

α 1-antitrypsin deficiency

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ABSTRACT

α_1 -antitrypsin deficiency (A1ATD) is an inherited disorder caused by mutations in *SERPINA1*, leading to liver and lung disease. It is not a rare disorder but frequently goes underdiagnosed or misdiagnosed as asthma, chronic obstructive pulmonary disease (COPD) or cryptogenic liver disease. The most frequent disease-associated mutations include the S and Z alleles of *SERPINA1*, which lead to the accumulation of misfolded α_1 -antitrypsin in hepatocytes, ER stress, low circulating levels of α_1 -antitrypsin and liver disease. Currently, no cure for severe liver disease exists and the only management option is liver transplantation, when liver failure is life threatening. A1ATD-associated lung disease predominately occurs in adults and is caused principally by inadequate protease inhibition. Treatment of A1ATD-associated lung disease includes standard therapies that are also used for the treatment of COPD, in addition to the use of augmentation therapy (infusions of human plasma-derived, purified α_1 -antitrypsin). New therapies targeting the misfolded α_1 -antitrypsin or attempting to correct the underlying genetic mutation are currently under development.

α_1 -antitrypsin is a serine proteinase inhibitor, produced principally by hepatocytes but also by neutrophils, monocytes and epithelial cells of the lung and gut. It is a major circulating antiprotease in humans and its key function is the regulation of the proteolytic effects of neutrophil elastase in the lung, which can lead to a range of consequences including inflammation and changes in the extracellular matrix (ECM). In addition, α_1 -antitrypsin is also an acute phase protein with anti-inflammatory and immuno-modulatory properties.

α_1 -antitrypsin deficiency (A1ATD, Online Mendelian Inheritance in Man (OMIM) entry 107400) is a disorder caused by mutations in the *SERPINA1* gene and is inherited in an autosomal and codominant pattern, indicating that the two copies of the gene (alleles) are active and contribute to the genetic trait.¹ This gene locus was previously called the protease inhibitor (PI) locus and this nomenclature is still used to describe the different genotypes linked to mutation in *SERPINA1*. Over 150 mutations in *SERPINA1* have been described. The most common, non-disease causing and so-called 'normal' allele is referred to as the 'M' allele; the most frequent (>95% of patients with A1ATD) disease-associated *SERPINA1* mutations, are referred to as 'S' and 'Z' alleles. The Z allele leads to severe disease symptoms and is the most widely studied variant. S and Z alleles lead to aberrantly folded α_1 -antitrypsin and reduced circulating levels of α_1 -antitrypsin. Patients with A1ATD can be classified based on their genotype, which can be heterozygous (patients who have 2 different *SERPINA1* alleles, for example M and Z alleles (PI**MZ* and PI**MS* genotypes)) or homozygous (patients who have 2 identical *SERPINA1* alleles, for example 2 Z alleles (PI**ZZ* genotype)) for the disease alleles. Each copy of the allele will contribute to the disease phenotype.

A1ATD predisposes to liver and lung disease (Figure 1), but patients with A1ATD might also be affected with asthma, granulomatosis with polyangiitis and panniculitis. A1ATD-associated liver disease is the consequence of accumulation of misfolded α_1 -antitrypsin in the ER of hepatocytes leading to ER stress. Currently, no cure for severe liver disease exists aside from liver transplantation. In contrast, A1ATD-associated lung disease is due to the consequence of low or undetectable circulating α_1 -antitrypsin levels leading to inadequate proteinase inhibition resulting, for example, in

damage to the protective layer in the lung. Additionally, polymers of misfolded α_1 -antitrypsin, in particular the Z-type α_1 -antitrypsin in the circulation and in lung tissue, and endoplasmic reticulum (ER) stress in monocytes and neutrophils have a role in the inflammation observed in A1ATD-associated lung disease. A1ATD-associated lung disease shares many characteristics of emphysema, but the pathology differs. A1ATD is also a genetic cause of chronic obstructive pulmonary disease (COPD) and is responsible for 1-2% of COPD cases. Moreover, the PI**MZ* genotype is a risk factor for the development of COPD. Individuals with A1ATD-associated lung disease receive many standard therapies that are used for the treatment of COPD, in addition to augmentation therapy using human plasma-derived, purified α_1 -antitrypsin to supplement the low levels of active α_1 -antitrypsin. New therapies that target the misfolding of mutant forms of α_1 -antitrypsin, or attempt to correct the underlying genetic mutation are being developed.

