients with the PiZZ, PiSZ, and PiMZ phenotypes were 58, 66, and 72.5 years, respectively; this suggested a later onset of the liver disease in heterozygotes. At the time of diagnosis, the hepatic condition was usually advanced (Rakela et al 1987). According to Massi (1996), cirrhosis may be accelerated by incorrect repair of hepatic connective structures damaged by inflammatory proteases More recently, Rudnick and Perlmutter (2005) have proposed a model whereby the accumulation of ZA1AT in the ER activates a number of ER stress responses but apoptosis is blocked at terminal steps, generating a population of globule-containing hepatocytes that are 'sick but not dead'. A trans-signal generated by these cells stimulates proliferation of adjacent globule-devoid hepatocytes. A cancer-prone state is thus engendered by some cells that are unable to die and others that are chronically dividing in an inflamed milieu.

It is relatively uncommon for hepatic and pulmonary disease to co-exist in the same individual. Liver disease is thought to be caused by the retention of the mutant, presumably hepatotoxic, ZA1AT molecule in the ER of liver cells, as previously explained, with only 10–15% of PiZZ individuals developing clinically significant liver disease. With the use of fibroblast cell lines from PiZZ patients with liver disease ('susceptible' hosts) compared with those from PiZZ individuals without liver disease (''protected'' hosts), it was found that more efficient ER degradation of retained mutant ZA1AT correlated with protection from liver disease (Teckman et al 2001a). A detailed elucidation of the mechanisms by which mutant aggregated ZA1AT is degraded in the ER is essential for understanding how the quality control apparatus of the ER works in general and for understanding the specific issue of how a subgroup of A1AT-deficient individuals become susceptible to liver injury and carcinogenesis. There are three main methods of degradation. The first is proteasomal degradation (Teckman et al 2001b) in a ubiquitin-dependent or ubiquitin-independent manner (Teckman and Perlmutter 2000), and there is also evidence that nonproteasomal mechanisms may contribute in part to ER degradation of some substrates. In the case of A1AT the main non-proteasomal method of degradation seems to be autophagy (Teckman and Perlmutter 2000).

The consistent overt liver disease in newborns in comparison with the occasional occurrence in young adults may be explained by the lower capability of the liver cells of infants to degrade the polymerized protein (Carrell and Lomas 2002). Other factors can also predispose A1AT-deficient individuals to liver disease such as male sex and obesity (Bowlus et al 2005). The role of hepatitis is less clear. A study in Austria looking at A1AT-deficient patients with chronic liver disease found that of those with cirrhosis 62% were HCV positive, 33% showed evidence of HBV infection, 41% had a history of alcohol abuse, and 12% had features of autoimmune liver disease. Out of 53 cirrhotic A1AT-deficient patients, only 5 had no co-existing liver disease. These authors suggested that the risk for chronic liver disease is increased in patients with the PiZ gene, because they may have increased susceptibility to viral infection or additional factors (Propst et al 1992). Another study looking patients with end-stage liver disease found that the prevalences of PiMZ and PiMS were 7.3% and 8.2%, respectively, compared with 2.8% and 4.2% in the control population. The odds of having a heterozygous Z phenotype were significantly increased in patients with hepatitis C virus, primary hepatic malignancy, and cryptogenic cirrhosis compared with the control population. Patients with hepatitis C or B virus were 3.6 times more likely to have a heterozygous Z phenotype than a normal phenotype compared with patients with diseases of autoimmune aetiology (Eigenbrodt et al 1997). However, some studies have found no association between hepatitis C infection and A1AT deficiency (Elzouki et al 1997). In particular, a study looking at the PiMZ phenotype found that the prevalence of A1AT PiMZ was no greater in hepatitis C patients than in the general population—2% compared with 4% in the Northern European population. Furthermore, there was no difference in the prevalence according to the degree of fibrosis on liver biopsy. Since PiMZ is common it was expected that PiMZ would be over-represented in either the group with fibrosis or with cirrhosis if it was a major co-factor with HCV (Scott and Egner 2006). Other co-morbidities such as autoimmune liver disease, alcoholic cirrhosis and non-alcoholic steatohepatitis are all factors that can enhance the phenotypic expression of liver disease in PiMZ heterozygotes (Banner et al 1998; Bell et al 1990; Bergwitz et al 2002; Bowlus et al 2005; Czaja 1998).

