Alpha-1 antitrypsin deficiency: current therapy and emerging targets

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Abstract

Introduction: Alpha-1 antitrypsin deficiency (AATD), a common hereditary disorder affecting mainly lungs, liver and skin has been the focus of some of the most exciting therapeutic approaches in medicine in the past 5 years. In this review, we discuss the therapies presently available for the different manifestations of AATD and new therapies in the pipeline.

Areas covered: We review therapeutic options for the individual lung, liver and skin manifestations of AATD along with approaches which aim to treat all three. Along with this renewed interest in treating AATD come challenges. How is AAT best delivered to the lung? What is the desired level of AAT in the circulation and lungs which therapeutics should aim to provide? Will treating the liver disease increase the potential for lung disease? Are there treatments to target the underlying genetic defect with the potential to prevent all aspects of AATD-related disease?

Expert opinion: With a relatively small population able to participate in clinical studies, increased awareness and diagnosis of AATD is urgently needed. Better, more sensitive clinical parameters will assist in the generation of acceptable and robust evidence of therapeutic effect for current and emerging treatments.

Keywords: alpha-1 antitrypsin deficiency, emphysema, COPD, cirrhosis, panniculitis, gene therapy, siRNA, gene editing, protein folding

Article highlights

- Alpha-1 antitrypsin deficiency is a genetic condition caused by mutations in the SERPINA1 gene.
- We discuss the lung, liver, and skin manifestations of this under-diagnosed condition and highlight improvements in detection and diagnosis.
- Treatment options have been historically limited for the lung, liver and skin manifestations, with lung and liver transplantation a common outcome.
- Current treatments for AATD-related lung disease are similar to treatments for non-AATD lung disease, with the notable exception of augmentation therapy.
- We discuss new and innovative therapies in development, including some that tackle the underlying genetic defect, and discuss future challenges for newer treatment approaches.

1. Introduction

Alpha-1 antitrypsin (AAT) is a glycosylated protein produced mainly in the liver [1]. It first came to prominence in the 1960s when Laurell and Eriksson, in Malmo, Sweden discovered that a lack of this protein was associated with an increased risk for emphysema [2]. Further work showed that the major role of AAT in the lung was to inhibit neutrophil elastase (NE), an omnivorous protease produced by neutrophils, which is capable of digesting many structural components of the lung in addition to proteins involved in immunity and inflammation [3]. This led to the development of the protease-antiprotease theory of emphysema in which the antiprotease protection in the lung, mainly provided by AAT, is markedly reduced, either functionally, in theory by cigarette smoke, or quantitatively by AAT deficiency (AATD), leading to the unopposed action of NE and subsequent lung destruction [4-6]. Further work elucidated the liver disease associated with AATD [7] and determined that the major cause of low levels of AAT in the blood and lungs of people with AATD was due to polymerization and retention of misfolded AAT protein in the liver [8,9].

SERPINA1 Variant	Molecular Basis	SNP Number	Cellular Effect	Disease Association
S	p.Glu264Val	rs17580	Polymerization, impaired secretion, reduced antiprotease activity	Lung & liver
Z	p.Glu342Lys	rs28929474	Polymerization, impaired secretion, reduced antiprotease activity	Lung & liver
I	p.Arg39Cys	rs28931570	Polymerization, impaired secretion, reduced antiprotease activity	Lung & liver
F	p.Arg223Cys	rs28929470	Reduced antiprotease activity	Lung
M_{malton}	p.Phe52del	rs775982338	Polymerization, impaired secretion, reduced antiprotease activity	Lung & liver
Null (Q0)	Premature termination codon	n/a	Family of mutations that block production of AAT protein	Lung

Table 1. Deleterious SERPINA1 variants frequently identified in Irish AATD Targeted Detection Programme. SNP: single nucleotide polymorphism. Quoted amino acid sequence does not include the 24 amino acid signal peptide which is not present in the mature AAT protein.

We know now that AATD is caused by harmful mutations within the serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 1 (SERPINA1) gene that encodes the AAT protein.[10] The most commonly encountered harmful mutation is called Z (p.Glu342Lys, rs28929474) but many other pathological mutations have been described (**Table 1**). The normal or wild type SERPINA1 gene is referred to as M, with S (p.Glu264Val, rs17580) another common but low risk variant. The majority of individuals with severe AATD are homozygous for the Z mutation and referred to as ZZ. The Z AAT protein leads to endoplasmic reticulum (ER) stress, liver inflammation and reduced secretion of AAT into the circulation resulting in insufficient quantities to protect the lung [1,11,12]. Thus, in the lung disease associated with AATD the problem is due to a lack of function while in AATD liver disease there is a gain of toxic function, i.e. too much abnormally folded AAT in the liver, leading to liver inflammation. Further studies over the following years described three major clinical manifestations of AATD: emphysema, liver disease and panniculitis [13] (**Figure 1**).