In this Primer, we summarize the epidemiology of A1ATD, discuss the pathophysiology of A1ATD-associated lung and liver complications and review current research in this field. We also consider existing treatment options for A1ATD and future developments that might further improve the outlook for patients with A1ATD.

[H1] Epidemiology

A1ATD is a relatively common, disorder^{2,3}. Although most prevalent in European (including Scandinavia, Spain and Portugal) and North American countries, A1ATD occurs world-wide. A1ATD has been reported in virtually all racial subgroups studied in almost 70 countries in 11 geographic regions around the world⁴. The global number of individuals with the PI**MS* or PI**MZ* genotype is 116 million and PI**ZZ*, PI**SZ*, or PI**SS* is 3.4 million. The prevalence of A1ATD has been estimated using two detection strategies: population-based screening and case reports (also called targeted detection). Many population-based screening studies for A1ATD have been performed^{4, 146}, the largest two were performed in newborn infants in Sweden and Oregon and estimated a prevalence of PI**ZZ* of 1 per 1,639 individuals in Sweden ($n=200,000$) and 1 per 5,097 individuals in Oregon ($n=107,038$)⁵. Data from one study suggests that approximately 100,000 individuals in the United States have A1ATD characterized by severely decreased serum levels⁶. Some targeted detection studies have assessed the prevalence of

A1ATD in individuals with several diseases. For example, one study estimated the prevalence of severe A1ATD in individuals with COPD as between 0–12%, with a mean value of 3.6%. In general, all data regarding the prevalence of A1ATD is likely confounded by the method of ascertainment and by general under-recognition of A1ATD (see Diagnosis, Screening and Prevention).

[H2] Genetic and environmental risk factors

A genome wide association study tightly linked circulating α_1 -antitrypsin levels in a general population sample to the *SERPINA1* gene cluster⁷. Also, the detrimental role of the exposure to cigarette smoke on the clinical phenotype of A1ATD has been demonstrated⁸. In a Swedish study, all 35-year-old individuals with PI*ZZ had normal liver and lung function but smoking frequency was significantly lower among patients with A1ATD, compared with controls⁹. Never-smokers with A1ATD also had abnormal chest X rays and lung function¹⁰. Nevertheless, the wide spectrum of clinical phenotypes associated with A1ATD could be caused by interactions between genetic and environmental factors other than *SERPINA1* and smoking alone. Indeed, single studies have identified potential genetic modifiers of COPD phenotypes in patients with severe A1ATD. For example, genetic variations in *MMP1/MMP3* and *TNF* have been associated with air transfer and chronic bronchitis, respectively, in patients with A1ATD^{11, 12} and polymorphisms in *IL10*, *CHRNA3* and *IREB2* are associated with lung function in individuals with the PI*ZZ genotype^{13, 14}. The expression of the lung disease for example, ranges from asymptomatic to severe emphysema in individuals with PI*ZZ. This heterogeneity is likely the result of genetic predisposition to lung disease and some environmental factors. For example, interplay between cigarette smoke-induced oxidative stress and Z-type α_1 -antitrypsin polymerization can impact on cellular inflammation and cytokine expression¹⁵. Regarding the role of the environment on A1ATD few data are available suggesting that outdoor air pollution can worsen respiratory status and predict lung function decline in individuals with PI*ZZ^{16, 17}. Another study revealed a significant interaction ($P<0.0001$) between the PI*MZ genotype and high levels of exposure to vapours, gases, dusts and fumes on annual change in FEF25–75% levels¹⁸. A separate study noted a significant interaction ($P=0.03$) between high-level vapours, gases,

dusts and fumes and annual changes in FEV₁/FVC¹⁷. Overall, larger annual declines in lung function in PI**MZ* carriers, compared to individuals with PI**MM* was associated with outdoor particulate matter of ≤10 μm and FEF25-75% decline associated with vapours, gases, dust and fumes was observed only in ever smokers with the PI**MZ* genotype¹⁸. Unlike smoking¹⁹, environmental or passive tobacco smoke exposure is not a risk factor for PI**MZ* individuals¹⁸.

