

Priorities for the alpha-1 community: The physicians perspective

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1. Introduction

Alpha-1 antitrypsin (AAT) deficiency is a clinically under-recognized hereditary disorder with multi-system manifestations, most prominently in the lungs and liver. A rare skin manifestation is also described. The AAT protein is synthesized in the liver and to a lesser extent in macrophages and neutrophils. AAT is the physiological inhibitor of a variety of proteases most notably neutrophil elastase (NE). Unopposed, NE and other proteases attack the lung matrix causing structural damage and markedly impairing host defence. In the commonest form of AAT deficiency the mutated Z AAT is improperly folded, polymerises and aggregates in the liver. As a result less AAT is secreted into the bloodstream and gets to the lungs. This results in liver disease due to AAT aggregation in the liver and pulmonary damage due to the deficiency in the lung rendering it unable to protect against NE-mediated damage. In this review we will discuss AAT deficiency in detail, outlining the pathogenesis, and the clinical manifestations of the condition.

2. The alpha-1 gene and subsequent deficiency disease state

AAT deficiency was first described by Laurell and Eriksson in 1963 who noted a clinical link with emphysema [1]. Just over 6 years later Sharp et al. first associated the protein deficiency with liver disease [2].

Since then, our understanding has advanced significantly. The AAT gene, located on the long (q) arm of chromosome 14 is a member of the serpin gene family which encode a group of serine protease inhibitors. Inheritance is monogenic and autosomal co-dominant where products of both alleles are expressed. There are numerous AAT

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Table 1
Table showing genotype variants in alpha-1 antitrypsin protein level & function

Variant	Allele or mutation	
Normal	<i>M</i>	Normal plasma levels (>20 $\mu\text{mol/L}$); 95% of alleles in Caucasian populations
Deficient	<i>Z</i> or <i>S</i>	Common deficiency variants: 'Z' (5–6 $\mu\text{mol/L}$) & 'S' (8–11 $\mu\text{mol/L}$) plasma levels
Null	<i>QO_{Lisbon}</i> , <i>Thr68Ile exon II</i>	No detectable circulating protein; no associated liver disease
Dysfunctional	<i>Met358Arg</i>	Unique Pittsburgh mutation (3): converts protein into thrombin rather than elastase inhibitor

alleles, and their nomenclature is based on their migration on electrophoresis, with those exhibiting high isoelectric points being allocated letters from the beginning of the alphabet, and those with low isoelectric points conferred letters from the end of the alphabet. At least 100 alleles have been identified; however they can be widely classified into four main clinical groups, based on serum levels and function (Table 1).

Both null and dysfunctional mutations are rarely encountered in clinical practice with the majority of patients with AAT deficiency either homo- or heterozygous for 'Z' or 'S' variants which subsequently correlate with severity of pulmonary or hepatic manifestations of disease. The highest disease incidence is within Europe [4,5].

Base substitutions, in-frame and exon deletions and frame-shift mutations can all occur within the AAT gene. The most common and clinically important variants (S, Z) result from single amino acid substitutions: a valine for glutamate at position 264 (Glu264Val) in the case of the 'S' variant and lysine for glutamate at position 342 (Glu342Lys) for the 'Z' variant [6]. The S protein is less polymerogenic than Z and results in reduced accumulation of S AAT within hepatocytes, yielding a milder serum deficiency. However, if the S variant is inherited with the rapidly polymerising Z variant, the two forms interact within hepatocytes, forming inclusions and in some cases, cirrhosis [7–10].

Hepatic polymerisation of Z AAT results in minimal secretion into the bloodstream and hence the lung. Consequently, a pulmonary protease-antiprotease imbalance occurs where proteases such as NE are relatively unopposed in causing lung destruction and hampering immune responses by effects on complement receptors [11, 12], immunoglobulins [13], ciliary motility [14], and antiproteases such as secretory leucoprotease inhibitor (SLPI) and elafin [15,16]. The polymerisation of Z AAT in the liver also goes some way to explaining liver disease associated with the condition. Normally cells have a mechanism by which abnormally folded proteins are recognised and removed. In AAT deficiency there is an imbalance between protein folding load and the cell's ability to process this load. The result is endoplasmic reticulum (ER) stress which manifests in a variety of individual but not exclusive intracellular responses including the ER overload response (EOR), the unfolded protein response (UPR) and apoptosis [17].