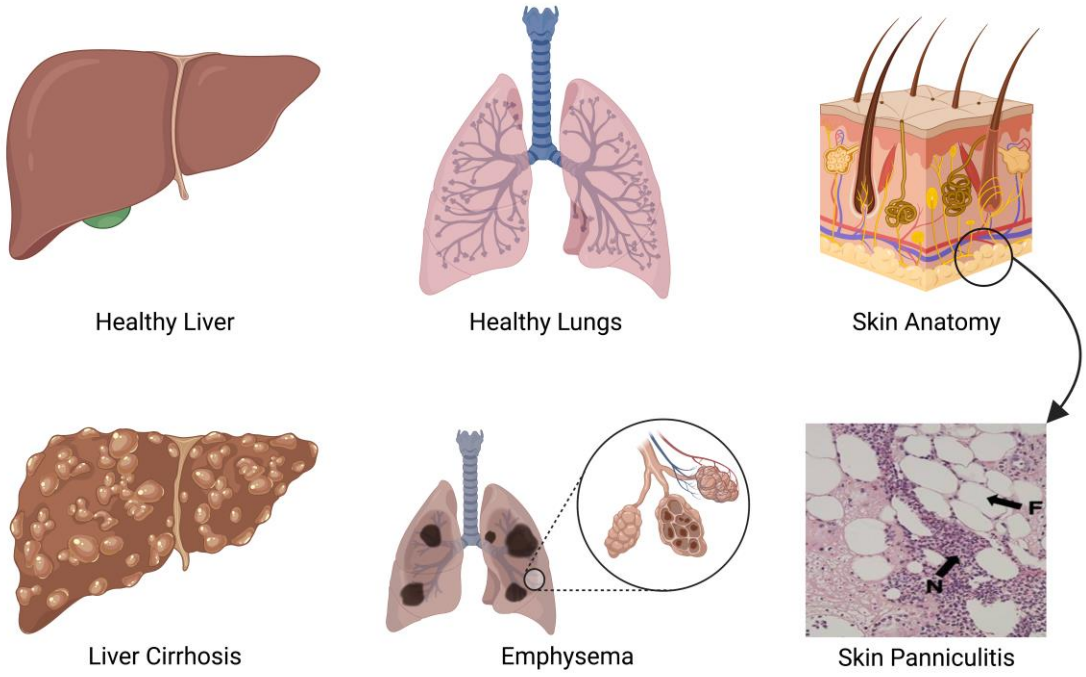

Figure 1. Clinical manifestations of AATD. Of note, the skin biopsy from a ZZ individual demonstrates neutrophilic inflammation (N) and fat necrosis (F), hallmarks of panniculitis. Created with BioRender.com.

The major clinical manifestation of the lung disease associated with AATD is early, progressive chronic obstructive lung disease with emphysema as a hallmark, which can occur even in the absence of cigarette smoking [14], but is greatly accelerated and exacerbated by smoking [15]. Although initially described as mainly lower lobe in distribution, emphysema can manifest in the upper lobes and is generally indistinguishable from nonhereditary emphysema [16]. This has led to considerable under-diagnosis and misdiagnosis of the condition [17]. This is a major problem which can delay treatment and lifestyle modifications, such as smoking cessation, leading to substantial lung damage and morbidity. In the US National Heart, Lung, and Blood Institute registry for severe AATD, although the average age at diagnosis was 46 years, the mean forced expiratory volume in 1 second (FEV1) and diffusing capacity of the lung for carbon monoxide

(DLCO) were 47% and 50% predicted, respectively, suggesting severe irreversible lung damage at an early age [18].

AATD is also associated with liver disease. The seminal study of Sveger et al [19] evaluated 200,000 newborns and identified 127 ZZ and 54 SZ (individuals who inherit Z with the mild S mutation) infants (**Table 2** for a description of the major AAT genotypes). Of the ZZ individuals, 14 had prolonged obstructive jaundice and 9 had severe clinical and laboratory evidence of liver disease. Approximately 15% of persons with cholestatic jaundice had progression to juvenile cirrhosis [20]. The risk of death from liver disease among children with the ZZ genotype was 2 to 3%, but none of the surviving cohort had clinical symptoms of liver disease at 12 years of age. In adults, clinically significant liver fibrosis has been shown on biopsy in 35% of adults with ZZ AATD [21], and a large European analysis using non-invasive assessment showed clinically significant liver fibrosis in 20 to 36% of ZZ AATD individuals. Overall, ZZ homozygous adults have a 10-20 times increased chance of developing advanced liver fibrosis (fibrosis stage ≥ 3) compared to adults without a polymerogenic AAT mutation such as Z [22]. There are however risk factors that influence the incidence and progression of liver disease in ZZ individuals such as male sex, age ≥ 50 years, alcohol misuse, obesity, diabetes mellitus, viral hepatitis or metabolic syndrome [21-24]. Surprisingly smoking habits and the development of hypertension were not considered as significant risk factors for AATD associated liver cirrhosis [24].This capacity to develop advanced liver disease is not limited to individuals homozygous for the Z mutation. Risk of liver disease exists in Z heterozygous adults, particularly in the setting of a "second-hit" such as alcohol misuse, diabetes, or non-alcoholic fatty liver disease [25].

AAT Genotype	**Mean AAT Level (g/L)***	**AAT Deficiency Status**	**Epidemiology in General Population#**	**Interpretation**
MM	1.48 +/- 0.44	None	n/a	Does not have the disorder – has 2 normal copies of the AAT gene.

MS	1.19 +/- 0.32	Mild	1 in 10	No evidence of increased risk of lung or liver disease but does carry 1 altered AAT gene.
MZ	0.88 +/- 0.18	Moderate	1 in 25	Significantly increased risk of lung disease in smokers. Increased risk of liver disease.
SS	0.90 +/- 0.16	Moderate	1 in 341	Presumed increased risk of lung disease in smokers. No evidence for increased risk of liver disease.
SZ	0.65 +/- 0.18	Moderate	1 in 424	Significantly increased risk of lung disease in smokers. Increased risk of liver disease.
ZZ	0.27 +/- 0.09	Severe	1 in 2104	Significantly increased risk of lung disease in smokers and ever smokers. Increased risk of liver disease.

Table 2. Common AAT genotypes. *Mean AAT levels are calculated from the Irish National Targeted Detection Programme which has tested over 22,000 individuals for AATD (mean AAT presented as +/- standard deviation; unadjusted for CRP). #Epidemiology data from [26].

