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The role of proteases, endoplasmic reticulum stress and *SERPINA1* heterozygosity in lung disease in alpha-1 antitrypsin deficiency

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ABSTRACT

The serine proteinase inhibitor alpha-1 antitrypsin (AAT) provides an antiprotease protective screen throughout the body. Mutations in the AAT gene (*SERPINA1*) that lead to deficiency in AAT are associated with chronic obstructive pulmonary diseases (COPD). The Z mutation encodes a misfolded variant of AAT that is not secreted effectively and accumulates intracellularly in the endoplasmic reticulum (ER) of hepatocytes and other AAT-producing cells. Until recently it was thought that loss of antiprotease function was the major cause of ZAAT-related lung disease. However the contribution of gain of function effects is now being recognised. Here we describe how both loss and gain of function effects can contribute to ZAAT-related lung disease. In addition we explore how *SERPINA1* heterozygosity could contribute to smoking-induced COPD and consider the consequences.

Keywords: alpha-1 antitrypsin deficiency, serine antiprotease, serine proteases, endoplasmic reticulum stress, *SERPINA1* heterozygosity, augmentation therapy.

1. Alpha-1 antitrypsin (AAT) and AAT deficiency

AAT is a serine protease inhibitor (serpin) that inhibits its targets neutrophil elastase (NE), proteinase-3 (PR-3) and cathepsin G via a suicide substrate-like inhibition mechanism. AAT is produced chiefly by the liver from where it diffuses into the circulation to provide an antiprotease protective screen throughout the body and importantly in the lungs. AAT is also produced by monocytes, neutrophils and airway epithelial cells albeit in less abundant quantities than hepatocytes [1-3]. It is the most abundant endogenous serpin and in addition to its direct anti-protease effects, AAT like other serpins, can impact on a range of biological processes including inflammation, innate immunity and apoptosis.

AAT deficiency is a genetic disorder associated with mutations in the *SERPINA1* gene (previously called the 'protease inhibitor' or 'PI' locus). AAT deficiency is characterised by decreased levels of AAT in the circulation (5-11 μM or 0.26-0.572 g/l) compared to non-AAT deficient individuals (20-53 μM). Deficiency classically predisposes to liver, lung or rarely skin manifestations. The most common disease-causing mutation, termed ZAAT, occurs as a result of a single nucleotide polymorphism encoding a glutamic acid to lysine substitution at position 342 of the mature protein (Glu342Lys). This leads to misfolding, intracellular polymerisation and accumulation of ZAAT [4, 5]. In addition the mutation modifies the protein's reactive centre loop leading to a reduction of antiprotease function.

Native AAT contains 3 β -sheets (A-C) and nine α -helices and exists in a meta-stable state. Its energy is released by cleavage of the reactive centre loop (RCL) which presents a pseudosubstrate to its target protease e.g. NE. Upon interaction with NE a Michaelis complex is formed and cleavage of the RCL by NE results in its incorporation to form a fourth strand in β -sheet A [5]. The Z mutation in AAT occurs at the base of the RCL at the head of the fifth strand of the A-sheet. The mutation causes a conformational change leading to formation of an unstable intermediate that is characterized by partial insertion of the RCL and opening of the β -sheet. This enables the parent β sheet A to accept the loop of another molecule and form a dimer; a process which can then extend to form a polymer. Accumulation of large

polymers can lead to the formation of aggregates that are detectable as diastase-resistant periodic-acid Schiff-positive hepatic inclusions in ZAAT deficient individuals [4, 6].

1.1 Epidemiology of AAT deficiency

AAT deficiency was first described in Sweden in 1963 by Laurell and Eriksson who linked the absence of the 'alpha-1' band on serum protein electrophoresis to emphysema. The oldest reported case of the disorder is in a six-year old girl discovered in the Alaskan permafrost after remaining frozen for 800 years [7]. The highest incidence of AAT deficiency is in Europe but the frequency of the Z allele fluctuates widely across countries, geographical regions and ethnic groups. It is important to note that many studies investigating the incidence of AAT deficiency are undermined by several factors, including cohort selection, sample size and diagnostic assay limitations, making accurate predictions of individuals at risk difficult. Above all, the continuing lack of awareness and under-diagnosis of this condition remain the biggest impediments to a true picture of its epidemiology.

The two most common *SERPINA1* mutations associated with AAT deficiency are the S (Glu264Val) and Z mutations, however, the vast majority of AAT deficient individuals with emphysema are ZZ homozygotes. It is estimated that approximately 3-4% of people of European descent are heterozygous for the Z allele [8]. The frequency of the Z allele in America is similar to the lowest frequencies in Europe. AAT deficiency is rarer in the Asian, African, and Middle Eastern populations [9]. The Z allele is also rare in Japan, where AAT deficiency is more often the result of the Siiyama mutation (Ser53Phe) [10]. The frequency of the Z mutation, which causes the most severe plasma deficiency, is highest in northwest Europe with a mean gene frequency of 0.014, and its distribution gradually decreases along a north-west to south-east gradient [11]. The Z allele is thought to have arisen from a single origin 66 generations or 2,000 years ago [12]. The high Z allele frequency in southern Scandinavia suggests that the mutation may have arisen in this region and was subsequently distributed across Europe by major population movements [13]. A recent study

of Swedish and Latvian ZZ and MZ individuals estimated the age of the mutation to be 2,360 years old in Sweden but 2,900 years old in Latvia [14].

The S variant occurs at a frequency of 0.02-0.03 and is associated with mild reductions in serum AAT levels. In northern Europe it is estimated that approximately 6% of people carry the S allele [8] however the S allele is more common in the US.

1.1.1 SERPINA1 heterozygosity.

There are at least 116 million carriers for AAT deficiency (principally MS and MZ) worldwide [9]. It is estimated that there are approximately 7 million MZ individuals in the United States and 10 million in Europe [11] however based on our unpublished data these numbers are likely to be much higher. Ireland's national targeted detection program estimates that the frequencies of the Z and S alleles occur at 0.022 and 0.054 in the Irish population, respectively. This translates to a prevalence of 3,000 ZZ individuals on the island of Ireland who are at increased risk of developing emphysema and a further 14,000 SZ and 250,000 MZ heterozygotes. This is one of the highest frequencies in the world.

AAT deficiency is an under-recognised cause of genetic emphysema. Screening studies demonstrate a much larger number of individuals to have AAT deficiency than is clinically recognised with only about 5% of such individuals diagnosed [15]. The diagnosis of AAT deficiency is most often made following the detection of COPD, liver disease or the detection of a family member with the condition. A delay in diagnosis is a major clinical problem and there are often long delays between symptom onset and diagnosis with one study demonstrating a mean delay from the onset of the first attributable symptom and disease diagnosis of 5.6 years [16]. Given the large numbers of heterozygotes in the general population this becomes an important public health issue.