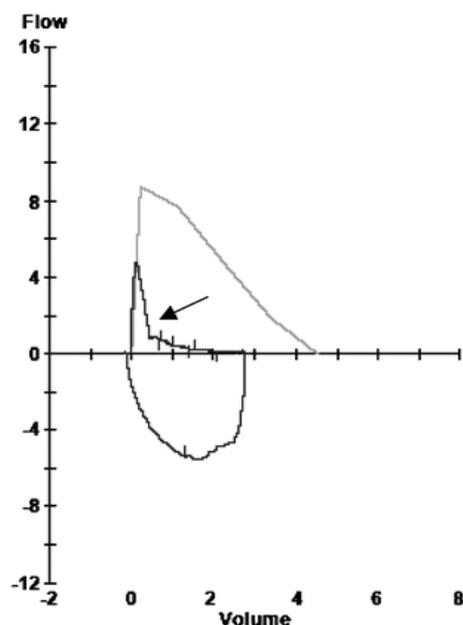


Fig. 1. Flow-volume loop of a patient with Alpha-1 antitrypsin deficiency. Coving of the expiratory portion of the curve is seen (arrow), reflecting flow limitation. A normal expiratory curve is also shown.

3. Clinical manifestations of AAT deficiency lung disease

The hallmark of AAT pulmonary disease is early onset panacinar emphysema in a predominantly basal distribution [18,19]. Obstructive lung disease presents at a mean age of 32 to 41 in subjects with a current or previous history of smoking [20–24]. In a study of 246 PI*ZZ individuals, chronic obstructive pulmonary disease (COPD) was present in 74.8% at a median age of 52 years [25]. True natural history of this condition remains uncertain; many individuals with AAT deficiency remain undiagnosed or misdiagnosed as non-hereditary emphysema. Most individuals with AAT deficiency have a smoking history (NHLBI Registry). Smoking is estimated to shorten survival of AAT-deficient individuals by up to 20 years [20].

The National Heart, Lung and Blood Institute Registry (NHLBI) of individuals with AAT deficiency utilised standardized symptom questionnaires to quantify symptom frequency. Exertional dyspnoea was predominant (84%), followed by self-reported wheezing (65%), cough with phlegm (50%) and coughing alone (42%). Frequency of wheeze is important as 35% of subjects self-reported a history of asthma, while 50% had a significant bronchodilator-associated reversibility of airflow obstruction on serial testing [26]. Eden et al. assessed for the presence of asthma through wheezing, bronchodilator responsiveness, atopy, and increased serum IgE. They demonstrated the presence of three or more of these markers in 22% of AAT-deficient patients compared with 5% of COPD patients without AAT deficiency [27].

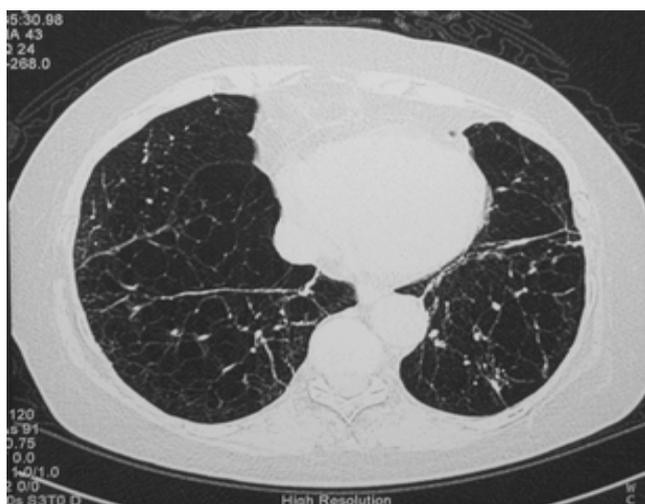


Fig. 2. High Resolution Computed Tomography (HRCT) of lung bases from patient with Alpha-1 antitrypsin deficiency. Widespread emphysematous changes are seen bilaterally.

Pulmonary function in established disease shows decreased expiratory flow rates (FEV1 & FEF_{25–75}) but preserved forced vital capacity (FVC). Flow volume curves show “classic coving” on expiration (Fig. 1).