The frequency and invasiveness of determining the progression of AATD liver disease should be determined on an individual basis. Whether liver disease has been established and its severity, factors such as age, sex, and presence of comorbidities should also be taken into consideration. Non-invasive tracking and stratifying methods are preferable [21,22]. These approaches include indirect blood tests (fibrosis-4:FIB4 or AST-to-platelet ratio index:APRI) or direct laboratory biomarker panels (such as enhanced liver fibrosis (ELF) or FibroTest), along with non-invasive scans such as vibration-controlled transient elastography (VCTE) and liver ultrasound. While these methods do help determine the presence and progression of AATD-related liver disease, a liver biopsy remains the gold standard with regards precise staging of disease. However, due to the invasiveness and inherent risks associated with biopsy (pain, bleeding, infection and damage to the surrounding structures), it is rarely a first-line tool.

In practice an adult with confirmed ZZ AATD would receive genetic counselling and undergo liver function tests (alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), and bilirubin) performed as well as indirect blood testing i.e. APRI. These test would be accompanied by VCTE and a standard liver ultrasound (US) to outrule radiologically obvious liver pathology such as cirrhosis and hepatocellular carcinoma (HCC). If the patient has no signs of liver pathology then they should be monitored in intervals in accordance with patient preference and risk factors and referred to an AATD-specific centre. In some centres, for individuals without signs of cirrhosis, liver function tests (LFTs) are to be reviewed at least every 6–12 months, liver fibrosis is to be assessed every 1–2 years, and liver ultrasound to investigate for HCC once a year in non-cirrhotic individuals. However, in the case of persistently raised LFTs or evidence of fibrosis or other liver pathology such as HCC, biopsy is recommended. If the biopsy confirms advanced cirrhosis individuals should be assessed for liver transplant, screened for other hepatological sequelea such as oesophageal varices, and receive HCC screening every 6 months [27].

AATD is also associated with an inflammatory skin condition called panniculitis [28]. This was originally presumed to be extremely rare affecting approximately 0.1% of people with AATD in the 1997 NHLBI registry study [18] and then only among severe deficiency genotypes. A closer look at the literature would suggest otherwise. In one cohort, AATD was present in up to 15% of all cases of biopsy-proven panniculitis [29]. A more recent meta-analysis revealed 117 reports of AATD-related panniculitis [13]. Thus, AATD-associated panniculitis may be more prevalent than previously thought, can occur in forms of AATD other than ZZ individuals, with a severe presentation and a significant risk of death if not recognized. The pathological mechanisms implicated in AATD-related panniculitis are not fully elucidated but include a protease-antiprotease effect in skin similar to that postulated for the lung. This is supported by several case reports that show intravenous plasma purified AAT can achieve complete resolution of refractory panniculitis. In addition, marked clinical improvements were observed in AATD-related panniculitis following liver transplantation with an AAT-sufficient liver [32]. The diverse immunomodulatory roles of AAT, in particular regarding neutrophil signalling, and the fact that polymers of Z AAT (which are neutrophil chemoattractants) have been demonstrated in panniculitis lesions, implies that the characteristic neutrophilic inflammation in non-AATD panniculitis may be amplified in AATD panniculitis.

With these varying presentations it is obvious that different approaches to treatment are required. However, the first problem is achieving a diagnosis of AATD. This has been improved by moving away from older diagnostic paradigms and towards targeted detection of AATD based on World Health Organization (WHO), European Respiratory Society (ERS) and American Thoracic Society (ATS) guidelines (**Figure 2**)[33-36].

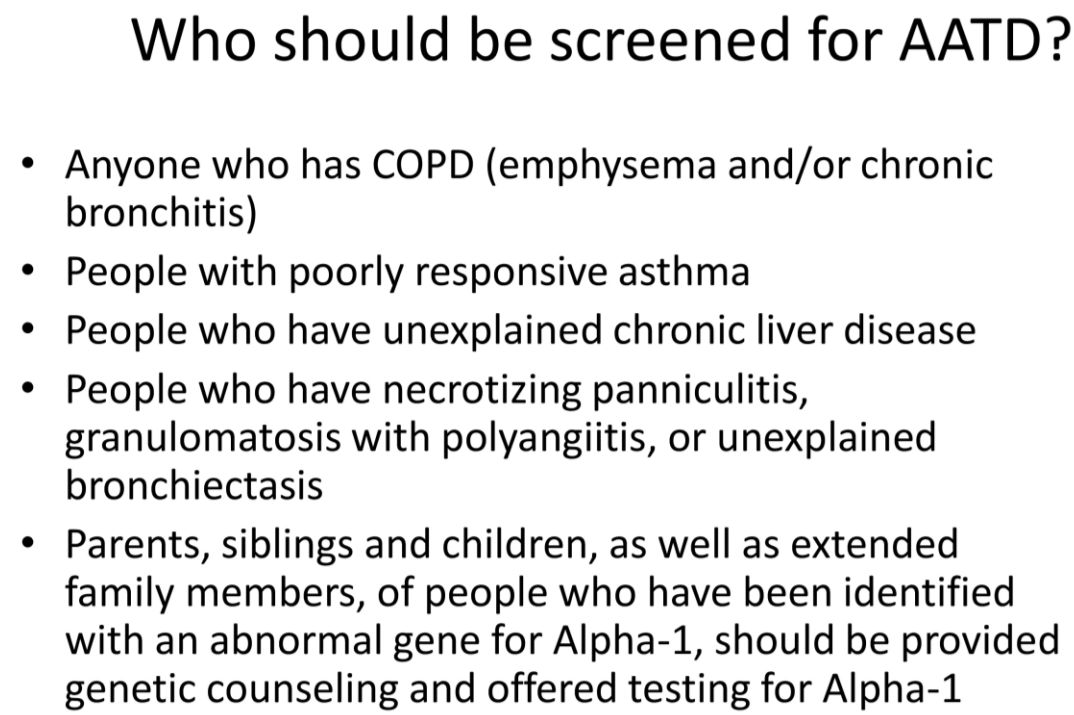

Figure 2. Groups recommended for targeted detection of AATD as per World Health Organisation, American Thoracic Society, and European Respiratory Society guidelines.