2. Clinical Manifestations of ZAAT deficiency

2.1 Lung

2.1.1 Emphysema and Chronic Obstructive Pulmonary Disease (COPD)

AAT deficiency increases the risk of lung diseases such as emphysema and COPD. Approximately 1% of patients with COPD are reported to have AAT deficiency [17] however this number could be much higher due to the lack of awareness and under diagnosis of ZAAT deficiency. Furthermore the role of *SERPINA1* heterozygosity, in particular MZ and SZ, in COPD sufferers remains to be determined. A major hypothesis for the emphysematous lung disease evident in AAT deficient individuals is the disturbed intrapulmonary protease-antiprotease imbalance. The lack of AAT in the lungs leads to unchecked serine protease activity such as that of NE which has been shown to cause lung destruction and impair immune responses by cleaving complement receptors and immunoglobins [18, 19], interfering with ciliary motility [20], and inactivating anti-proteases such as secretory leucoprotease inhibitor (SLPI) and elafin [21, 22]. The protease-antiprotease imbalance in AAT deficient individuals is aggravated by smoking and lung infection and may be due to a functional AAT deficiency as AAT is oxidised and potentially inactivated by cigarette smoke.

The classic presentation of AAT deficiency-related emphysema in susceptible patients is severe, early-onset panacinar emphysema with a basilar predominance in the fourth or fifth decades of life. This is distinctly different from the centriacinar emphysema observed in MM individuals with cigarette smoking-related COPD [23]. However, diffuse distribution and upper lobe-predominant emphysema has also been reported [24]. AAT deficiency-associated COPD rarely develops before the age of 30 years [25]. In a Swedish study of 246 ZZ individuals, COPD was present in 74.8% at a median age of 52 years [26].

Dyspnea is generally the most common symptom, but chronic cough and wheeze are well-described symptoms too [27]. In diagnosing patients with AAT deficiency with COPD, a detailed assessment of environmental and occupational exposures such as smoking or occupational dust is important. Pulmonary-function testing including spirometry, lung volumes and diffusing capacity for carbon monoxide remains the hallmark to evaluate the severity of COPD (Figure 1). In a study performed in the National Heart, Lung and Blood Institute (NHBLI) Registry, the mean forced expiratory volume in 1 second (FEV1) of 1129

participants was 43% predicted, and mean age was 46 years [27]. Chest radiography (CXR) is a useful diagnostic tool to identify basilar-predominant emphysema, however it is not as sensitive as thoracic computed tomography (CT thorax). One study revealed that only 20% of CXRs in AAT deficiency patients showed the distinctive pattern of bibasal emphysema [28]. Another study revealed basal and apical predominant emphysema in 64% and 36%, respectively [24]. CT thorax is more sensitive in picking up emphysema and bronchiectasis (Figure 2). Lung densitometry has recently been used in longitudinal studies demonstrating that the latter is more sensitive than FEV1 in detecting the progression of emphysema [29].

The precise risk of developing emphysema in *SERPINA1* ZZ homozygotes is not known. While smoking remains the most important risk factor for the development of emphysema in *SERPINA1* ZZ homozygotes, epigenetics also has an important role to play. Tobin *et al.* assessed the risk of developing emphysema in ZZ siblings of index cases [30] and found radiological emphysema was present in 90% of smokers compared with 65% of non-smokers. Smoking remains the biggest influence on survival rates with a clear correlation demonstrated between decline in FEV1 and smoking status in ZZ individuals [31]. In a study of 124 AAT deficiency individuals, smoking was estimated to reduce survival by up to 20 years [32]. Genetics variations which appear to modify the development of COPD in alpha-1 antitrypsin deficiency include interleukin-10 [33] and tumour necrosis factor (TNF)- α [34]. Factors reported to be associated with increased mortality in alpha-1 antitrypsin deficiency include evidence of emphysema, older age, lower education, lower FEV1 predicted and lung transplant [35, 36]. Besides conventional treatment with inhaled beta-agonists, all patients with AAT deficiency should be counselled regarding smoking cessation including those without documented lung disease. Pneumococcal and influenza vaccinations are recommended despite the lack of compelling data for their efficacy for COPD [37].

2.1.2 Bronchiectasis

Bronchiectasis is a well-established phenomenon in COPD patients. A study with 110 non-AAT deficient COPD patients revealed that bronchiectasis was noted in 29% of their high-resolution CT thorax (HRCT) [38]. Bronchiectasis, although uncommon, is also an established phenomenon in pulmonary manifestations of AAT disease. It can occur with or without concomitant emphysema [39]. An early study noted bronchiectasis in 11.3% of 246 ZZ patients [26]. However, the NHLBI registry reported bronchiectasis in only 2% of 1129 participants [40] and in a case control study, no excess frequency of AAT deficiency in patients with bronchiectasis [41]. A more recent study of 74 patients evaluated the CT thorax phenotype of ZZ patients [39]. This found evidence of bronchiectatic changes in the majority of patients and clinically significant bronchiectasis (i.e. radiologic bronchiectasis in 4 or more bronchopulmonary segments together with symptoms of regular sputum production) in 27% of the patients [39]. It has been argued that the similarity in the frequency of bronchiectasis and emphysema in this study indicates that bronchiectasis is predominantly an end-product of emphysema; however this study also revealed that severe bronchiectasis co-existed with relatively mild emphysema. A case control study to compare the prevalence of bronchiectasis in emphysema patients with and without AAT deficiency is needed to determine a direct causal effect of AAT deficiency in the etiology of bronchiectasis. Microbiology culture studies should also support specific infections which may cause or propagate bronchiectasis. One study with 100 patients revealed that the prevalence of non-MM AAT phenotypes was 27% in patients with primary bronchiectasis due to rapidly growing mycobacteria [42]. This frequency is 1.6-fold times the estimated prevalence of mutant AAT alleles in the U.S. population suggesting that AAT may be an anti-mycobacterial host-defense factor, and that AAT deficiency could constitute a risk factor for pulmonary disease due to rapidly growing mycobacteria.

2.1.3 Asthma

Thirty five percent of the AAT deficient participants in the NHLBI registry were reported to have asthma. Partial reversibility of airflow obstruction, as indicated by a 12%

and 200 ml rise in the FEV1 post bronchodilator, was evident in 61% of NHLBI registry participants tested with up to three serial spirometries [27]. The same registry reported that symptoms and signs of asthma are common in AAT deficiency and although it does not lead to an accelerated FEV1 decline, the clinical features associated with asthma commonly start at the age of most rapid FEV1 loss [43]. Also, this study showed that elevated total IgE levels are associated with a history of allergy and wheezing, indicators of atopy. A prospective study of 43 patients with the PiZ phenotype and emphysema revealed that the greatest bronchodilator reversibility are associated with a more rapidly decline in FEV1 [44]. However, the prevalence of airflow reversibility in AAT deficient patients depends on the criteria used. In another smaller study, asthma associated with atopy was more common in subjects with emphysema and severe AAT deficiency than in those with COPD without the deficiency [45]. Interestingly, AAT heterozygous phenotypes have also been shown to be associated with bronchial hyperresponsiveness [46].