[H1] Mechanisms/pathophysiology

The misfolding and accumulation of mutant forms of α₁-antitrypsin within the ER of α₁-antitrypsin-producing cells can lead to toxicity; this primarily affects the liver and to a lesser extent the lungs. The accumulation of misfolded α₁-antitrypsin causes cellular damage through two principal mechanisms; the perturbation of homeostasis within the lumen of the ER and the assembly of polymers (especially of Z-type α₁-antitrypsin) in the circulation, lumen of the lung or within tissues that can cause chemotaxis and/or activation of inflammatory cells²⁰. Retention of mutant α₁-antitrypsin in α₁-antitrypsin-producing cells leads to low or undetectable α₁-antitrypsin levels, which can cause defects in other tissues owing to a lack of proteinase inhibition, especially in the lung.

[H2] Genetic basis of disease

α₁-antitrypsin is encoded by the *SERPINA1* gene (Figure 2). Different α₁-antitrypsin transcripts are generated in different tissues owing to different transcription initiation sites, alternative splicing of untranslated exons²¹⁻²³ and tissue-specific proinflammatory cytokines in particular IL-6 and leukaemia inhibitory factor and the acute phase mediator oncostatin-M²⁴⁻²⁸. A specific quantitative reverse-transcriptase PCR test has been developed to quantify the expression of the different *SERPINA1* transcripts, to improve understanding of the regulatory mechanisms controlling *SERPINA1* expression²⁹.

The *SERPINA1* gene is highly polymorphic and mutations in this gene cause a hereditary co-dominant autosomal disorder. Pathological *SERPINA1* α₁-antitrypsin variants are classified as either 'deficient' or 'null'. Deficient variants occur as a result of

a point mutation that causes retention of the α_1 -antitrypsin protein in hepatocytes and other α_1 -antitrypsin-producing cells, resulting in low levels of α_1 -antitrypsin in plasma. Null mutations generally occur due to the presence of a premature stop codon and patients with these mutations have no detectable α_1 -antitrypsin in serum. The most common severely deficient variant is Z-type α_1 -antitrypsin and is caused by a single nucleotide polymorphism (SNP) in *SERPINA1* that results in the substitution of glutamic acid for lysine at residue 342 (Glu342Lys, reference SNP cluster ID (rs)28929474), which is found at a frequency between 2–5% in Caucasian individuals of European descent. At least 40 other deficient variants have been identified over the last few decades; these variants are rare, the molecular mechanism by which they cause disease varies and they can be prognostic for either liver and lung diseases^{30,31}. Similarly, up to 34 null alleles have been characterized to date³², most of which are extremely rare.

[H2] Mutant α_1 -antitrypsin

α_1 -antitrypsin's primary target protease is neutrophil elastase, but it can also inhibit other proteases. α_1 -antitrypsin uses the characteristic serpin inhibitory mechanism to irreversibly inhibit the activity of neutrophil elastase³³.

The Z-type of α_1 -antitrypsin is retained within the ER of hepatocytes as ordered polymers that become sequestered in inclusions, which are Periodic Acid Schiff-positive, diastase-resistant on histological examination^{34, 35}. This same process underlies the severe α_1 -antitrypsin plasma deficiency and intrahepatic inclusions of other protein variants of α_1 -antitrypsin; Siiyama (Ser53Phe)³⁶, Mmalton (Δ Phe52)³⁷, and King's (His334Asp)³⁵. Polymerisation also underlies the deficiency of the mild S (Glu264Val), I (Arg39Cys), Queen's (Lys154Asn) and Baghdad (Ala336Pro)³⁸ alleles of α_1 -antitrypsin. However the rate of polymer formation, which is proportional to the destabilising effect of the mutation on the protein³⁹, is much slower for the **I, S, Queen's and Baghdad alleles** and explains the absence of liver disease and the association with only mild plasma deficiency.