Innovative strategies to increase detection have been attempted that use electronic prompts or red-flags on pulmonary function, medical record, and laboratory IT systems [37-40]. The number of harmful SERPINA1 mutations causing AATD is also expanding with increased access to sequencing technology [41-43]. As a result, not only are more people with AATD being detected, but they are being diagnosed earlier, and at an age when lifestyle advice and specific treatments can be initiated in a more effective fashion. The aim of this review is to discuss and review the therapies presently available for the lung, liver and skin manifestations of AATD as

well as explore the new therapies and treatment approaches along with their associated challenges that are currently in the pipeline.

2. Treatments for lung disease associated with AATD

The treatment options for AATD related lung disease may be divided into general treatments as for non-hereditary COPD [44] and specific treatments aimed at the underlying pathogenesis of AATD [45] (Table 3). However, the best treatment option for every person with AATD is lifelong avoidance of cigarette smoking or immediate smoking cessation. This is especially true as we now have a much greater understanding of the lung disease risks associated with the various AATD genotypes. In this regard, it is imperative to offer early and effective smoking cessation advice. People with the severe ZZ form of AATD have an increased risk for COPD even if they do not smoke. People with the more common moderate AATD genotypes such as MZ and SZ types do not have an increased risk for COPD if they do not smoke but their risk for COPD is increased if they smoke [46-49] (Table 2). Of note for the SZ group, the rate of decline in lung function post smoking cessation is the same as a never smoking SZ if airway obstruction has not manifested prior to stopping smoking, highlighting the need for smoking cessation programs in this cohort [47]. The diagnosis of AATD of itself is a deterrent to smoking. In a recent study of people with AATD, current smoking was uncommon (2.5% vs. 17% vs. 16% for ZZ, SZ and MZ respectively) with ZZ individuals being significantly less likely to be current smokers [50]. In addition, in people with AATD, it was reported that parental smoking is associated with ever-smoking status, higher cumulative tobacco consumption and more quit attempts to achieve smoking cessation among former smokers [50]. Therefore, a diagnosis of AATD within a family can result in improved health outcomes for subsequent generations.

In addition to smoking cessation, influenza and pneumonia vaccination is recommended, as is use of inhaled bronchodilator and anti-inflammatory treatments, similar to those used in non-hereditary COPD. Evidence exists to suggest that people with severe AATD have a greater risk of lung damage in the event of SARS-CoV-2 [51-54] infection. This further emphasises the need for vaccination in this cohort and given the proteolytic nature of many bacterial infections it could be argued that timely initiation of antibiotic therapy is necessary in this group. The data from the MZ and SZ AATD cohorts would suggest that within the present body of knowledge

there is no rationale for augmentation therapy in these groups. The data for the ZZ phenotype is different. Never-smoking ZZs can develop significant lung disease, but this is a process dramatically accelerated by smoking [55].

2.1 Augmentation therapy with plasma purified alpha-1 antitrypsin

The only approved treatment specific for AATD-related lung disease is augmentation therapy with plasma purified alpha-1 antitrypsin. This was first approved in 1987 following a number of seminal papers which showed that intravenous augmentation therapy with plasma purified AAT given at a dose of 60mg/kg once a week could raise levels of AAT in plasma and lung epithelial lining fluid above a putative protective threshold of AAT [56,57]. This was followed by further studies evaluating different dosing regimens and aerosol delivery of AAT [58]. The main thrust of these papers was to show a biochemical effect of augmentation therapy, i.e. a restoration of the antiprotease protection in blood and perhaps more importantly in the lung. It was accepted that showing clinical efficacy would be more difficult given the relative rarity of the ZZ genotype and the problems with using forced expired volume in one second (FEV1) as a clinical endpoint [59].

Despite the challenges, a number of studies did suggest a clinical effect of augmentation therapy with intravenous AAT on FEV1 in specific subgroups of the ZZ population [60,61]. The study by Seersholm et al evaluated annual change in FEV1 in severe AATD in a treated German group compared to an untreated Danish group [61]. Overall, there was a difference in FEV1 change per year, but this was only significant in those with an FEV1 between 31% and 65% predicted. The US NHLBI registry showed similar results with significant treatment effect in those with FEV1 between 35-49% predicted [60]. This study also showed an effect of augmentation therapy on survival. Neither of these were randomized control trials and so clinical efficacy data continued to prove elusive. This process changed with the advent of lung density as measured by CT scan to estimate progression of emphysema in ZZ AATD.

The first of these studies by Dirksen et al evaluated a relatively small Danish-Dutch cohort (n=56) with patients randomized to either AAT (250mg/kg) or albumin (625mg/kg) at 4-week intervals for at least 3 years [62]. This study showed an almost significant (p=0.07) difference

between the AAT treated and albumin-treated groups. Enough data was generated to derive a power statistic to determine the number of AATD individuals required for future trials to demonstrate a relevant clinical endpoint. It was estimated that approximately 130 patients with ZZ AATD would be required in a randomized placebo controlled trial to show significant protection against loss of lung tissue (1.07g/L) as measured by CT lung density, but in contrast, to demonstrate a corresponding (i.e. 50%) correction of FEV1 decline, 550 AATD individuals would be required [62]. This led to increased interest in studies utilising lung density as an outcome measure in AATD. The EXAcerbations and Computed Tomography scan as Lung End points (EXACTLE) trial [63] followed which was a multi-centre, randomized, placebo-controlled trial evaluating 60mg/kg plasma-purified AAT given weekly over two years, compared to placebo. Several statistical approaches were used in this study with p-values ranging from 0.049-0.084 but all trending towards efficacy in reducing progression of emphysema.

