CHAPTER 9

Alpha-1-antitrypsin deficiency

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Affecting one in 3,000–5,000 individuals, α1-antitrypsin deficiency (AATD) is one of the most common hereditary disorders in Caucasians. AATD is characterised by low serum levels of α1-antitrypsin (AAT), a predisposition to early-onset emphysema and, less commonly, liver disease, including neonatal jaundice and adult cirrhosis and hepatoma. In recent years, in order to overcome the significant challenges that exist when investigating uncommon disorders, a concerted effort has been made to bring together all relevant information on research into the underlying pathophysiology and the clinical management of AATD, as illustrated by the establishment of a substantial number of registries for the disorder (e.g. www.aatregistry.org and www.alphaone.org). Although much remains to be elucidated, progress continues to be made. Moreover, it has become clear that advances in AATD research can contribute to the understanding of other diseases and that approaches developed for other medical areas are now being applied to AATD. The Alpha One International Registry, formed in 1998, is a multinational organisation involving nearly 20 countries, with clinical scientists working on research and clinical development relating to AATD [1].

History of alpha-1-antitrypsin deficiency

In 1963, seminal work on AATD by Carl-Bertil Laurell [2], Head of the Dept of Clinical Chemistry in Malmö, Sweden, has led to progress in medicine extending far beyond AATD. An observation in a clinical laboratory in Sweden 40 yrs ago has opened up new fields in biology and medicine, and has done so long before the significance was recognised by science as a whole. Numerous avenues of research and clinical progress can be traced to Laurell’s observation of the absence of the α1-band among the electrophoretic abnormalities of lung patients. Not only is AAT the archetype of the serpin supergene family, but it also has been the prototype for an entire category of disorders, the conformational diseases [3]. The serine proteinase inhibitors, or serpins, are a superfamily of proteins that are found in organisms from plants to viruses to animals. In humans, the family includes, in addition to AAT, proteins as diverse as α1-antichymotrypsin, C1 inhibitor, antithrombin, and plasminogen activator inhibitor-1, which have key regulatory functions in the inflammatory, complement, coagulation and fibrinolytic cascades [4]. The serpins all possess an exposed 14 amino acid residue mobile reactive centre loop that acts like a mouse trap for serine proteinases and enables the serpins to inhibit the serine proteases irreversibly. The design of this reactive loop makes serpins very effective antiproteinases, but also leaves them susceptible to conformational transitions that can cause disease.
The \( \alpha_1 \)-antitrypsin protein

In individuals with the PiZ (proteinase inhibitor) phenotype, a single nucleotide mutation causes the AAT molecules to accumulate in the endoplasmic reticulum (ER) of hepatocytes rather than be secreted. In the ER, there is a rapid sequential interaction between the reactive centre loop of one AAT molecule and the \( \beta \)-sheet A of a second (fig. 1). The consequence is the formation of long polymers of Z AAT, with each molecule linked by its reactive loop to the sheet of the next [5]. Characteristic inclusion bodies in the liver, which are readily recognised on periodic acid-Schiff staining, develop over time. The accumulation of aberrant forms of individual proteins rather than a failure to produce the protein is the common theme in conformational diseases. Conformational diseases, such as the common dementias, Alzheimer’s disease, prion diseases and the serpinopathies, are all caused by structural rearrangements within a protein that transform it into a pathological species. Recently, in a study of familial neurodegenerative disease, inclusion-body formations containing neuroserpin, a brain-specific serpin, were found sufficient to cause neurodegeneration [6]. The onset and severity of the disease was found to be associated with the rate and magnitude of the neuronal protein aggregation.

Efforts are underway to develop small peptides that can selectively inhibit polymerisation of the Z allele of the AAT protein.

Fig. 1. – Tertiary structure of \( \alpha_1 \)-antitrypsin.
The gene

The gene encoding for AAT is localised on chromosome 14. The AAT gene has a length of 12.2 kb and consists of seven exons [7]. The genes of the serpins are partly clustered on chromosome 14 on location 14q32.1. The gene of AAT is very polymorphic. In the European population, >75 different alleles have been described [7]. The most frequent mutations are the AATD Z- and the S-mutations, mutations in exon 5 and 3, respectively. There are also more rare mutations (null mutations), by which no protein is being formed in the liver. Finally, polymorphisms have also been described in the enhancer sequence of the AAT gene, mutations that are involved in the regulation of expression of AAT.