2.2 Liver

The liver disease associated with ZAAT is due to the intracellular accumulation of misfolded protein rather than a plasma deficiency, and there are several overwhelming lines of evidence to support this gain of toxic function mechanism - null alleles, which produce no AAT, do not get cirrhosis [32]; the overexpression of ZAAT in animal models results in liver damage [47, 48]; and purified plasma ZAAT can form chains of polymers when incubated under physiological conditions [6]. Finally, the involvement of ZAAT polymerisation *in vivo* was confirmed by the finding of AAT polymers in inclusion bodies from the liver of a ZAAT homozygote with cirrhosis [6].

The Z mutation affects post-translational folding of the protein and induces a conformational change, causing it to accumulate as inclusions in the rough ER of the liver [49]. These inclusions predispose ZZ homozygous individuals to juvenile hepatitis, cirrhosis [50] and hepatocellular carcinoma [51]. Sharp and colleagues first described cirrhosis associated with AAT deficiency in 10 children from six families and later reported intra-

hepatocyte periodic acid–Schiff diastase-resistant inclusions, which occur owing to ZAAT polymer formation in the ER [52].

Hepatic disease associated with AAT deficiency is most common in children, but the natural history of the condition remains poorly defined. Of 127 newborn ZZ infants studied by Sveger *et al.* all showed increased liver enzyme concentrations, and 22 had manifested clinical signs of liver disease in infancy [50]. Eleven percent had prolonged neonatal jaundice, the most common presentation of AAT deficiency in early childhood, and among this cohort a further 29% developed cirrhosis. Assessments performed on 121 subjects from the original study showed elevated liver enzymes in 17% of ZZ adolescents at age 16 and 12% of ZZ adolescents at age 18 [53].

In adults, liver damage can manifest as chronic hepatitis, cirrhosis or hepatocellular carcinoma with reported incidences of the latter ranging from 5-30% [54-56]. Heterozygotes are a subset of AAT deficiency patients also at potentially increased risk of liver disease [57]. *SERPINA1* heterozygosity is implicated as an important co-factor in the aetiology of chronic liver disease. It is a modifier for hepatitis C virus, end-stage liver disease, cirrhosis and hepatocellular carcinoma. A study of 19 adult patients with AAT 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 age of the ZZ patients was 58 years when liver disease was diagnosed [58]. Liver disease can occur even in old age, with or without concomitant lung disease [59]. The reasons for the broad variation in liver disease associated with AAT deficiency remain unclear, and suggest the possible involvement of modifier genes or infection. For example, single nucleotide polymorphism-mediated translational suppression of the human ER mannosidase 1 gene can accelerate the onset of the end-stage liver disease associated with AAT deficiency [60]. Interestingly, a study looking at the differences in distribution of genotypes in patients with cystic fibrosis liver disease versus patients without liver disease showed that the *SERPINA1* Z allele is a risk factor for liver disease, and patients who carry the allele are at greater risk of developing severe liver disease with portal hypertension [61]. However conversely, cystic fibrosis

transmembrane conductance regulator (CFTR) gene heterozygosity as a modifier for liver disease in AAT deficiency patients has not been proven.

2.3 Other Manifestations

As well as lung and liver disease, AAT deficiency is associated with risks for the development of cutaneous panniculitis [62], anti-cytoplasmic antibody positive (ANCA) vasculitis [63], Wegener's granulomatosis [64], arterial aneurysm [65], lung cancer [66], glomerulonephritis [67], fibromuscular dysplasia [68] and pancreatitis [69]. However, even though AAT deficiency panniculitis and ANCA vasculitis are clear recognized manifestations, the associations with some of the other diseases listed above are much weaker. Associations with some neuropsychological conditions have been suggested such as anxiety disorder and bipolar disorder [70]. One study described how low serum AAT in family members of individuals with autism correlates with the MZ genotype [71]. AAT polymorphisms which affect iron, lipid and copper metabolism may affect early events in nervous system development, function and response to environmental exposures [72]. These associations may also explain linkage of bipolar disorder to 14q near AAT locus [73] and medical co-morbidity of reactive airway disease noted in bipolar patients [74]. Also, due to the fact that polymers of ZAAT are pro-inflammatory and can act as potent neutrophil chemoattractants, it is possible that they underlie the exuberant inflammation described in different organs of AAT deficiency individuals. To date, there have been no studies investigating the role of ZAAT polymers in these less prevalent conditions.

3. Loss of function effects in the lung: Pulmonary protease/antiprotease imbalance

AAT is the most abundant endogenous serine protease inhibitor in the blood and its major function is the inhibition of human NE [75]. The dominant model for the pathogenesis of emphysema in AAT deficiency is the protease-antiprotease imbalance, extrapolated from animal models where excessive protease activity, particularly NE, has been shown to cause lung damage, affect innate immunity and inhibit specific anti-proteases [76] (Figure 3). Lung

proteases include serine, aspartyl and metallo-proteases that function both intra- and extracellularly to regulate processes such as tissue remodelling, neutrophil chemotaxis, microbial killing and mucin production. In order to counterbalance overexuberant and potentially harmful pulmonary proteases, an array of antiproteases exist. These include AAT, SLPI [21], elafin [22], monocyte/neutrophil elastase inhibitor (MNEI) [77], and tissue inhibitors of metalloproteases (TIMPs) [78].

The degradation of extracellular matrix proteins such as elastin, collagen, fibronectin, laminin and proteoglycans is central to the tissue destruction observed in emphysema in AAT deficiency. In addition, degradation of collagen yields the proinflammatory chemotactic peptide proline-glycine-proline (PGP) which is synergistically augmented with increased NE activity. PGP is produced by the combined activities of matrix metalloproteinases MMP-8, MMP-9 and the serine protease prolyl endopeptidase [79, 80]. NE has also been shown to cleave coagulation factors such as fibrinogen, plasminogen, and complement proteins and their receptors [81]. Moreover NE possesses the ability to inactivate the naturally occurring protease inhibitors of MMP-9 and MMP-2 [82], and other protease inhibitors such as cystatin C [83], SLPI [21] and elafin [22].

Regulated protease activity has an important role in the killing of microbes and the modulation of innate immunity. For example it has been previously demonstrated that protease-deficient mice are highly susceptible to infection with *P. aeruginosa* [84], *E. coli* and *K. pneumonia* [85]. Another example is the degradation of the host antimicrobial protein lactoferrin by the lysosomal cysteinyl cathepsins in *P.aeruginosa*-positive sputum of cystic fibrosis patients [86]. Via the cleavage of the cell surface chemokine receptor CXCR1, excessive airway NE is also implicated in impairing pulmonary bacterial killing by neutrophils [87]. NE can also decrease responsiveness to lipopolysaccharide (LPS), resulting in a decreased ability to respond to invading pathogens [88].

A number of *in vitro* studies have shown that unopposed NE activity can facilitate neutrophil migration in response to inflammatory stimuli [74]. Increased NE activity is evident in the AAT deficient lung where excessive neutrophil recruitment can be seen. The other

AAT-regulated proteases PR-3 and cathepsin G are similarly increased amounts [89]. There is also evidence demonstrating that NE, and possibly other serine proteases can transcriptionally regulate expression of other classes of proteases. For example, NE upregulates expression of MMPs and cathepsins and neutralization of NE activity with AAT can reduce the overall burden of proteases [90]. Increased levels of active MMP-9 can lead to the increased production of chemotactic peptides that contribute to airway remodelling and inflammation [91].