The most definitive trial to date in this area is the RAPID study [64]. Unlike the previous studies, the RAPID study was powered sufficiently to show a therapeutic effect of augmentation therapy on CT lung density. The RAPID study showed that intravenous administration of plasma-purified AAT at a dose of 60mg/kg slowed down emphysema progression, as measured by CT lung density at total lung capacity, by 33%. In RAPID and the RAPID-OLE (open label extension) study [65], it was shown that when individuals who had received placebo in the RAPID study were switched over to active therapy their rate of lung tissue density loss was slowed. This was the first study to show definitively a clinical effect of augmentation therapy on lung in AATD. It did not however arrest the decline in lung tissue density. In addition, some of the data suggested that perhaps larger doses might be more effective, an area which is being actively pursued in upcoming studies. Furthermore, at the present time some licensing agencies are reluctant to approve this therapy based on CT-derived evidence alone.

2.2 Therapeutic aerosol delivery of AAT to the lung

Given that the standard dose of AAT given intravenously is 60mg/kg and there is not an unlimited supply of plasma-purified AAT, investigators have evaluated other forms of AAT and other routes of delivery. The initial work with recombinant AAT was disappointing as it quickly became obvious that non-glycosylated AAT (for example AAT derived from yeast-based

systems) had a very short half-life after intravenous administration [66]. Later work would show that non-glycosylated AAT had a less effective anti-inflammatory profile [67,68]. Transgenic AAT as produced initially in “Dolly” the sheep also suffered from glycosylation differences compared to humans, with negative effects on half-life. With this as background, investigators turned to aerosol delivery of AAT as an alternative with lower doses required, the ability to dose more frequently simulating what occurs in “real life”, and less concerns about glycosylation impacting upon half-life [69,70]. To date there has been one randomized control clinical trial on aerosolised AAT in AATD. The results showed no effect on time to first exacerbation [71].

2.3 Gene therapy for AATD lung disease

As AATD is a monogenic disorder, gene therapy would seem to provide a potential therapeutic option. The most commonly employed gene-delivery vector to date is the adeno associated virus (AAV), a single stranded DNA virus which can infect a variety of cells with low immunogenicity and stable long term expression. In the first human study in AATD, an AAV vector containing the AAT cDNA was administered intramuscularly. The level of AAT achieved was well below the putative protective threshold (PPT). Patients developed neutralizing antibodies against the AAV capsid but the expression lasted for up to one year [72]. A subsequent trial using a herpes-simplex virus-1 helper system showed a 10-fold higher level compared to the first trial but AAT levels remained below the PPT. This study required multiple intramuscular injections. Subsequent evaluation showed sustained AAT transgene expression of around 3% of the PPT with other downstream effects consistent with an increased antiprotease screen in blood [73].

Other routes of AAV-gene transfer include pleural administration targeting mesothelial cells and liver targeting [74,75]. An advantage in recent AAV-driven gene therapy is the use of the rhesus AAVrh10 serotype that circumvents the problems associated with human immunity to AAV that impact upon repeat administration [75,76]. A number of liver-targeted trials have been proposed. Liver targeting, while attractive in its ability to express high levels of AAT, presents specific difficulties - chiefly the intrinsic liver susceptibility in AATD which might be exacerbated by liver-directed AAV-vector administration. Other potential problems with AAV-usage is the possibility of insertional mutagenesis [77], which was noted in neonatal mice receiving AAV via

injection [78]. Despite the fact that the rate of integration of AAV genomes in the cells of non-human primates or humans is very low, integration remains a concern.

Additional viral vector systems include the adenovirus (Ad). This vector system has many desirable qualities; it is non-integrative, can work in both dividing and non-dividing cells, has a large DNA-insertion capacity and is relatively easy to produce in high quantities [79]. In conditions such as cancers where the adenovirus vector can target cancer cells or in vaccine development [80] where its ability to express antigens which can induce humoral responses, adenoviral vectors may have decided advantages [81]. However, in AATD, transient gene expression and the potential for harmful liver inflammation has dampened enthusiasm for this approach. A potential solution is to modify the Ad vectors to alter tropism, for example the targeting of recombinant Ad vectors to respiratory epithelial or liver cells. Incorporating myeloid binding peptide (MBP) into the Ad results in increased tropism for pulmonary endothelium and reduced tropism for hepatic cells. This increases lung levels of AAT while mitigating liver inflammation [82]. Recent research has shown that the interaction between hexon (Ad major capsid protein) and factor X are the key mediators of Ad liver transduction after intravenous administration [83,84]. This was the genesis for a new method to mitigate against liver inflammation using hexon-swapping or hypervariable region-swapping to reduce liver sequestration of Ad [85,86]. Even when the question of tropism is answered, the problem of limited duration of transgene expression is a major impediment to Ad-mediated gene therapy in the context of AATD. Various methodologies have been employed including use of non-human primate serotypes which have low sero-prevalence in humans and may be expected to have increased duration of expression with the opportunity for re-administration [87].

2.4 Surgical intervention in AATD

Lung transplantation in AATD, is unfortunately still a commonly required end stage treatment for AATD. To put this in context, of the 50,000 lung transplants performed in the United States between 1995 and 2015, 2568 (5.1%) were because of AATD-related COPD [88]. In Sweden around the same period, 19% of patients with AATD-related COPD underwent lung transplantation compared to 1% with non-AATD-related COPD. The post-transplant FEV1 decline, severity of acute rejection and survival in patients with severe AATD is generally

similar to that of non-AATD patients with COPD. One Swedish series showed that patients with ZZ AATD who had received a double lung transplant have an approximately doubled survival time after lung transplant compared with recipients with usual COPD [89].. Unfortunately, a major limitation of several large transplant studies is the broad classification of "AATD" without further stratification by AATD genotype [90,91].