The clinical course of liver disease within siblings with PiZZ A1ATD is not clear. Some studies have demonstrated varying patterns of disease progression within siblings (Cox and Mansfield 1987; Psacharopoulos et al 1983). Hinds and colleagues (2006) retrospectively analysed 29 families in which more than one child was diagnosed with PiZZ A1AT deficiency and

compared the pattern of liver disease between affected siblings: 72% of PiZZ siblings of the probands had liver disease, which was equally severe in 29% of cases, while 28% had no liver involvement. Also, 5 of 7 children requiring liver transplantation had siblings with no persistent liver dysfunction, suggesting that there is a variable degree of liver involvement in siblings with ZA1AT-related liver disease and that environmental and genetic factors are likely to be involved in determining disease severity.

Polymerization of ZA1AT is accelerated with increasing temperature. As A1AT is an acute-phase reactant its expression also is regulated by temperature. Thus febrile episodes lead to increased A1AT synthesis and, in the case of ZA1AT, a likely increase in polymerization. Changes in temperature have the potential to affect multiple steps in the pathways by which ZA1AT is translocated through secretory and degradative pathways. The variability in expression of liver damage may be explained in part by individual variations in episodes of systemic inflammation and the concomitant increase in temperature. This was recently also shown *in vitro* by Lawless and colleagues (2004), who reported an increase in ER stress in a model system of ZA1AT expression in the presence of increased temperature.

Autophagy

Autophagy is the primary means for the degradation of cytoplasmic constituents within lysosomes and is the process by which cells recycle cytoplasm and dispose of excess or defective organelles. Morphological changes associated with autophagy, including marked expansion and dilatation of the ER, are characteristic of fibroblasts overexpressing ZA1AT and liver cells from ZA1AT individuals (Teckman et al 2004). ZA1AT molecules have been detected in autophagosomes by electron microscopy and intracellular degradation of ZA1AT can be partially reduced by chemical inhibitors of autophagy, showing that ER retention of ZA1AT is associated with a marked autophagic response. It has also been reported that the autophagic response induced by ER retention of ZA1AT involves the mitochondria, with specific patterns of both mitochondrial autophagy and mitochondrial injury seen in cell culture models of A1AT deficiency, in PiZ transgenic mouse liver, and in liver from A1AT-deficient patients (Perlmutter 2002; Teckman and Perlmutter 2000; Teckman et al 2002). Although the majority of PiZZ individuals are protected from liver injury by efficient mechanisms of intracellular degradation of ZA1AT, it has been suggested that patients susceptible to liver injury may have inefficient mechanisms to deal with the aberrant accumulation of misfolded ZA1AT. This may lead to a net increase in ER accumulation of ZA1AT, and thus chaperone dysfunction may have a role in susceptibility to development of A1AT-associated liver disease. Calnexin, Grp78, Grp94, and Grp170 have all been shown to interact with ZA1AT. Approximately 85% of ZA1AT forms heterogeneous soluble complexes with multiple chaperones, with the other 15% forming large polymers or aggregates devoid of chaperones (Schmidt and Perlmutter 2005).