Plain chest radiography (CXR) in early disease shows minimal change. Changes are seen earlier and are more easily quantified on computed tomography (CT) scans of the thorax (Fig. 2). There has been an increased use of CT scanning in AAT deficiency for two main reasons; to detect early disease before appreciable loss of lung function and to assess efficacy of therapeutic interventions such as augmentation therapy. This has led to the detection of other associated lung conditions such as bronchiectasis. Guest et al. [28] demonstrated that in an AAT deficiency population, radiological features consistent with bronchiectasis were observed in 41%. King et al. [29] demonstrated a similar prevalence of bronchiectasis (43%). An early series however, by Larsson et al. [21] showed that bronchiectasis was present in just 11.3% of 246 PI*Z homozygotes investigated.

Arterial blood gases (ABG) show normoxia or hypoxia at rest although hypercapnia is seen in advanced disease associated with markedly decreased FEV1 [26].

4. Clinical manifestations of AAT deficiency liver disease

Sharp and colleagues [2] first described cirrhosis in AAT deficiency in 10 children from six families and later reported intra-hepatocyte periodic acid-Schiff diastase-resistant inclusions, which occur owing to polymer formation of Z AAT in the ER

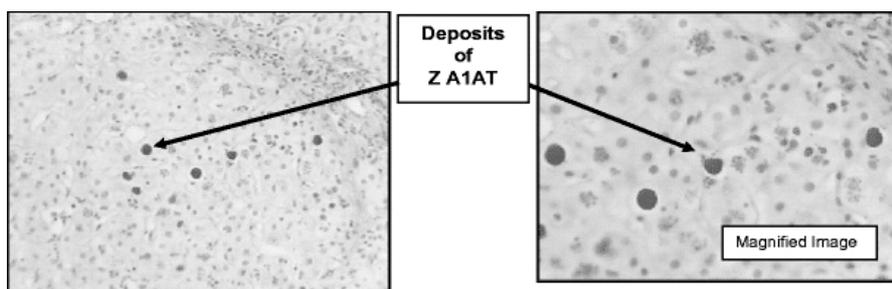


Fig. 3. Diastase-resistant periodic acid-Schiff stained liver section from a ZA1AT-deficient individual showing A1AT deposits.

(Fig. 3). In PiZZ individuals, 10–15% show clinically significant liver disease in their first 20 years of life [30].

Hepatic disease associated with A1AT deficiency is most common in children. Of the 127 newborn PiZZ infants studied by Sveger et al. [31], all showed increased liver enzyme concentrations. Eleven percent had prolonged neonatal jaundice (the most common presentation of AAT deficiency in early childhood), of whom 29% developed cirrhosis. Sveger et al. [31] also showed that infants with a PiSZ phenotype had no signs of liver disease. A follow-up study [30] performed at age 16 of neonates screened in Sweden showed elevated liver enzymes in 17% of PiZZ and 8% of PiSZ adolescents. Adults with liver disease in infancy were later clinically healthy [30].

In adults, liver damage can manifest as chronic liver disease or hepatocellular carcinoma with reported incidences of the latter ranging from 5–30% [30,32]. Five to ten percent of AAT-deficient patients over 50 will develop cirrhosis. 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. Mean age of the patients with the PiZZ, PiSZ, and PiMZ phenotypes were 58, 66, and 72.5 years when liver disease was diagnosed, suggesting later onset liver disease in heterozygotes [33].

5. Other clinical manifestations of AAT deficiency

An association has been described with Wegener's granulomatosis which is not surprising given that anti-neutrophil cytoplasmic antibody; the hallmark of this condition is raised against proteinase 3 which is naturally inhibited by AAT. There exists several case series [34,35] describing an excess of AAT phenotypes in cohorts with ANCA positive vasculitis. Necrotizing panniculitis is the characteristic skin lesion described in AAT deficiency. Clinical responses to augmentation therapy and dapsone have been described [36–38].

Reports of aneurysms (intra-cranial/abdominal) and fibromuscular dysplasia demonstrating predilections for AAT deficient patients exist [39] but formal association remains unproven. Renal disease has been implicated in AAT deficiency,

with a heterogenous series of manifestations ranging from IgA nephropathy to membranoproliferative glomerulonephritis. There is also a reported association with the development of renal disease in patients with cirrhosis [40,41].