In chronic inflammatory lung disease, proteases have the capability to activate key inflammatory receptors such as Toll-like receptors (TLRs). TLRs are expressed on immune cells such as neutrophils, monocytes, macrophages but also on lung epithelial cells. TLR activation occurs through binding of ligands such as lipopeptides, LPS, bacterial flagellin and viral and bacterial double- and single-stranded RNAs and DNAs. When activated, these receptors can augment neutrophil chemokine IL-8 levels, found in abundance in AAT deficient lungs and other chronic neutrophilic lung diseases [92]. NE itself induces increased expression of IL-8 [93], MMP-2 and cathepsin B through TLR-4 [94] in bronchial epithelial cells and macrophages, respectively. Additionally, activation of protease activated receptors (PAR 1-4) which are all expressed in epithelial, alveoli, smooth muscles and leucocytes are increased when there is unopposed protease activity [95]. Proteases that activate PAR in the lung include trypsin (PAR1, 2, 4) and cathepsin G (PAR4). In an AAT-deficient lung, these activated receptors result in the production of proinflammatory cytokine production e.g. IL-8 [96].

Epidermal growth factor receptors (EGFRs) are also implicated in the production of IL-8 in lung inflammation [97]. Activation of the EGFR pathway by NE has been demonstrated to occur by two different mechanisms involving either activation of dual oxidase 1 (DUOX-1) leading to production of reactive oxygen species [98] or direct activation and release of the metzincin, meprin α [99]. Both pathways lead to increased expression of IL-8. Increased NE activity also induces the generation of mucin in airway epithelial cells,

involving reactive oxygen species mediated activation of transforming-growth factor α (TGF- α) via the EGFR pathway [100, 101].

3.1 Therapeutics targeting the protease/antiprotease imbalance.

The dysregulation between protease activity and inhibition by antiproteases in AAT deficiency represent a key process for therapeutic intervention. In contrast to the liver disease in AAT deficiency which is a gain of function due to accumulation of polymerized ZAAT in the endoplasmic reticulum, therapeutic strategies aimed at the protease-antiprotease imbalance are more appropriate to treat AAT-related lung disease

Theoretically AAT augmentation therapy is a strategy designed to re-establish physiological levels of AAT within the lung. Gadek *et al.* first partially purified AAT from pooled human plasma and devised an intravenous infusion protocol of once weekly administration of purified AAT to ZAAT deficient individuals [102]. Significant amounts of AAT with full anti-elastase activity diffused into the lower respiratory tract and consequently, infusion of purified human plasma AAT (60mg per kilogram of body weight per week) to achieve serum levels $\geq 11\mu\text{mol/L}$ was approved by the FDA and is now widely used in Europe and North America to treat AAT deficiency [103]. Although infusion of AAT is safe and well tolerated, the clinical benefits have yet to be fully characterised [104, 105]. Augmentation therapy can decrease the rate of lung function decline and possibly mortality but only in patients with an initial FEV₁ of 30-45% predicted [35, 106]. Using Computed tomography (CT) lung density measurements a trend towards a slower rate in loss of lung density has been observed in patients receiving intravenous augmentation therapy [107, 108]. Tonelli *et al.* further clarified these findings by reporting that augmentation therapy was only effective in ex-smoking AAT deficient individuals with and FEV₁ <50% [109], whilst Parr *et al.* [110] using PD15 (the 15th percentile lung density), a specific CT densitometric index, showed an improvement in lung density in response to AAT augmentation therapy when the lower zones alone were assessed. A further study [111] collating data from two separate

underpowered clinical trials 10 year's apart, that each individually showed a trend towards improvement, used the statistical/endpoint analysis method to report a significant reduction in the decline in lung density. Later a meta-analysis of FEV1 data from five trials with 1509 patients reported that the decline in FEV1 was slower by 23% among all patients receiving augmentation therapy [112]. The overall protective effect was only evident in patients with a baseline FEV1 30-65% of predicted; other patients showed no improvement. Nonetheless it concluded that augmentation can slow lung function decline in patients with AAT deficiency and that patients with moderate obstruction are most likely to benefit. This meta-analysis was somewhat flawed as outlined by McCarthy and Dimitrov [113]; 60% of the patients were from a single non-randomised retrospective study, in another of the trials patients were used as their own controls with only two FEV1 measurements taken pre- and post-therapy. In addition introduction of newer treatment modalities in the 9-year period intervening the various trials made differences difficult to interpret.

Aerosolisation of AAT has been shown to increase AAT levels and restore anti-NE capacity in lung epithelial lining fluid of both AAT deficient and cystic fibrosis patients [114]. Also found to play a role in neutrophil-mediated killing of *Pseudomonas* spp. [115], AAT prevents cleavage of neutrophil complement receptors by serine proteases [87]. Griese *et al.* examined the effect of four weeks of plasma purified AAT inhalation on lung function, protease-antiprotease balance and airway inflammation in cystic fibrosis patients [116]. Post treatment, levels of NE activity, numbers of infiltrating neutrophils, pro-inflammatory cytokines levels and the numbers of bacteria (*P. aeruginosa*) were all reduced. In a later study, this group described the effect of aerosolized AAT in improving the killing of *Pseudomonas* by neutrophils with the restoration of CXCR1. Aerosolised AAT has also been shown to abrogate NE-induced expression of cathepsin B and MMP-2 *in vivo*, thus indirectly protecting key anti-inflammatory and antimicrobial peptides such as SLPI and lactoferrin, respectively from cathepsin-mediated proteolysis [87].

Another therapeutic strategy aimed at the protease-antiprotease imbalance is the administration of either natural (elafin/trappin-2 and SLPI) or synthetic antiproteases. Elafin,

and its precursor trappin-2, is an elastase-specific inhibitor found in the lung that has been demonstrated to be produced under inflammatory conditions. Increased elafin levels diminish neutrophil infiltration and LPS-induced monocyte chemotactic protein-1 (MCP-1) [117]. Elafin also possesses anti-microbial activity against both *P. aeruginosa* and *Staphylococcus aureus* [118]. However, despite its anti-protease, anti-inflammatory and anti-microbial attributes, *in vivo* oxidation and inactivation by neutrophil-derived oxidants and NE itself have been described, with possible negative impacts upon the clinical efficacy of elafin.