With regards lung volume reduction surgery, there is a paucity of data in this area, with only 5 published series of lung volume reduction surgery (LVRS) in AATD and 3 published series on endoscopic lung volume reduction (ELVR)[92]. The data are conflicting but most studies show that the improvement in forced expired volume in one second (FEV1) was not sustained and that results in AATD patients are inferior both in magnitude and duration to the improvements seen in non-AATD emphysema. One series also reported higher mortality in the AATD group [93]. There are 2 published series on the use of endobronchial valves in AATD which focused mainly on the feasibility of performing the procedure and patient safety [94], but the numbers studied are quite small [95].

3. Treatment of AATD liver disease

Ideally the primary intervention for AATD liver disease would occur when the patient is first diagnosed with AATD before any signs of irreversible liver failure have developed. Individuals should be educated on the increased risks of developing steatosis and be advised to modify lifestyle habits such as dietary choices and alcoholic intake [96]. Currently, in practice, the mainstay of treatment in AATD-liver disease is liver transplant and it is usually reserved for those with end-stage liver disease, suffering from the complications of cirrhosis. However, ZZ AATD patients with liver disease have a low threshold for liver transplant evaluation due to the potentially rapid progression of liver disease caused by the severe portal hypertension seen in the ZZ patient population [97].The ideal candidate for liver transplantation should have preserved lung function to improve overall post-transplant survival outcomes [98]. Although liver transplant is the only curative treatment for AATD-liver disease there are a number of upcoming treatment options not only for the liver, but the liver and lung combined, which are currently in early phase studies. All these approaches however require detailed evaluation before they can be introduced into clinical practice. The means of determining the efficacy of these early phase

studies depends on the intervention being assessed. If the intervention is aimed at promoting secretion of Z AAT from the liver, it would be expected to increase circulating AAT levels and anti–NE capacity in plasma and BAL, and decrease desmosine and isodesmosine (biomarkers of lung elastin degradation) respectively, in addition to having anti-inflammatory effects. Measuring liver fibrosis can be assessed by vibration controlled transient elastography (VCTE) in advanced stages (F3, F4), or potentially via the live enzyme GGT in determining early stage fibrosis. However, if the intervention was to "knock-down" Z AAT production via silencing-RNA without concomitant intravenous augmentation therapy, this would decrease systemic and lung AAT levels and antiprotease protection. Systemic and BAL evaluations may be required to determine efficacy and ensure no increase in lung inflammation (1). As mentioned previously, the early therapeutic attempts in AATD liver disease were aimed at increasing AAT secretion from the liver or accelerating the intracellular degradation of abnormal Z AAT. Chemical chaperones such as 4-phenylbutyric acid were evaluated in the belief that they would improve folding of Z AAT and augment secretion. This approach was effective in animal models [99] but in human studies there was no significant effect on circulating AAT levels [100]. Other attempts using 4-mer and 6-mer peptides engineered to block polymerization have also been evaluated in vitro but with no in vivo success [101,102]. Enhancing autophagy is another means of decreasing the Z AAT burden in the liver [103]. A variety of such approaches have been used in animal models [104,105] but these would require excessive dosages to produce a clinical effect in vivo. Carbamazepine has been trialled in human subjects with ZZ AATD but no clinical data is available at present (ClinicalTrials.gov Identifier: NCT01379469).

Another option is to selectively knock-down the production of Z AAT in the liver using RNA interference (RNAi). Two strategies to achieve this are with small interfering RNAs (siRNAs), which are short double-stranded RNA fragments, and antisense oligonucleotides (ASO), which are single stranded [106]. The initial studies in this area borrowed from experience with hepatitis B virus infection [107]. Various groups used liposome particles, or PEGylated nanoparticles to deliver siRNA but with little success. More recently a polymer-based system, which included an amphipathic endosomolytic polymer that was reversibly masked and only active in the acidic environment of the endosome has been explored [108]. A targeting ligand N-acetylgalactosamine (NAG) was attached to the masked polymer resulting in hepatocyte-specific delivery via the asialoglycoprotein receptor on hepatocytes. This allowed endosomal escape and cytoplasmic

delivery of siRNA. The initial construct involved covalent attachment of the siRNA to the polymer via a bio-degradable disulphide link. This was changed on subsequent iterations so that the targeted polymer is co-injected with a liver tropic-siRNA conjugated to cholesterol (chol-siRNA)[109]. In preclinical studies, this type of siRNA approach has shown silencing of Z AAT production in ZZ mice and has decreased serum AAT levels in normal healthy volunteers. In the first-in-human study this strategy showed knockdown of hepatic AAT production based on reduced serum AAT in healthy controls and people with AATD. The study was terminated prematurely because of inflammation related to the delivery vehicle in a non-human primate study [110]. Since then, this group has presented interim results from a phase-2 open label study using a different delivery construct which holds further promise.

A recent clinical trial examined the efficacy of ARO–AAT, an RNA interference (RNAi) therapeutic to silence Z AAT mRNA expression, thus reducing Z AAT protein synthesis [111]. Sixteen ZZ patients with varying degrees of liver fibrosis (mostly ranging from F2-F3) were divided into 3 cohorts; Group 1: 4 patients received ARO-AAT, Group 1b: 4 patients received only 100mg and Group 2: 8 patients also received 200mg. These patients were assessed for both liver and serum AAT, liver enzymes, Pro-C3, with transient elastography and liver histology performed. Patient biopsies were taken at baseline and the post baseline points of 24 weeks for group 1a/b and 48 weeks at group 2. At 24-48 weeks there was a 72-100% decrease in hepatic Z AAT and a 79-99% reduction in Z AAT in serum. Of the remaining 14, 6 (all of whom received 200mg ARO-AAT) had, as per MATAVIR score, 1 point or more improvement in liver fibrosis, while 6 had no change from baseline. All patients demonstrated a decrease in Z polymer inclusions by periodic acid-Schiff staining with diastase digestion (PAS-D), a histologic hallmark of liver disease associated with AAT deficiency. The PAS-D total globule burden reduced to a mean score of 2.5 from a mean of 7.3 at baseline. ALT (decreased by 42 to 56%) and GGT levels decreased (by 33 to 54%) to normalised levels post treatment. While this data suggests that silencing Z AAT mRNA expression can lead to an improvement in liver fibrosis, this was not reflected in any clinically significant improvement in mean FEV1 at follow up at week 40 and week 72. This phase 2 clinical study did demonstrate valid outcomes that should encourage regulators that RNA-silencing therapeutics have a role in AATD-associated liver disease, in particular with those with liver fibrosis of F2 and above, however it has yet to be

approved for AATD liver disease. A number of ASO-based drugs have been approved for different disorders; their use in AATD-liver disease is still at the pre-clinical stage.