6. Current and future translational and therapeutic aspects to disease

In 1987 Wewers et al. [42] demonstrated that plasma purified AAT could be safely administered by intravenous infusion to AAT deficient individuals maintaining plasma levels above a putative protective threshold of 11 μM . This protective level was based on typical levels of AAT in SZ individuals, who if non-smokers are at minimal risk of developing emphysema [43]. Additionally, there were concomitant increases in both AAT and anti-NE capacity on the lung's epithelial surfaces. This evidence of biochemical efficacy is as yet unaccompanied by evidence of clinical efficacy. A number of studies suggest that intravenous AAT augmentation therapy may have some beneficial clinical effects. Seersholm et al. [44] compared a group of Danish ex-smokers with AAT deficiency to a similar German group who received augmentation therapy. In individuals with forced expired volume in one second (FEV1) ranging from 31–65% predicted there was a significant difference in FEV1 decline of approximately 21 cc/year between those receiving augmentation therapy and those who did not. Similar results were found on evaluation of the NHLBI registry data [45]. These data additionally showed a mortality risk reduction in those receiving AAT augmentation therapy. Further work by Dirksen et al. [46] evaluated radiographic changes (CT) in those receiving augmentation therapy and although no significant difference ($p = 0.07$) was observed the study provided adequate information to develop a power statistic and determine how many AAT individuals were needed in future trials to conclusively show a clinical effect by CT scan. Consequently, spirometry measurements are now regarded as secondary efficacy endpoints. Once monthly and bi-weekly administration of AAT has also been evaluated but most data presently supports a once-weekly regimen [47].

Worries about potential transmission of infectious agents by a human plasma-derived product have led to the development of transgenic and recombinant sources of human AAT. Transgenic production of human AAT protein has been achieved in goats [48] and sheep [49], and human AAT has also been produced in yeast using recombinant technology [50]. Unfortunately, all these proteins are cleared more rapidly than plasma purified AAT from the circulation following intravenous administration. Consequently, the inhaled route has been investigated for these products. These have an acceptable half-life on the epithelial surface following aerosolisation. The conceptual concern with this route is that while it can be argued that increasing the level of AAT in blood and subsequently measuring it in epithelial lining fluid (ELF) gives some reassurance that the interstitium of the lung is being protected the same argument is not necessarily applicable with aerosolisation. A number of gene therapeutics for AAT deficiency has been developed. For example,

the normal AAT gene has been successfully introduced into striated muscle cells in animals using an adeno-associated virus vector [51] but clinical trials are awaited.

In terms of hepatic manifestations, liver transplantation provides the only effective intervention. Liver transplantation achieves successful serum conversion and acceptable survival rates of over 80% in both adults and children. Its benefit and application is hampered by the lack of donors and negative aspects of life-long immunosuppressive therapy. With regards potential medical interventions Miller et al. [17] have shown that the bile acid tauroursodeoxycholic acid (TUDCA) targets the newly delineated apoptotic pathway in the liver and as such may hold therapeutic promise in promoting survival of hepatocytes in Z AAT expressing cells.

7. Key challenges and opportunities faced by AATD patients worldwide

There are a number of major questions, challenges and opportunities facing AAT deficient patients worldwide and these include;

1. What is the natural history of the condition?
2. How to diagnose individuals before appreciable organ damage?
3. How to determine whether current treatment modalities are effective and if not to investigate more effective therapeutics?
4. How to determine whether MZ carriers are at increased risk of lung disease?
5. How to utilise lessons from over 50 years research in AAT deficiency to better understand the pathogenesis of non-AAT related lung and liver disease?

7.1. The natural history of AAT deficiency

The natural history of this condition still requires elucidation. We do not know how many people there are with the condition and how many will be clinically affected if they do not smoke or have other significant liver disease. Most published data suggesting a bleak outcome are based mainly on symptomatic index cases [52]. With new larger pan-national registries and targeted detection programs inclusive of studying family members, a clearer picture should emerge in the coming decades.

7.2. Diagnoses of individuals before appreciable organ damage

This has been a major problem particularly in the early years of the condition. The diagnostic algorithms for lung disease have tested individuals with obstruction at an early age. The new diagnostic algorithms widen the net considerably (Table 2) to include first degree relatives, poorly responsive asthmatics, and individuals with cryptogenic cirrhosis and vasculitis, inevitably leading to earlier detection rates.