SLPI is expressed by lung epithelial cells [119]. It has anti-protease activity against a variety of serine proteases including NE, cathepsin G, trypsin and chymotrypsin [120]. SLPI also exhibits an array of anti-inflammatory properties including the inhibition of MMPs production by monocytes [121] and suppression of nitric oxide and TNF- α production by macrophages in response to LPS [122]. Animal studies have shown that intravenous recombinant SLPI can augment antineutrophil elastase defense [123] however, delivery of SLPI to the lungs has been difficult [124] and relatively more recombinant SLPI than AAT is required to suppress NE activity in the lungs of CF patients [125]. Whether augmentation therapy with SLPI is likely to benefit individuals with ZAAT deficiency remains to be shown. Other alternative therapies such as synthetic and semi-synthetic engineered elastase inhibitors and development of anti-protease chimeras [126] have demonstrated anti-NE capacity, however, preliminary data has only been generated from animal models [127], and their effect in humans remains to be explored.

4. Gain of function effects in the lung

As AAT deficiency is characterised by aberrant folding of the AAT protein it belongs to a class of genetic conditions collectively termed conformational disorders [128]. The accumulation of aberrantly folded Z protein within the ER lumen has the potential to cause the phenomenon of ER stress – the three classical features of ER stress are the unfolded protein response, the ER overload response and apoptosis. A detailed explanation of these responses is beyond the scope of this review however a number of recent review articles

describe them in depth [129-131]. Together they can be considered gain of function effects leading to, for example, translational attenuation of global protein synthesis, transcriptional induction of specific gene subsets including proinflammatory genes and apoptosis [132-136].

AAT is known to be produced by cells other than hepatocytes. For example in the circulation AAT is expressed by monocytes [135] and neutrophils [137] and also locally within the lung by airway epithelial cells [134] and more than likely alveolar macrophages. Expression of ZAAT by these different cell types can lead to a variety of gain of function proinflammatory effects due to accumulation of misfolded ZAAT in the lumen of the lung or within the ER of monocytes and airway epithelial cells (Figure 4).

4.1 Chemotaxis

It has been shown by a number of investigators that AAT is present in airway epithelial lining fluid in ZAAT deficient individuals [138-140] and that this ZAAT is in the polymerized form. Whilst some of this ZAAT may diffuse in from plasma it is no doubt also expressed locally by bronchial epithelial cells or pulmonary alveolar macrophages [134, 135]. Not only is this polymerized ZAAT unable to inhibit NE but it also can have a proinflammatory effect by acting as a neutrophil chemoattractant. Polymerized ZAAT is as potent a neutrophil chemokine as IL-8 [139].

4.2 Cytokine expression

Expression of ZAAT in 16HBE14o- human bronchial epithelial cells has been shown to lead to IL-8 and IL-6 production [132]. This was the first evidence that misfolded ZAAT may have the potential to affect the lung disease associated with AAT deficiency by leading to an aberrant inflammatory response. The reason for this increased cytokine expression is most likely due to activation of ER stress responses within the airway epithelial cells overexpressing the mutant Z transgene. A more striking example of this however is the effect of endogenous ZAAT on the cytokine expression profile of basal or LPS-stimulated ZZ

homozygous peripheral blood mononuclear cells [135]. Not only do ZAAT monocytes secrete higher than normal levels of IL-6, IL-8 and IL-10, amongst others, but their ZAAT can be seen by confocal microscopy to be retained in the ER in association with the ER resident protein glucose-responsive protein 78 (GRP78). This ER accumulation leads to activation of the transcription factors NF κ B and XBP-1 which can have multiple effects on signalling within a cell [130].

4.3 Autophagy

With respect to removal of misfolded AAT, there are at least two pathways for degradation of ZAAT that accumulates in the ER, the proteosomal and autophagic degradative pathways. Soluble ZAAT is degraded by the proteasome in a process termed ER associated degradation (ERAD) [141-143] These processes have been well characterised in liver cells however less is known regarding ZAAT removal in monocytes and airway epithelial cells.

Autophagy is a normal cellular process by which cells manage the disposal and recycling of cytoplasmic and membrane constituents and excess or defective organelles [144-146]. It involves *de novo* formation of a vesicle called the phagophore that wraps around a misfolded protein and becomes enveloped in a double-membraned autophagosomal vesicle. This fuses with a lysosome to generating an autophagolysosome facilitating access of hydrolytic enzymes to the vesicle contents. Degradation products are recycled and reused for energy and biosynthesis of new molecules and organelles.

In diseases characterised by aggregate-prone proteins autophagy has a role in managing misfolded proteins but in other contexts it has also be implicated in the processes of infection, repair and apoptosis [144, 146, 147]. In ZAAT deficiency in particular it is known that there are three autophagy gene products ATG5, ATG6 and ATG16 that are particularly important for the digestion of aggregated ZAAT [148] and although much is known regarding autophagy in ZAAT-related liver disease [149-152] it is not a phenomenon that has been intensively studied in ZAAT-related lung disease to date.

5. Effect of *SERPINA1* heterozygosity on susceptibility to lung disease

COPD is a complex trait, likely influenced by genetic factors but also gene-by-environment interactions. A number of genetic polymorphisms have been associated with specific pathological processes which influence individual sub-phenotypes of the disease. Oxidant stress from cigarette smoking upregulates genes encoding pro-inflammatory cytokines. Inflammation plays an important role in the pathogenesis of COPD. IL-13 over-expression in mice results in induction of MMPs and the development of emphysema, whereas TNF- α knock-out mice are relatively protected from emphysema [153]. The protease-anti-protease balance in patients with *SERPINA1* ZZ homozygosity has an important role in the development of early emphysema whereas *SERPINA1* MZ and SZ heterozygosity has been inconsistently associated with COPD. Approximately 1-3% of all cases of chronic obstructive pulmonary disease are caused by severe AAT deficiency (ZZ), which is the only definitely identified genetic risk factor, smoking being the most important environmental risk factor [154]. Wood *et al.* [155] also reported an association with AAT deficiency and mutations in surfactant protein B suggesting that variations within genes involved in inflammatory pathways may also have a role. However the risk of developing COPD in heterozygous individuals (MZ/SZ) with intermediate levels of AAT remains uncertain. MZ individuals have AAT levels approximately 60% of MM levels. If there is a correlation between plasma levels of AAT and lung disease it seems plausible that intermediate levels of AAT would confer an increased risk of emphysema [156].

Over a 100 studies have attempted to assess the risk of COPD in MZ individuals, yielding conflicting and controversial results. A meta-analysis of 22 of these studies concluded that there was a small increase in risk of COPD in all MZ individuals. The summary odd's ratio (OR) for COPD in MZ individuals compared with MM individuals was significantly increased at 2.31 (95% confidence interval (CI); 1.6 to 3.35). The results from this meta-analysis are consistent with a small increase in the risk of COPD but variability in

study design and quality limits the interpretation with those studies adjusting for cigarette smoking having a lower OR than those that did not (OR 1.61, 95% CI 0.92 to 2.81 versus OR 2.73, 95% CI 1.86 to 4.01)[157]. The St. Louis AAT study, carried out by Silverman *et al.* found a trend towards lower FEV1 in MZ relatives of symptomatic ZZ subjects with airflow obstruction [158]. Patients with the MZ phenotype appear to be at increased risk of hospital admission for COPD if they are first degree relatives of ZZ index cases [157].