Another option is the use of genome editors as a knock-out method where the gene of interest is completely removed as opposed to the gene expression being merely silenced as seen in RNAi treatments. The clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) system introduces a DNA double strand break at a specific locus targeted by a guide RNA (gRNA) sequence. These DNA strand breaks are repaired either by nonhomologous end-joining (NHEJ) or homology-directed repair (HDR) [112]. Using this system, a single dose of CRISPR/Cas9 with a gRNA targeting human SERPINA1 delivered by an Ad vector normalized the pathologic liver phenotypes of ZZ transgenic mice [113]. The obvious drawback of this approach is that while it may effectively decrease the liver disease in AATD, it also decreases the circulating AAT levels increasing the potential risk for lung disease.

4. Therapies aimed at both lung and liver disease in AATD

The pathogenesis of the lung and liver disease associated with AATD is different, therefore a single therapeutic approach aimed at both organs is rare. The initial concept was that enhancing secretion of Z AAT from hepatocytes would increase plasma and lung concentrations of AAT, while simultaneously relieving liver stress. Intravenous administration of *Salmonella typhi* increased serum AAT in people with ZZ AATD but that approach was not considered viable as a long term therapeutic option [114]. Synthetic estrogen such as diethylstilbestrol can stimulate hepatocytes to increase secretion of AAT in MM individuals but not in ZZ individuals[115]. Potential for use was further hampered by the associated oestrogenic side effects. Later, a synthetic androgen, danazol was evaluated in this context. Danazol did increase AAT secretion in people with ZZ AATD but only by approximately 37% over pre-treatment levels [116]. None of these therapeutic approaches were evaluated for their effects on AATD liver disease and may have the potential to exacerbate liver symptoms.

The early attempts at preventing Z polymerization in the liver using targeted peptides have not been evaluated in humans. More recent developments, such as CRISPR/Cas9 may be of benefit. In early studies in AATD, CRISPR/Cas9 was aimed at targeting SERPINA1 and decreasing

production of Z AAT. However, as previously noted this will not alleviate and may increase the risk for lung disease. Recent approaches have used a dual approach inducing insertion and deletion mutations by NHEJ and gene correction by HDR at the SERPINA1 locus of the ZZ mouse. This latter correction occurs at a much lower level with about 2-5% of the liver corrected to the normal M AAT while insertion-deletion rates were 5 times higher [116,117]. With present technology, it is likely that the focus will be on HDR efficiency for production of M protein, perhaps reducing the NHEJ frequency to prevent loss of function of the targeted gene, despite the fact that this could also lessen the hepato-protective elements of the approach.

Current Treatments for AATD	**Emerging Therapies for AATD**
Pharmacological	Gene therapy with adeno-associated virus (AAV)
Bronchodilators	Gene therapy with adenovirus
Inhaled medications	Autophagy enhancers
Vaccinations (influenza, pneumonia, SARS-CoV-2)	RNA interference
Long-term oxygen therapy	Gene editing with clustered regularly interspaced short palindromic repeats (CRISPR/Cas9)
Positive pressure ventilation	Protein-folding correctors
Augmentation therapy (for both emphysema and panniculitis)	
Aerosol AAT	
Dapsone (for panniculitis)	

Non-Pharmacological

Pulmonary rehabilitation

Smoking cessation

Surgical Interventions

Lung and liver transplantation

Lung volume reduction surgery (LVRS)

Table 3. Current treatments and emerging therapeutic approaches for AATD disease.

A novel approach first employed in in cystic fibrosis utilises correctors of abnormal protein folding [118]. This technology is now being employed in the treatment of Z AAT with the aim of correcting Z AAT folding and increasing its secretion into the blood stream. A number of correctors have been trialled in ZZ individuals with early evidence of proof of concept and the results of newer correctors are eagerly awaited [119]. Another group using folding correctors has started a phase 1, double-blinded, randomized, placebo-controlled, single ascending and repeat dose trial evaluating healthy MM controls and MZ individuals. The compound being trialled, (ZF874), acts as a molecular 'patch' for the Z protein, allowing it to fold correctly and be secreted, simultaneously relieving the liver burden of polymer accumulation and providing Z AAT in the circulation to protect the lungs. In a recent study, a systematic library search identified a small molecule compound (GSK716) which was able to selectively correct Z AAT misfolding, decrease polymer formation and increase serum levels in ZZ mice [120]. This study failed to show any effect on intrahepatic AAT inclusions.

Other approaches have targeted hydrophobic cavities in the Z AAT with the intent to prevent polymer formation, while others have evaluated intracellular antibody fragments (intrabodies). Classically these are single chain variable fragments, one heavy and one light chain variable domain linked by a synthetic peptide. In vitro evaluations have shown that this approach has the

ability to reduce intracellular polymerization of Z AAT and increase secretion, whilst maintaining antiprotease activity [121]. The use of ataluren and gentamicin to treat severe AATD caused by premature stop codons (knowns as Null mutations) has led to enthusiasm for evaluating this in ZZ AATD with some encouraging results, most likely due to mRNA stabilization, but no human evaluations have taken place to date [122,123]. The varying therapeutic approaches together illustrate the cardinal question in AATD therapeutics. How much AAT do you need to get out of the liver to protect the lung and does the amount required vary according to physical state?