Therapeutics for the liver disease

Currently liver transplantation provides the only effective means of intervention for A1AT-deficient patients with liver disease. Whilst transplantation has been shown to successfully achieve A1AT serum conversion, its usefulness as a treatment is confounded by a lack of suitable donors and concomitant immunosuppressive therapy. There is a 70–80% survival rate in children, and up to 70% in adults. One-year survival rates have improved over the past several years to approximately 90% with the development of improved immunosuppressive drugs. Xenogenic hepatocyte transplantation from living donors is under investigation as an alternative to full liver transplantation. In stem cell research, allogenic and autologous stem cell transplants are also under development. It has been suggested by Novoradovskaya and colleagues (1998) that proteasome inhibitors such as lactacytsin, an agent that binds covalently to the active-site N-terminal threonine residue in certain beta-subunits of the proteasome, may increase delivery of ZA1AT to the extracellular milieu and provide a potential treatment for ZA1AT deficiency and other diseases associated with misfolded proteins. Unfortunately, others failed to detect an increase in secretion of ZA1AT from fibroblasts, hepatoma cells and HeLa cells treated with lactacystin and furthermore observed a marked increase in the formation of insoluble aggregates of ZA1AT, which lessens enthusiasm for this agent as a therapeutic (Teckman et al 2001b). Chemical chaperones can reverse the cellular mislocalization or misfolding of several mutant plasma membrane, lysosomal, nuclear, and cytoplasmic proteins. Compounds such as trimethylamine oxide (TMAO), 4-phenylbutyric acid (4PBA) or glycerol have potential to reverse the cellular mislocalization or misfolding of ZA1AT. TMAO can stabilize both M

and Z A1AT in an active conformation, but rather than aiding the refolding of denatured A1AT instead enhances its polymerization (Devlin et al 2001). 4PBA alters secretion of ZA1AT without apparently increasing its *de novo* synthesis or decreasing ER degradation (Sharp et al 2006), while glycerol and erythritol, trehalose and glucose can all decrease the rate of ZA1AT polymerization but are unable to refold the misfolded conformer. The mechanism by which this occurs remains unclear, but it is not thought to be due to an increase in viscosity, rather to act via a specific interaction between glycerol, for example, and ZA1AT that can slow down conformational transitions of the protein (Burrows et al 2000).

As ZA1AT deficiency occurs owing to a single gene defect, inhibiting expression of the ZA1AT gene represents a promising therapeutic strategy. Ribozyme-mediated specific gene replacement is a dual therapy that aims to treat the manifestations of A1AT deficiency by inhibiting the expression of the mutated gene with a ribozyme at the same time as replacing the defective gene the with a normally functioning A1AT gene in the liver (Ozaki et al 1999). Unfortunately, this approach has not been successful to date. Although antisense technology held much promise initially, its usefulness *in vivo* for diseases other than A1AT has been confounded by the inherent instability of antisense molecules. Second- and third-generation oligonucleotides based on a peptide nucleic acid backbone are now available that have considerably improved stability and recent advances in gene knockdown technology (also known as RNA interference or RNAi) have superseded these other approaches to some extent. There are hopes that RNA, using siRNAs targeting A1AT may have therapeutic potential for A1AT deficiency (Cruz et al 2007).

Prevention of polymerization of ZA1AT may result in the release of mutant ZA1AT and relieve ER perturbations (Mahadeva et al 2002). Parfrey et al (2003) designed a synthetic peptide capable of inserting into a hydrophobic cavity of the A1AT molecule and preventing polymer formation. Obstacles that must be overcome for the future development of such inhibitors include efficient intracellular delivery systems and the ability to reversibly remove the bound peptide from ZA1AT. Several imino sugar compounds have also been suggested to be useful for chemoprophylaxis of the liver disease. For example, castanospermine, kifunesine and deoxymannojirimicin have been shown to have positive effects in mediating an increase in secretion of ZA1AT protein (Marcus and Perlmutter 2000).