The most recent study attempting to assess the risk of airflow obstruction in MZ heterozygotes compared two large populations, a case-control study from Norway (n = 1,669) and a multicentre family based study from Europe and North America (n = 2,707). The results suggest a slightly increased susceptibility to COPD in MZ individuals, with MZ patients having an FEV1/FVC ratio 3.5% lower in the case-control study and 3.9% lower FEV1/FVC ratio in the family study (p = 0.009). This was the first study to examine quantitative CT phenotypes in *SERPINA1* MZ heterozygosity wherein heterozygosity was associated with 3.7% more emphysema on chest CT scans (p = 0.003) [159]. Although the *SERPINA1* genotype is an important determinant of AAT levels, a complex interplay between genetic and environmental factors contributes to disease phenotype in MZ heterozygotes.

The only scientific evidence of a fundamental immune derangement in MZ individuals comes from a study of non-smoking asymptomatic MZ subjects without airflow obstruction demonstrating significant IL-8-related neutrophilic inflammation in the airways when compared to MM subjects. This study demonstrated that the mean (SD) neutrophil count was higher in PiMZ subjects [$84.5 (22.2) \times 10^4/\text{ml}$] compared with healthy controls [$55.0 (8.7) \times 10^4/\text{ml}$]. IL-8 levels were also higher in PiMZ subjects [828.5 (490.6) ng/ml; median 1003.0 ng/ml; range 1260–100 ng/ml] versus healthy age matched controls [3.5 (0.5) ng/ml; median 3.5 ng/ml; range 4.5–2.5 ng/ml]. There was a significant positive correlation between IL-8 concentration and neutrophil counts in PiMZ subjects (r = 0.66; p = 0.036). [160].

The *SERPINA1* S allele is more common than the Z allele, however the Z allele appears to be a more important determinant of disease with higher rates of COPD in SZ than in MS individuals. Early initial studies have indicated an increased risk of COPD in SZ individuals [161] however the SZ genotype appears to be less important than the ZZ phenotype in the development of emphysema [30]. A small study of 59 subjects demonstrated that the risk of COPD in SZ individuals appears to be influenced by smoking. SZ individuals who are either ex- or current smokers have similar rates of airflow obstruction as ZZ subjects. This was a small study of 59 patients with no distinction made between index and non-index subjects [162]. Another study which distinguished between index and non-index subjects showed that a lower proportion of PiSZ index (46%) and non-PiSZ index (15%) patients showed visible emphysema on CT scans compared with matched PiZZ index (91%; $p < 0.001$) and non-PiZZ index (61%; $p = 0.011$) patients. PiSZ subjects also had less airflow obstruction than matched PiZZ subjects [163]. A meta-analysis assessing the risk of the S allele showed that the OR for COPD in SZ heterozygotes is significantly increased at 3.26 while the risk of COPD in MS individuals is 1.19. Thus the SZ genotype appears to be a significant risk factor for COPD while the MS genotype is not [164]. It is important to consider that acquisition bias may influence this data.

Currently the most important therapeutic intervention in heterozygotes involves risk factor modification, primarily smoking cessation [165]. Heterozygous individuals typically have serum AAT levels ranging from 20-60% of normal (between 11 and 20 $\mu\text{mol/L}$), while those with severe deficiency (ZZ homozygotes) have serum levels $< 11 \mu\text{mol/L}$ [166]. A putative plasma AAT threshold level of 11 μmol is thought to protect against the development of emphysema based on population studies [167]. Alpha-1 antitrypsin augmentation therapy is currently not recommended for heterozygous individuals. Demonstration of an associated disease risk in heterozygotes patients will have a significant impact not only on the potential need for AAT augmentation therapy in this group but also on the current protective plasma threshold level required for ZZ homozygotes [165]. A large

population based study including careful control for age, sex, ethnicity and cigarette smoking is required to determine if SERPINA1 heterozygosity confers an increased risk of COPD.

6. EXPERT COMMENTARY

The American Thoracic Society and European Respiratory Society recommend that AAT deficient individuals with symptomatic lung disease be treated similarly to the standard treatments for non-AAT deficient COPD individuals [167]. Smoking cessation and avoidance of exposure to cigarette smoke are also highly recommended. In contrast to non-AAT COPD individuals however, therapeutic strategies aimed at restoring the correct protease-antiprotease balance exist specifically for the treatment of the lung disease in AAT-deficient individuals. AAT augmentation therapy is FDA approved in the US but is not a standard therapy throughout Europe. Intravenous augmentation therapy with plasma purified AAT is the current gold standard for treatment of the lung manifestations however it has yet to be proven to have clinical efficacy. Currently its most important effect appears to be the ability to decrease the rate of lung function decline in a subset of AAT deficient individuals with an FEV1 of 30-45% predicted [35, 106-109]. Recent data indicates that in addition to restoring AAT levels, augmentation therapy can also have positive effects on neutrophil-mediated inflammation in AAT deficient individuals, specifically by regulating neutrophil chemotaxis induced by soluble immune complexes, IL-8 and LTB4 [39]. Current studies are comparing the effects of intravenous (IV) versus aerosol administration routes. IV infusions are known to result in diffusion of AAT from the circulation into the lung interstitium, with a decreasing concentration gradient evident in plasma versus bronchoalveolar lavage fluid. Whether aerosolised AAT delivered to the airway lumen can gain access to the lung parenchyma has yet to be determined. Thus there are potential problems with the efficacy of aerosol administration. Currently recombinant and transgenic AAT proteins that have been generated cannot be safely administered to humans. The recombinant forms are non-

glycosylated whilst transgenic AAT has poor pharmacokinetics. In addition both forms co-purify with contaminating factors which can induce allergic reactions [168].

Gene therapies to treat both liver and lung manifestations for AAT deficiency are currently at various stages of development. For the liver disease, approaches that have been considered include ribozymes, antisense, peptide nucleic acids and small-interfering RNAs; all designed to inhibit expression of the mutant gene [reviewed in 169]. MacNab *et al.* used a small DNA fragment (SDF) approach to repair the Z mutation in ex vivo peripheral blood monocytes from ZAAT deficient individuals [170]. For the lung disease gene therapy studies have been carried out using non-viral, lentiviral and adeno-associated viral approaches to express the normal gene either locally or intramuscularly [171-176]. New approaches are focused on coupling haematopoietic stem cell therapy coupled with A1AT-lentiviral gene therapy [171, 177].

An important emerging theme in the field of AAT deficiency research involves the role of endoplasmic reticulum stress-induced responses. As intracellular gain of function effects mediated by ER accumulation of misfolded AAT are unlikely to respond to augmentation therapy alone, strategies designed to promote intracellular degradation of ZAAT and inhibit aberrant signalling cascades could prove more effective. Therefore newer treatments should be aimed at selectively modulating ER stress responses to enhance protein folding and/or disposal. For example the recent demonstration that promoting autophagy and proteosomal degradation in murine liver cells using carbamazepine can decrease the hepatic load of ZAAT and reduce hepatic fibrosis [178, 179] prompts the question of whether a similar strategy would be successful to reverse the toxic gain of function effects in airway epithelial cells and monocytes. It would also be worthwhile to investigate in more detail the ER stress-relieving properties of selenium supplementation [180], administration of bile acids [133] or chemical chaperones [reviewed in 130].