Table 2

Guidelines for diagnostic testing of AAT deficiency (adapted from ATS AAT Task Force Recommendations); **A:** Genetic testing is recommended **B:** Genetic testing should be discussed and could reasonably be accepted or declined

<p>Type A recommendation for diagnostic testing Symptomatic adults with emphysema, chronic obstructive pulmonary disease (COPD), or asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators. Symptomatic adults with emphysema, chronic obstructive pulmonary disease (COPD), or asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators. Individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g. cigarette smoking, occupational exposure) Adults with necrotizing panniculitis</p>
<p>Type B recommendation for diagnostic testing Adults with bronchiectasis without evident etiology Adolescents with persistent airflow obstruction Asymptomatic individuals with persistent airflow obstruction and no risk factors Adults with C-ANCA-positive (anti-proteinase 3-positive)</p>

7.3. Determine efficacy of current treatments

We are still uncertain whether augmentation therapy has any clinical effect. This disappointing fact, twenty years after the original Wewers et al. paper [41] is not surprising given the numbers of patients who would have to be studied to prove efficacy. It is estimated that approximately 550 AAT deficient individuals would need to be studied over a 2 year period to show a significant impact of intravenous AAT augmentation therapy on spirometry. However only 130 would have to be studied to show impacts on CT based indices of emphysema. This has been one of the major breakthroughs in recent years and such parameters are now accepted by the Food and Drugs Administration (FDA) [53].

7.4. Are MZ carriers at increased risk of disease manifestations?

Many studies of the PI MZ population and COPD risk have been performed with inconsistent results. In general, studies comparing COPD with healthy controls have found an excess of PI MZ individuals among COPD cases, but studies assessing FEV1 in PI MZ and PI MM subjects from population-based samples have not found significant differences [54]. Our group in collaboration is currently assessing this question. We are performing this in the setting of National Registries and in specific studies utilising the MZ population detected by our targeted detection program.

7.5. Utilise lessons from over 50 years of research to understand the pathogenesis of non-AAT related lung and liver disease

AAT deficiency is the only hereditary condition directly related to development of COPD. As such it gives invaluable insight into COPD pathogenesis and its treatment. This deficiency has inspired studies into the effects of oxidants from cigarette

smoke [55–58] and their ability to inactivate normal M AAT causing a functional deficiency in the lungs of smokers [59]. This suggests possible therapeutic options such as antiproteases or antioxidants. While hepatic manifestations have been less studied many of the mechanisms elucidated in AAT liver disease can be applied to other conditions such as viral liver disease and haemochromatosis. Most recently our understanding of AAT deficiency as a conformational disorder has paved the way to a better understanding of pathogenesis of conditions as disparate as Alzheimer's, Parkinson's and Huntington's disease.

8. Examples of physician initiatives in the EU and/or internationally

There have been increasing numbers of physician-inspired initiatives within the realm of AATD in the last decade. For example within the European Union several augmentation and lung transplant programmes exist along with the alpha-1 international registry (AIR). The Alpha One Foundation and the University of Florida screening programmes in the United States provide further evidence of this trend. For the purposes of this chapter, we will highlight our national targeted initiative that has been developed over the last five years: "The National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme and Registry".

In the Republic of Ireland, we established in 2004 a national targeted detection programme for suspected alpha-1 antitrypsin deficiency patients and a website (<http://www.alpha1.ie/>) that provides a resource for physicians, patients and the general public.

This programme is directly funded by the Department of Health and provides testing for AAT deficiency at no cost to patients at risk according to ATS guidelines.

Based on a genetic screening study on 1000 random samples by our group, we estimate that 3,000 Irish citizens have the deficiency and up to 700,000 are carriers, yet only a fraction of these has been identified to date. The targeted detection program is into its fifth operational year and during the period we have tested 3,000 individuals throughout Ireland. Thus far, we have identified over 90 severely deficient individuals (ZZ, SZ) and over 700 moderately deficient individuals (carriers, mainly MZ).

Following diagnosis, the foundation provides multiple ancillary services to patients including counselling, expert advice through clinics, information packs and leaflets for patients and relatives. Through this initiative, we also offer patients opportunities for enrolment in clinical trials including an augmentation therapy trial and membership of the alpha-1 patient support group. In the last year, we have established a National Alpha-1 Registry to track the health status of patients with the deficiency throughout Ireland. The type of information collected includes height, weight, gender, genotype, pulmonary function, liver tests, hospitalisations and complications related to lung and liver disease manifestations. Such information will help clinicians and researchers to identify new trends, design clinical trials and improve care delivery for patients.

9. Conclusion

Alpha-1 antitrypsin deficiency is more prevalent in Ireland than was previously thought, even after making allowances for the targeted symptomatic population investigated through our national initiatives. The importance of an early and accurate diagnosis cannot be over-emphasised as the consequential medical follow-up and lifestyle changes help to prevent or at least postpone the development of related lung and liver disease.

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