Lung disease

The hallmark of AATD is the development of early-onset, severe lower zone emphysema in individuals with the PiZZ genotype. AAT is the major defence in the lung against neutrophil elastase. In AATD, the uninhibited action of neutrophil elastase on lung parenchyma results from a deficiency of AAT and leads to alveolar destruction. Severe AATD is the only defined genetic risk factor for chronic obstructive pulmonary disease (COPD), and individuals with susceptible genotypes are at risk for severe, early-onset COPD. Cigarette smoking markedly increases the risk and rate of development of emphysema in patients with severe AATD. The role of exacerbations on the course of COPD is under intense study. The occurrence of exacerbations in AATD can be defined as "a sustained worsening of the patient’s condition, from the stable state and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD" [8]. The criteria for COPD exacerbation proposed by ANTHONISEN et al. [9] are still widely accepted: breathlessness, sputum volume and sputum purulence. Increased numbers of exacerbations show a relationship to a faster decline in lung function in individuals with AATD [10]. Exacerbations have been associated with bacteria and viral infections, pollution, airway irritability/obstruction, and increased temperature, all of which need further studies in delineating the contribution of each of these factors.

A study by HILL et al. [11] demonstrated that individuals with AATD have increased elastase activity at both the start and resolution of the exacerbation when compared with other COPD patients. Moreover, their study showed that both at the start and resolution of an exacerbation, airway secretions of AATD individuals contain more myeloperoxidase, leukotriene B4 and interleukin (IL)-8. Increased exacerbation frequency has also been reported in those AATD individuals with chronic sputum expectoration [12]. Exacerbations last approximately twice as long in AATD patients as in other COPD patients who have an equivalent degree of lung function impairment. They do not occur in 40–50% of AATD individuals each year, but those AATD individuals who do experience exacerbations have 2.4 on average. Although this predictor is not clear-cut, lower forced expiratory volume in one second (FEV1) appears to be associated with an increased risk of exacerbations. One study has indicated that patients on augmentation therapy have fewer exacerbations (i.e. the number of infections per year) compared with those not on replacement therapy [13].

Lung densitometry with computerised tomography (CT) scanning is the most sensitive noninvasive method to diagnose progression of emphysema. Quantitative CT has shown good correlation with the pathological extent of emphysema, lung function tests, diffusion capacity of the lung especially, and, to a lesser extent, measures of airway obstruction, such as FEV1 [14]. Quantitative CT is reproducible and more sensitive than
lung function testing in monitoring the progression of emphysema and also correlates with the patient’s health status. However, a major confounder in the use of quantitative CT is the depth of inspiration. The lung density almost doubles from full inspiration to full expiration and, therefore, correction for volume of air in the lung is needed using specific models [14].

**Role of phenotype**

The gene locus specifying the AAT protein is polymorphic, and >70 variants have now been identified. However, the M, S and Z alleles are the most prevalent. The PiM allele, the predominant allele, results in normal AAT levels (fig. 2). The PiS allele accounts for 2–3% of the alleles and is associated with mildly reduced AAT levels, whereas the PiZ allele accounts for only 1% of alleles but is associated with severely reduced AAT levels (16% of normal) and the characteristic emphysema of AATD. The risk of developing COPD in heterozygotes is still not clearly delineated and has been a subject of controversy. A longitudinal study of 9,187 participants in the population-based Copenhagen City Heart Study for up to 18 yrs found that the 451 PiMZ heterozygotes had 31% lower levels of AAT than PiMM genotypes [15, 16]. Their rate of FEV1 decrease was 19% greater than the average values in persons with the PiMM genotype, and they had a 30% increased risk of airway obstruction, a 50% increased risk of COPD and a 50% increased risk of hospitalisation or death from COPD [15, 16].

The prevalence of the PiSZ genotype in most European countries is one in 1,000. The genotype is more prevalent in Spain compared with other European countries [17]. The prevalence in the USA is estimated to be one in 1,270. However, few individuals of this genotype are in the various registries, and, therefore, the natural history of lung disease has not been extensively studied. In the Copenhagen City Heart Study, airway obstruction was increased in PiSZ individuals (40%) compared with PiMM (15%) [16]. A Swedish study of 94 PiSZ individuals revealed that only a small fraction of subjects with the PiSZ phenotype are at an increased risk of developing pulmonary emphysema, and at an older age than subjects with the PiZ phenotype [18]. A cohort study of 50 individuals demonstrated that in nonsmokers PiSZ may confer little or no added risk [19]. PiSZ never-smokers seem to carry no increased risk of lung disease, while PiSZ smokers may carry a little additional risk. Life expectancy in PiSZ individuals appears to be normal.