Hidvegi *et al.* [136] generated a mouse model with inducible ZAAT-liver expression however a suitable animal model of ZAAT deficiency-related lung disease does not exist. Attempts have been made to generate ZAATD mice however as they have five AAT

genes/pseudogenes generation of a mutant has been very difficult. This is a stumbling block for researchers studying ZAAT-related lung disease. Currently there are no commercially available cell lines from ZZ individuals however recent advances in induced pluripotent stem cell technology has generated a transgene-free lung-specific cell line [181] which will be very useful for basic science and therapeutic studies.

7. Five-year view

There are a number of important clinical questions to be addressed and answered in the short-term. Key amongst these are (i) whether IV and/or aerosol augmentation therapies are clinically effective and (ii) which routes of administration, doses and durations of therapy are optimal. With respect to gene therapies clinical trials currently underway will go a long way to proving how effective this approach can be.

Within the next five years AAT deficiency researchers will have determined whether *SERPINA1* heterozygosity represents an increased risk of developing COPD and if AAT augmentation therapy can reduce this risk. There are currently no planned clinical trials for AAT replacement therapy in patients with *SERPINA1* heterozygosity. Before such a clinical trial is undertaken it is crucially important to clarify the risk of COPD in *SERPINA1* heterozygotes in a well designed large population based study which removes ascertainment bias observed in previous studies. If *SERPINA1* heterozygosity is associated with a significant risk for emphysema this would have a very significant impact not only on the potential need for augmentation in these populations but also on the level of augmentation therapy required for *SERPINA1* homozygotes. The design of any such clinical trial in augmentation therapy would have to take into account these findings and would also have to determine whether computerised tomography or spirometry would be the most useful in evaluating efficacy. Ultimately, if therapy prove to be worthwhile for heterozygotes this will have important consequences. Foremost will be determining the correct mode of administration to achieve therapeutic levels (yet to be determined for heterozygotes) to potentially vast numbers of individuals. This would force rapid advances in the development

of recombinant and transgenic AAT formulations given the inadequate global supply of plasma-derived AAT. Given the close-relatedness of the lung disease in AAT-deficiency and COPD, from the lessons learned to date, it should be strongly considered whether augmentation therapy holds promise for COPD sufferers.

Clearly we also need a better understanding of the pathogenesis of COPD. In addition to dysfunctional NE-AAT ratios, other risk factors such as modifier genes undoubtedly play a role. We know this because of the marked variability in the development of lung disease in ZZ individuals. Indeed single-nucleotide polymorphisms (SNPs) in the IL-10 promoter have been identified that are significantly associated with airflow obstruction in AAT deficient individuals [33], others are also likely to exist [34] including surfactant protein B [182] and future studies should focus in this area. As a corollary to this SNPs at the alpha-nicotinic acetylcholine receptor (CHRNA 3/5) locus have been identified in a genome-wide association study of COPD, with the hedgehog interacting protein (HHIP) locus on chromosome 4 also believed make a significant contribution to the risk of COPD [183]. Reciprocal studies to determine whether these loci are important in risk and severity of illness in AAT deficiency are needed. With respect to the processes of ER stress, ERAD and autophagy, SNPs in genes involved in these responses could potentially impact on ZAATD also. Indeed Pan *et al* [60] have reported a SNP in ERMan1, a protein involved in ER associated degradation, that predisposes ZAATD individuals to liver disease.

Other areas ripe for investigation include enhancing our understanding of the role of proteases in COPD based on what we have learned from AAT deficiency and expanding our knowledge of the role of oxidants, not just in AAT deficiency and COPD, but also in other chronic and acute lung diseases such as acute respiratory distress syndrome, cystic fibrosis and pneumonia. Studies such as these may lead to a point where we consider prescribing AAT augmentation therapy for these diseases. Finally whilst we have made significant advances in our basic understanding of the role of ER stress in liver and lung disease in AAT deficiency, there will be intensive research in this area over the coming years. A particularly exciting aspect of this will be the prospect that new therapeutics developed for AAT

deficiency may have application for the treatment of other conformational diseases characterised by ER stress such as serpinopathies.

8. Key issues

- Alpha-1 antitrypsin (AAT) is a serine proteinase inhibitor produced largely by hepatocytes, that provides an antiprotease protective screen throughout the body but importantly in the lungs. It inhibits that activity of serine proteases with neutrophil elastase (NE) being its primary target.
- Over 100 mutations have been identified in the AAT gene (*SERPINA1*, previously termed the Pi locus). The most important are those that lead to deficiency in AAT and are associated with chronic obstructive lung diseases (COPD), liver disease and other manifestations. AAT deficiency is characterised by decreased levels of AAT in the circulation (5-11 μM or 0.26-0.572 g/l). This is in contrast to the non-AAT deficient individual with serum levels of 20-53 μM .
- The Z mutation encodes a misfolded variant of AAT in which the glutamic acid at position 342 is replaced by a lysine (Glu342Lys). ZAAT is not secreted effectively and accumulates intracellularly in the endoplasmic reticulum (ER) of hepatocytes and other AAT-producing cells. ER function is compromised as a result leading to ER stress responses and inflammation. The Z mutation occurs in >95% individuals with AAT deficiency.
- The S mutation (Glu264Val) is more common in the US and Southern (or SW) Europe than northern Europe and is associated with milder reductions in serum AAT levels (15-33 μM) than the Z mutation.
- Until recently it was thought that loss of antiprotease function was the major cause of ZAAT-related lung disease. Decreased intrapulmonary AAT levels lead to unopposed NE activity which can cleave matrix proteins and protease inhibitors causing tissue

destruction, and activate other proteases and cell surface receptors leading to cytokine and mucin production.

- Gain of function effects due to accumulation of ZAAT polymers in the lumen of the lung or intracellularly within the ER of monocytes and airway epithelial cells are now recognised. Specific gain of function effects that have been recognised that can contribute to ZAAT-related lung disease include promoting neutrophil chemotaxis into the lung and aberrant cytokine production from macrophages.
- Augmentation therapy with AAT can restore the anti-NE activity within the lung and slow the rate of decline in lung function in AAT deficient individuals however it has no effect on the proinflammatory gain of function effects associated with intracellular accumulation of polymerised ZAAT in monocytes and bronchial epithelial cells. Whether augmentation can impair neutrophil chemotaxis into the lung in response to accumulation of ZAAT polymers on the epithelial surface remains to be shown.
- *SERPINA1* heterozygosity, in particular SZ, is believed to contribute to smoking-induced COPD however large rigorous studies are required to determine the actual risk of developing COPD in MZ and SZ individuals, particularly those who smoke.

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Figure legends.

Figure 1: Spirometry

Flow volume loop demonstrating an obstructive airways disease in a 62 year old lady with GOLD Stage IV (FEV1 = 29%) COPD secondary to alpha-1 antitrypsin deficiency. Note the concavity of the expiratory phase of the curve (open arrow) in comparison to the normal F-V loop demonstrated by an MM individual (closed arrow).