Lung disease: clinical manifestations

Patients with A1AT deficiency characteristically develop pulmonary disease in the third and fourth decades of life. Recent estimates suggest that 75–85% of patients with severe A1AT deficiency develop chronic obstructive pulmonary disease (COPD) (Ranes and Stoller 2005) and the majority of patients with A1AT deficiency have a history of cigarette smoking. In the National Heart Lung and Blood Institute (NHLBI) registry of A1AT deficiency, 8% were current smokers, 72% were ex-smokers and 20% never smoked (McElvaney et al 1997). Common symptoms include shortness of breath on exertion, wheezing (with or without respiratory tract infection) and chronic cough. Individuals are usually diagnosed on the basis of pulmonary symptoms, but a substantial percentage, up to 20%, may only be detected through family screening, as many people with A1AT deficiency are asymptomatic without lung function impairment (Silverman et al 1989). Occasionally individuals are identified as a result of abnormal chest radiographs or pulmonary function tests (2%), liver disease (2%) or blood screening tests (1%) (Gadek and Crystal 1983).

The clinical signs of A1AT-related lung disease are those of obstructive lung disease and emphysema. They include hyperinflation of the chest and reduced intensity of breath and heart sounds as well as wheezing in up to 20% of cases (Cox 1999; Tobin et al 1983). The association between A1AT deficiency and the development of emphysema was first described in 1963 (Eriksson 1963). The typical pattern shows lower zone predominance, although emphysema may affect all zones. The characteristic pulmonary pathological abnormality is diffuse emphysema. This contrasts with centrilobular emphysema characteristic of cigarette smoking, which predominantly affects the respiratory bronchioles in the central portion of the lobule. These pathological abnormalities are reflected in a characteristic appearance on plain chest radiographs. There is a hyperlucent appearance with a basal predilection and oligaemia because of destruction of the pulmonary parenchyma and progressive loss of vascularity (Brantly et al 1988; Gishen et al 1982).

High-resolution CT (HRCT) scan of the chest demonstrates widespread abnormally low-attenuation areas resulting from a lack of lung tissue. There are also increased air spaces and bullous formation (Guest and Hansell 1992). These changes predate the associated abnormalities of pulmonary function (McElvaney et al 1989; Simon et al 1989). In moderate disease the panlobular nature of the process and the

characteristic lower zone predominance are more obvious. Very severe forms may be indistinguishable from severe centrilobular emphysema (Fig. 4).

Pulmonary function in patients with established disease reveals evidence of reduced forced expiratory volume in 1 second (FEV_1), whereas the forced vital capacity (FVC) is generally preserved or modestly reduced (Brantly et al 1988). Both residual volume and total lung capacity are increased. The diffusing capacity of the lung for carbon monoxide is diminished and the alveolar-arterial oxygen gradient is widened. Flow–volume curves demonstrate coving of the expiratory portion of the curve, reflecting expiratory flow limitation (Fig. 5). Pulmonary function can be preserved until the fifth or even sixth decade of life in those who have never smoked and generally until the third or fourth decade of life in most other patients (Wall et al 1990). Airflow limitation seen on pulmonary function testing is not always fixed and the symptoms and signs in A1AT deficiency can be similar to features of asthma. Patients can often be given this diagnosis in childhood or early adulthood. Indeed, 15% of patients in a Swedish cohort identified by neonatal screening had been diagnosed as having asthma by the age of 22 years (Piitulainen and Sveger 2002).

Other than emphysema and airflow obstruction, patients may also have chronic bronchitis or bronchiectasis. Patients with chronic bronchitis tend to have more severe airflow obstruction and more extensive emphysema than those without chronic bronchitis, despite similarities in age and smoking history (Dawson et al 2002). Exacerbations occur more frequently in patients with chronic bronchitis, in index patients identified as a result of their lung disease, and in those with more severe disease as assessed by the GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria (Pauwels et al 2001). A tentative association with Wegener granulomatosis has also been suggested and a number of other conditions, including rheumatoid arthritis and hepatocellular carcinoma, have been reported to occur with increased frequency in patients with A1AT deficiency.