5. How much AAT is enough?

One of the major problems facing treatment of the lung disease in AATD is the uncertainty as to what the target AAT level should be. Much of the risk stratification has centred on the concept of a "putative protective threshold (PPT)" of 11μM (0.57g/L) in serum. The origins of the PPT are unclear. It has been variously attributed to the AAT level associated with the SS mutation and in other publications to SZ levels. In 1991 it was stated that 11μM approximated the lower 10^{th} percentile of the SZ range (10 to 23μM [124], but recent data would suggest that 11μM is nearer the 40^{th} percentile for SZs [IQR 9.6-13μM] when levels are measured in the absence of a concomitant acute phase event [47]. The only AATD genotype that straddles the 11μM level is SZ. The initial NHLBI registry of AATD used 11μM as the level for registry qualification [18]. Most studies of augmentation therapy have used this level as one to aim for and in the RAPID and RAPID-OLE studies, which showed significant effects of augmentation therapy by CT lung density, 60mg/kg once a week maintained serum AAT above the PPT [64,65].

However, recent studies show that people with the SZ form of AATD with levels lower than 11μM have no increased risk for AATD if they never smoke [47]. If they ever-smoke, SZ individuals display a significantly increased risk for COPD compared to normal risk (MM or MS) relatives who smoke a similar amount. The same data holds for MZ individuals who typically exhibit AAT levels far higher than 11μM who, if never smokers have no increased risk, but in the event of ever-smoking have a significantly increased risk of COPD compared to MMs who smoke a similar amount [46]. These data would suggest that the risk of COPD is not purely a manifestation of the AAT level but is also related to the AAT genotype and the inflammatory

burden, some of which can be attributable to the “gain of function” associated with Z AAT polymerization and retention in hepatocytes, monocytes, neutrophils and other cells. This might also explain why therapies aimed at restoring a PPT level of AAT may decrease rate of lung tissue loss but do not stop it.

It is also well established that levels of AAT increase significantly during infection which is the body’s natural acute phase anti-inflammatory response [125,126]. Recent data from this group shows that during COVID-19 infection, the levels of AAT in MM individuals rise very significantly. Nevertheless, this AAT elevation may be insufficient in the setting of significant systemic and local infection [53]. This would suggest that a single dose per week strategy does not replicate what happens in “real life” and may need boosting particularly during times of infection and inflammation. This is clearly shown by the inflammatory processes surrounding panniculitis. In this relatively rare complication of AATD, which can occur in most AATD mutations, albeit more commonly in ZZs, the response to intravenous AAT is striking but complete resolution often requires quite high levels of intravenous AAT (often in excess of 120mg/kg) which may need replenishing[13]. In these circumstances, aiming for a level of 11μM would be insufficient.

This raises the question of what is the ideal treatment for AATD. Ideally, this should be a treatment for both lung and liver disease, moving AAT out of the liver and into the circulation with the ability to mount an acute phase response in the setting of inflammation. New therapies aimed at this are already in trial. The questions for these trials are; how much AAT can they get out of the liver, how similar is this AAT to regular M AAT including during inflammation, can they mount a vigorous acute phase response without causing increased liver inflammation, and finally, is the AAT as good as M AAT in inhibiting NE and in mounting other anti-inflammatory responses? To date we have addressed some aspects of these questions, mainly aiming at the lung or liver disease in isolation but more recently approaches aimed at the underlying genetic defect have emerged which may treat both lung and liver at the same time while providing a normal acute phase response.

6. Expert opinion

There has been a massive expansion of knowledge regarding the epidemiology and pathogenesis of AATD. Recent research has elucidated the risks in heterozygotes and questioned the putative protective threshold theory in AATD, which has been used in the past to determine the susceptibility of developing the clinical manifestations of AATD and response to therapy. This will impact upon all therapeutic interventions. The old diagnostic paradigms, which identify people with AATD only when significant lung or liver disease has occurred have been altered. We are adopting targeted detection strategies with increasing awareness of AATD among specialist and primary care physicians. There has been a small but perceptible shift towards new improved non-invasive methods of demonstrating therapeutic efficacy in AATD but this will require more acceptance by regulatory authorities. The only recognized treatment option, intravenous augmentation therapy with AAT, is effective but needs improvement. What is the optimal dosing strategy? What is the optimal AAT concentration to confer protection for the lungs? Does dosing need to be increased during exacerbations? Is aerosol delivery as effective as intravenous administration? These are all questions that are being actively interrogated. Other questions remain. Can we make gene therapy effective at delivering AAT in sufficient quantities, perhaps with an inbuilt acute phase response to boost AAT levels when most needed? Can we knockout the Z AAT gene and knock in the M AAT gene to restore normality with the question of how long that effect will last? Will protein folding be the answer whereby we refold the Z AAT in the liver permitting it to escape in sufficient quantities to protect the lung while reducing liver inflammation?

Five years from now;

- We will know many people there are with AATD because in many countries we will have some version of neonatal screening.
- We will have determined the effectiveness or limits of plasma purified AAT augmentation therapy in slowing down or stopping progression of emphysema. We will know whether aerosol delivery of AAT is as effective as intravenous augmentation therapy in its effect on emphysema.

- We will have efficacy data on knockdown of Z AAT in the liver and whether this poses a risk for the lung
- We will have data from clinical trials of SERPINA1 gene editing
- We will see whether therapies focused on restoring correct AAT folding will treat both liver and lung disease simultaneously and if they can enable AATD individuals to mount a normal acute phase AAT response to infection
- We will have newer acceptable methods of determining clinical efficacy which will not require people with AATD to participate in placebo controlled trials for unacceptable periods of time

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**** of considerable interest**

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