Figure 2: High Resolution CT Scan (HRCT)

High resolution computerised tomography from a 33 year old patient with ZZ A1AT deficiency. Note the basilar predominant pan-acinar emphysema (A). Bronchiectasis is also observed in the basal segments of both upper lobes (B).

Figure 3. Loss of Function Effects

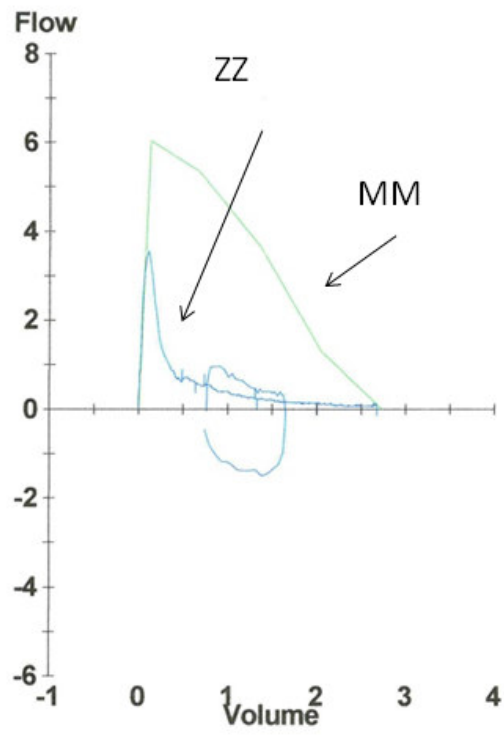
Loss of functional AAT facilitates unopposed NE activity leading to impaired bacterial killing due to 1. decreased LPS responsiveness resulting in a decreased ability to respond to invading pathogens and 2. cleavage of the IL-8 receptor CXCR1 on neutrophils thus disabling their bacterial killing capacity. NE can activate receptors including TLRs, EGFR and PARs leading to increased mucin expression, cytokine production and further neutrophil recruitment due to increased levels of IL-8. Protease inhibitors are cleaved by NE facilitating the unopposed activity of other proteases. This leads to damage to tissue and the generation of additional chemotactic factors

ADAM = A Disintegrin and Metalloproteinase; CXCR-1 = Chemokine Receptor-1; EGFR = Epidermal Growth Factor Receptor; IL = Interleukin; LPS = Lipopolysaccharide; MMP = Matrix Metalloproteinase; NE = Neutrophil Elastase; PAR = Protease Activated Receptor; PGP = Proline-Glycine-Proline; SLPI = Secretory leukocyte Peptidase Inhibitor; TIMP = Tissue Inhibitor to Matrix Metalloproteinases; TLR = Toll-like Receptor

Figure 4. Gain of Function Effects

Gain of function is associated with intracellular accumulation of polymerised ZAAT in the airway lumen. This form of AAT cannot effectively inhibit NE and is also a potent neutrophil chemoattractant. Accumulation of ZAAT in the ER of monocytes causes it to associate with GRP78 in the ER and leads to activation of the transcription factors NF κ B and XBP-1, resulting in increased expression of basal or LPS-induced expression of IL-6, IL-8 and IL-10. In bronchial epithelial cells ZAAT induces IL-6 and IL-8 expression. GRP78 = Glucose-Responsive Protein 78; IL = Interleukin; LPS = Lipopolysaccharide; NE = Neutrophil Elastase; NF κ B = Nuclear Factor kappa B; XBP-1 = X Box Binding Protein-1.

Figure 1



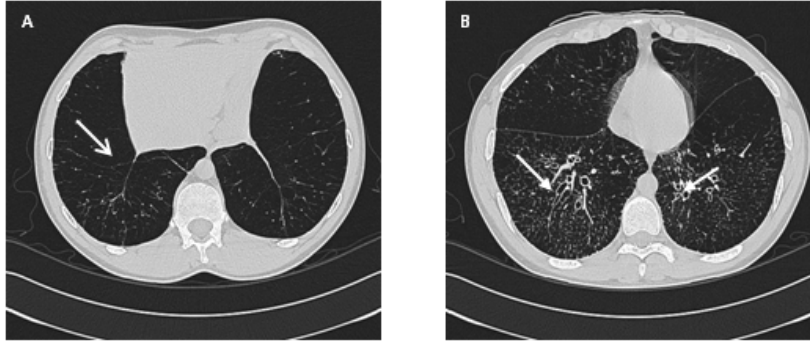


Figure 2: High Resolution CT Scan (HRCT)

High resolution computerised tomography from a 33 year old patient with ZZ A1AT deficiency. Note the basilar predominant pan-acinar emphysema [A: Open Arrow]. Bronchiectasis is also observed in both upper lobes [B: Solid Arrow].

Figure 3

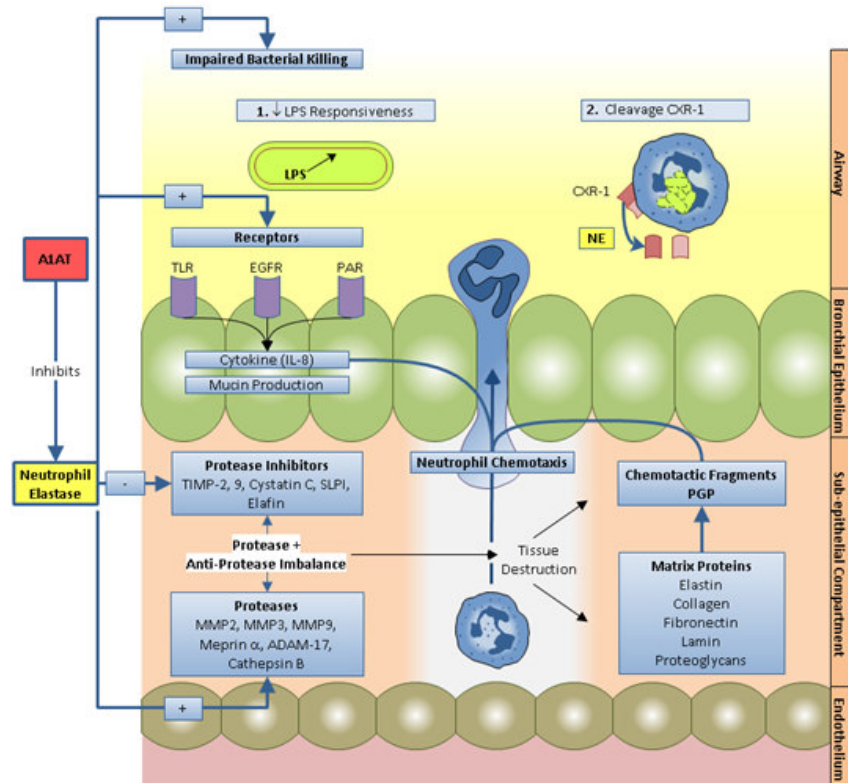


Figure 4