![Fig. 2. – Serum α1-antitrypsin (AAT) levels in various Pi phenotypes.](image-url)
Pathophysiology of lung disease

The pathogenesis of emphysema is still incompletely understood, and it is increasingly clear that complex, interacting pathways are involved in its initiation, progression and the failure of repair processes [20]. While the protease/antiprotease hypothesis is still "alive and well", animal models are elucidating how other cellular components and biochemical pathways can also contribute to the lung damage seen in emphysema (fig. 3). Emphysema can be modeled in many ways, and mice have become the predominant species for these studies. Three categories of experimental design are currently being pursued: 1) inducing emphysema in normal mice by challenge with exogenous agents; 2) studying airspace enlargement in naturally occurring genetic mutant mouse strains, such as the pallid mouse; and 3) creating targeted mutagenesis ("knockout") or transgenic mice. Transgenic and gene-related mouse models of emphysema are dominating research currently. Amongst the advantages are that many genes have been cloned, techniques for genetic manipulation of mice are well established and large numbers of mice can be generated quickly. However, it is still not clear how accurately these models reflect human biology and pathology. There are now many examples linking proteases and emphysema, and different proteases seem to be involved in different models. In addition to neutrophil elastase, other potential enzymes implicated are macrophage elastase, interstitial collagenase and cysteine proteases.

The role of macrophage matrix metalloproteinase (MMP)-12, also known as macrophage elastase, in inflammation and subsequent emphysema was highlighted in two recent reports [21, 22]. Mice lacking αV β6 integrin have high levels of lung MMP-12.
and develop emphysema. Transforming growth factor (TGF)-β1 is activated by binding to αV β6 integrin, and active TGF-β1 downregulates the expression of MMP-12 in macrophages. It is hypothesised that emphysema may occur in the αV β6-deficient mice due to overexpression of MMP-12 following a failure to activate TGF-β1 [21, 23]. In another mouse model of cigarette smoke-induced emphysema, MMP-12 was found to mediate smoke-induced inflammation by releasing tissue necrosis factor (TNF)-α from macrophages, with subsequent endothelial activation, neutrophil influx and proteolytic matrix breakdown caused by neutrophil-derived proteases [22]. These models are opening new directions for the study of human emphysema.

The role of cytokines in the pathogenesis of COPD is currently being unraveled [24]. Available evidence suggests that cytokines, such as IL-1, TNF-α and interferon-γ, can "start" emphysema and that macrophages, neutrophils and CD8 T-cells play a role. Cytokines, such as IL-8, the chemokine regulated upon activation, normal T-cell expressed and secreted (RANTES) and interleukin-13, derived from neutrophils, eosinophils and CD8 T-cells, appear to be involved in COPD exacerbations. Even less explored is the role of cytokines and growth factors, such as vascular endothelial growth factor, in lung haemostasis and repair. While there is not yet a clear picture of the varied roles of cytokines in emphysema, a growing number of models have demonstrated the proof of principle that cytokines do have a function at least in animal models in inducing emphysema [22, 25, 26]. Interestingly, the role of endothelial cells is also actively studied. An increase in pulmonary artery pressure is a predictor of poor clinical outcome in COPD patients, and pulmonary vascular abnormalities start at early stages of the disease [27, 28]. Endothelial dysfunction can lead to pulmonary hypertension. Smooth muscle cell proliferation, as well as elastin and collagen deposition, in the thickened intima of pulmonary arteries in moderate COPD patients and smokers has been reported [29]. Cigarette smoke products may initiate vascular changes by a direct effect on endothelium and/or an inflammatory mechanism.

Liver disease

PiZZ AATD is the most common genetic/metabolic cause of end-stage liver disease in children, accounting for ~10% of paediatric liver transplantation in Europe/USA. The liver in children has two peaks with liver failure, either within the first 2 yrs of life or in puberty. The most common presentation is neonatal hepatitis, and liver deposits of AAT have been demonstrated at as early as 17–20 weeks of gestation [30]. Physiological perinatal prevalence of hydrophobic bile acids may add to prolonged neonatal jaundice, but the majority of children who will not need liver transplantation clear their jaundice by the age of 6 months. As much as 78% of PiZZ infants with neonatal hepatitis syndrome will develop chronic liver disease, and 28% will either require a transplant or die.