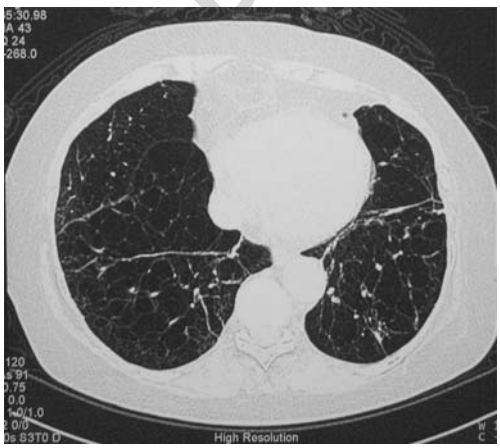


Fig. 4 Slice through lung bases from HRCT of the thorax of a 56-year-old woman with A1AT deficiency, showing widespread emphysematous change bilaterally

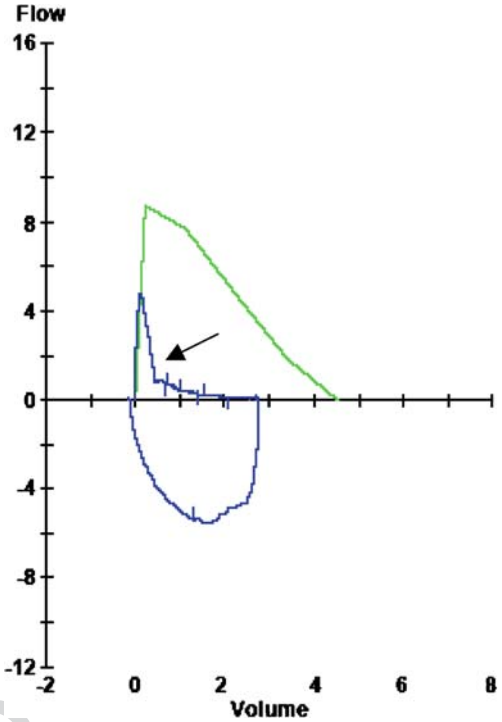


Fig. 5 Flow–volume loop of a 61-year-old man with A1AT deficiency. There is coving of the expiratory portion of the curve (see arrow), reflecting expiratory flow limitation. A normal expiratory curve is shown in green

Therapeutics for A1AT-related lung disease

A1AT has been purified from the plasma of healthy individuals and delivered intravenously to patients with A1AT deficiency since 1987 (Wewers et al 1987). This intravenous augmentation therapy used a dose of 60 mg/kg body weight weekly, and successfully raised levels of serum A1AT above the putative protective threshold of 11 μmol/L throughout the duration of therapy. Furthermore, serum anti-neutrophil elastase capacities increased from 5.4 ± 0.1 to 13.3 ± 0.1 μmol/L and there were concomitant significant increases in A1AT levels in BAL fluid. A number of plasma-derived intravenous augmentation products have been developed but, as yet, conclusive evidence of their effectiveness in preventing A1AT deficiency-

associated lung disease is lacking. As yet none has been evaluated in randomized placebo-controlled trials to show effectiveness in treating or preventing emphysema.

Alternative routes of administration of augmentation therapy, most notably delivery by inhalation, are being explored. The ease of administration compared with the intravenous route, as well as the use of smaller doses, makes delivery by inhalation an attractive option. It has been shown that the airways of individuals with A1AT deficiency are under a constant inflammatory barrage (Rouhani et al 2000) and that administration of exogenous inhaled A1AT can reconstitute the lower respiratory tract anti-protease screen and potentially reduce inflammation (Hubbard and Crystal 1990; Hubbard et al 1989). Polymerization of locally produced ZA1AT acts as a neutrophil chemoattractant in A1AT deficiency and is a contributory factor to the lung inflammation (Mulgrew et al 2004), thus standard anti-protease therapies alone may not address the problem fully.

There is increasing evidence that A1AT has anti-inflammatory activity independent of its anti-protease effects. This suggests that the administration of augmentation therapy may do more than simply restore the protease/anti-protease balance. Monocytes that have been induced to express surface PR-3 release significant amounts of biologically active IL-8 when exposed to either monoclonal anti-PR-3 IgG or IgG from Wegener granulomatosis patients with high titres of cANCA. Interestingly, this interaction is prevented by the addition of A1AT (Ralston et al 1997), suggesting that A1AT may indirectly regulate inflammation by suppressing the inflammatory cascade induced by cANCA. A1AT has also been shown to inhibit lipopolysaccharide-mediated human monocyte activation *in vitro*. (Janciauskiene et al 2004).