In contrast to PiZZ individuals, liver disease in PiSS and PiSZ individuals is much less well documented [31–33]. The PiS allele is more common than PiZ in some parts of Europe, such as the Iberian peninsula where the incidence may be as high as 28%. It has been reported that PiZZ patients with acute liver decompensation may exhibit a PiSZ-like pattern on electrophoretic phenotyping and this could lead to an erroneous exaggeration of the actual incidence of childhood liver disease associated with PiSZ [34]. A study conducted at King’s College Hospital in London (UK) that included nine PiSS and 20 PiSZ individuals reported that a variety of liver problems, including biliary atresia, glycogen storage disease type III, urea cycle defect and steato-hepatitis (Mauriac syndrome) can occur in both genotypes. However, after a mean follow up of 62 months (PiSS) and 24 months (PiSZ), there was no clinical evidence of liver disease, except in
relation to associated pathologies. It was concluded that possession of PiSS and PiSZ phenotype per se does not induce liver disease in childhood. Liver involvement in PiS individuals may be subclinical and prospective community-based studies in areas with high prevalence of the S allele are needed. Three studies that investigated the effect of rare Pi phenotypes on liver function have also been recently published [35–37] (fig. 4).

To date, there are no specific therapies for AATD. The mutant PiZ protein has a single nucleotide substitution that prevents it from folding properly. The AAT PiZ protein polymerizes and is retained in the ER rather than secreted into blood and body fluids. While the assumption has been that the retention of this aggregated PiZ glycoprotein elicits liver injury, the possibility exists that the accumulation of Z polymer per se does not cause liver disease. The mechanisms of liver injury, including the role of mutant AAT in triggering injury, are still poorly understood. For instance, the PiSaar mutation results in prolonged intracellular retention even though the proteins do not have polymeric properties [38]. The mapping of the flux of AAT PiZ through the cell from synthesis,
chaperone binding, polymerisation, retention and degradation, and even to the small amount that is secreted, is key to understanding liver pathophysiology. The glycoprotein ER-associated degradation system has been found to be a major component of the eukaryotic cell’s "biosynthetic quality control" system [39]. AAT is a glycoprotein that is transported to the ER by a molecular chaperone to undergo conformational maturation. The protein’s asparagine-linked oligosaccharide containing three glucose, nine mannoses and two N-acetylglucosamines serves a vital role in the protein’s ER transport, processing and proper folding. This oligosaccharide is covalently attached during translocation of AAT into the ER. The removal of two glucose molecules allows the binding of the chaperone protein calnexin, which then results in the shedding of the final glucose and the folding of the AAT molecule. The PiZ molecule cannot be folded properly and, therefore, is marked for degradation. The oligosaccharide chain has also been found to play an essential role in the signal for degradation. The removal of one mannose plus a non-native protein structure are both required for degradation.

Degradation of the misfolded or polymerised AAT molecules occurs primarily in the proteosome [40]. However, non-proteosomal autophagy may also be involved. In autophagy, cytosol and intracellular organelles, such as ER, are first sequestered from the rest of the cytoplasm, allowing them to be degraded subsequently within lysosomes. The concentration of the PiZ protein may determine whether it is degraded by proteosomes or lysosomes. Mitochondrial autophagy may play a role in the mechanism of liver cell injury in AATD [41], but mitochondrial injury is only one part of the story.

A mouse model study has demonstrated an altered response to stress, such as fasting, in the AATD liver, including an incapacity for further increase in activated autophagic response, a developmental state-specific rise in AAT-containing globules, and higher mortality [42]. The liver of AATD individuals could be very susceptible to perturbations and insults, and will suffer more injury than a normal liver for any given insult.

**Diagnosis and rare phenotypes of AATD**

In all countries in which AATD is prevalent, the majority of individuals with the disease have not yet been detected. In Sweden and Denmark, ~15–20% of all deficient individuals have been detected. However, in the remainder of the world that percentage is much lower. Of the >80,000 AATD individuals estimated to be in the USA, only 4,000–5,000 (5–6%) have been diagnosed.

From a World Health Organization meeting in 1996, recommendations were issued that all patients with COPD, all adults and adolescents with asthma and all individuals with a family history of AATD, as well as neonates, children and adults with unexplained liver disease, should be screened, and that those with abnormal results on screening should undergo phenotyping [43]. Three recommended categories of testing were immunoassay for serum levels of AAT, phenotyping based on isoelectrophoretic focusing of the AAT protein and genotyping using PCR to analyse DNA. At least two independent tests are required to establish a diagnosis of AATD and all approaches have their limitations.

Advances in the diagnostic testing are complicated by the presence of null alleles, which result in a total lack of circulating protein. Prior to the availability of DNA-based testing, null alleles could be identified with certainty only through familial patterns of inheritance. In PCR-based genotyping, primers for both the PiZ mutation and the normal sequence allow "counting" of the PiZ alleles present. Individuals with an allele, having normal sequence at residue 342, but only Z protein identified by phenotyping have a null allele. The prevalence of unusual AAT alleles in deficient individuals is ~6–10%,
i.e. 17 out of 182 (9.3%) USA patients and six out of 103 (5.8%) British patients have genotypes other than PiZZ [44]. However, unlike the PiZ mutation which results from a single specific mutation, null alleles can result from a number of mutations, including ones that result in T-splicing abnormalities, deletion of AAT coding exons and premature stop codons.