Worries surrounding the potential transmission of infectious agents by a human plasma-derived product have led to the development of transgenic/recombinant sources of human A1AT and the evaluation of synthetic inhibitors of neutrophils elastase (NE). Transgenic production of human A1AT protein has been achieved in goats (Ziomek 1998) and sheep (Wright et al 1991), and human A1AT has also been produced in yeast using recombinant technology (Casolaro et al 1987). Unfortunately, all these proteins are cleared rapidly from the human circulation. Those raised in yeast are non-glycosylated, with a resultant short plasma half-life, while those produced from transgenic animals have different glycosylation patterns also leading to alterations in half-life and making their intravenous use impractical. The inhaled route, however, is a possibility for the future. Several inhibitors of NE have been evaluated in humans but not in A1AT deficiency to date (Cadene et al 1997; Edwards and Bernstein 1994; Kawabata et al 1991; Luisetti et al 1996; Williams et al 1991).

Other treatments, although not all specific for A1AT deficiency emphysema, are under investigation. The administration of all-*trans* retinoic acid (ATRA) is being studied in relation to pulmonary emphysema in the general COPD population and may have a potential application in A1AT deficiency. Retinoids can activate genes involved in lung development and promote alveolar septation and growth. Clinical trials to date are disappointing, however (Mao et al 2002). Trials of inhaled hyaluronic acid in individuals with A1AT deficiency are based on the fact that animals administered hyaluronic acid are protected from exogenous NE-induced emphysema (Cantor et al 1995, 1998). Drugs with antioxidant potential are also being considered. A number of gene therapeutics for A1AT deficiency have been developed. For example, the normal A1AT gene has been successfully introduced into the striated muscle cells of animals using an adeno-associated virus vector (Song et al 1998; Flotte 2002). However, this approach does not address the problems associated with endogenous production of abnormal A1AT, making the role of gene-targeted therapies more appealing.

The future and A1AT deficiency

The past 40 years have seen a huge increase in our understanding of the molecular basis of the lung and liver manifestations of A1AT deficiency. This knowledge, in conjunction with significant technological and pharmacological advances, has led us to a point where it is possible to manage and relieve many of the clinical manifestations associated with this disorder. However, our goal as clinicians and scientists is to develop an effective cure, and as yet there are still many unanswered questions. Given the emerging complexity of signalling cascades regulating protein folding, intracellular stress responses and inflammation, it appears that the way to achieve this goal will be by gaining even greater insights into the key factors regulating the different aspects of this complex disorder. What is known to date regarding A1AT deficiency is likely to have implications for other conformational diseases, in particular the serpinopathies and neurological disorders associated with

aberrant protein folding. Reciprocally, it is likely that therapeutics developed for these diseases may, in turn, have use for A1AT deficiency.

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AUTHOR QUERIES

AUTHOR PLEASE ANSWER ALL QUERIES.

Q1. Please check if affiliation was captured correctly.
Q2. Should this author *also* be shown as RCS Ireland [affiliation for the work presented] since the Trinity address was given only as "current address"?
Q3. Only 1998 is listed: is that reference intended?
Q4. "substitution of a valine residue **for** glutamate … (Val264Glu) – OK? Should it be Glu264Val? Or "substitution of a valine residue **by** glutamate"? [Original residue precedes number and changed residue follows it? See Glu342Lys next.]
Q5. "Elliott 1998" was changed to "Elliott et al. 1998". Please check if appropriate.
Q6. Reference not listed.
Q7. Is this the reference that was intended?
Q8. Reference not listed.
Q9. What is meant here? Just that 29% had severe disease?
Q10. Is **this** the reference that was intended?
Q11. Year amended. OK?
Q12. Year amended. OK?