In Northern Italy, a unique screening programme has tracked rare AAT phenotypes and liver-test abnormalities during infancy [35]. The authors concluded that only a neonatal screening based on phenotyping can detect these rare carriers early in life.

**Augmentation therapy for lung disease**

The only available randomised trial of 56 ex-smokers with AATD receiving monthly therapy found that augmentation conferred no significant benefit overall in slowing FEV1 decline, but the analysis of lung density measured by CT showed a trend to slower decline [14].

The National Heart Lung and Blood Institute Registry study was a non-randomised comparison of 927 patients either receiving or not receiving AAT replacement. The rate of FEV1 decline was significantly less in those receiving augmentation therapy (66±5 versus 93±11 mL·yr⁻¹; p=0.03), but only in the subgroup with an FEV1 of 35–49% of predicted [45]. In that study, subjects receiving augmentation therapy also had a significantly decreased mortality risk. In another large nonrandomised cohort study comparing 198 German patients receiving weekly augmentation infusions and 97 untreated Danish patients, the mean annual decline in FEV1 was significantly less in treated patients, but only in the subgroup with an FEV1 of 31–65% of predicted (62 versus 83 mL; p=0.04) [46]. Both these studies are concordant in showing that augmentation therapy recipients experience a slower rate of decline in FEV1, especially in those individuals with a moderate degree of airflow obstruction.

Currently available augmentation products are administered i.v., but augmentation therapy via the inhalation route is an attractive strategy because AAT would be delivered directly to the lung. Studies to evaluate this concept are needed.

**Potential new therapeutic approaches**

Gene therapy and immortalisation of hepatocytes for replacement therapy are therapies currently being investigated to provide new approaches to treat AATD. A phase I AAT gene therapy clinical trial has been approved at the University of Florida College of Medicine, Gainesville, Florida, USA. The trial involves the intramuscular injection of the AAT gene using a recombinant adeno-associated virus (rAAV) vector. This safety study is an open-label, single-dose escalation study with four cohorts of three subjects each. The rAAV-2 is a nonpathogenic Parvo virus that is a common inhabitant of the upper respiratory tract. Gene therapy, as a direct extension of the protein replacement strategy, has the potential for single-dose treatment and may be able to provide stable plasma levels [47].

An approach in the preclinical investigation phase is the immortalisation of hepatocytes through the transduction of hepatocytes with the catalytic component of human telomerase, the telomerase reverse transcriptase (TERT) [48]. Telomeres are located on the tip of chromosomes. Each time a cell divides the telomeres shorten and eventually the cell dies. Telomerase lengthens telomeres, allowing the cell to divide indefinitely. Individuals with primary lung disease may merely require the transplantation of an adequate number of cells
to provide the AAT protein. Those with liver and lung disease will require enough cells to provide for adequate liver function as well as enough AAT protein for their lungs. Immortalised hepatocytes could potentially be used for direct liver transplantation or in a bioartificial liver. In preclinical studies, human foetal hepatocytes transduced with TERT have been in continuous culture for 1.5 yrs, compared to controls that went into senescence at ~100 days. The immortalised cells were not found to induce tumours in mouse studies.

Summary

Alpha-1-antitrypsin deficiency (AATD) is classically associated with the early onset of severe basal emphysema, but is also associated with the development of cirrhosis, primary carcinoma of the liver and possibly the vasculitides. All these decrease life expectancy, although the pulmonary condition dominates, as emphasised by the additive effect of cigarette smoking on mortality in patients with deficiency. In those aged over 50 yrs, a proportion of the deficient subjects may develop increasing airflow obstruction which was shown to be related to a history of wheezing and occupational exposure to irritants. This raises the possibility that asthma may be a predisposing factor to the development of permanent airflow obstruction in nonsmokers with AATD and supports one of the World Health Organization’s recommendations that all adults with asthma should be tested for this deficiency. Central to the pathology of AATD is the accumulation of aberrant forms of misfolded or polymerised $\alpha_1$-antitrypsin rather than a failure to produce the protein, and strategies to develop small peptides that can selectively inhibit polymerisation of the Z allele of the $\alpha_1$-antitrypsin protein in the liver is the common theme to repair this conformational disease.

Keywords: Alpha-1-antitrypsin deficiency, emphysema, liver disease, lung disease.

References