The initial description of the polymers of Z-type α_1 -antitrypsin described a linkage between the reactive centre loop and β -sheet A on adjacent Z-type α_1 -antitrypsin

proteins³⁴ (Figure 3a). Alternative linkages of α_1 -antitrypsin polymerisation have been described in the crystal structures of a dimer of antithrombin (linkage by a β -hairpin of the reactive centre loop and strand 5A (Figure 3b)⁴⁰) and a trimer of α_1 -antitrypsin (linkage by strands 1C, 4B and 5B (Figure 3c))⁴¹. The biophysical characteristics of polymers of α_1 -antitrypsin formed by refolding following denaturing by guanidine supports the β -hairpin and β -sheet A linkage⁴². Data from small-angle X-ray scattering suggests that trimers, tetramers, and pentamers of Z-type α_1 -antitrypsin all form ring-like structures, consistent with a linkage by strands 1C, 4B and 5B (Figure 3d)⁴³. In patients, ring-shaped polymers of α_1 -antitrypsin are rarely seen in inclusions from the livers of individuals with PI*ZZ³⁴.

[H2] Antitrypsin deficiency

Mutation in *SERPINA1* results in enhanced neutrophil elastase activity, which causes a plethora of effects that can contribute to the pathophysiology of A1ATD-associated lung disease. Enhanced neutrophil elastase activity has a range of consequences that lead to inflammation and an enhanced rate of neutrophil reactive oxygen species production (Figure 4). Excessive cleavage of molecules involved in the immune response (complement factors, immunoglobulins, antimicrobial peptides⁴⁴ and cell surface receptors such as C-X-C chemokine receptor type 1 (CXCR1) and monocyte differentiation antigen CD14^{45, 46}), result in a decreased activation of monocytes (owing to reduced responsiveness to lipopolysaccharide (LPS)) and a decreased efficiency of phagocytosis by neutrophils — one of the hallmarks of acute inflammation. Reduced expression of low affinity immunoglobulin γ Fc region receptor type III-B on the neutrophil surface and increased chemotaxis of neutrophils in response to IL-8 and soluble immune complexes⁴⁷, together with degranulation of secondary and tertiary granules further exaggerates the production of reactive oxygen species⁴⁸. Increased cleavage of coagulation factors and extracellular matrix or connective tissue molecules (elastin⁴⁹, collagen⁵⁰, fibronectin⁵¹ and proteoglycans⁵²), can also contribute to the pathology. Interestingly, neutrophil elastase can also induce gene expression of ECM modulators (matrix metalloproteases and cathepsins) by activation of disintegrin and metalloproteinase domain-containing protein 17 (ADAM17) and meprin A-mediated

epidermal growth factor receptor (EGFR) signalling⁵³⁻⁵⁶. Inactivation of tissue inhibitors of metalloproteases⁵⁷, secretory leucoprotease inhibitor (SLPI)⁴⁴, elafin⁵⁸ and cystatin-C⁵⁹ has also been reported. Other outcomes that occur either directly or indirectly due to decreased α_1 -antitrypsin levels in the lung, include goblet cell (mucus-secreting cells) hyperplasia, increased mucus secretion and impaired mucociliary clearance leading to defective bacterial clearance.⁶⁰ Moreover, reduced inhibition of caspase-3 can promote apoptosis of lung endothelial cells⁶¹. Emerging data have also indicated that leukotriene B4 production, and leukotriene B4 receptor 1 (BLT1) membrane receptor expression⁶² are increased in neutrophils, as are tumour necrosis factor (TNF)- α -mediated peripheral blood neutrophil apoptosis⁶³. Other evidence has demonstrated increased phosphorylation of p38 mitogen-activated protein kinases and NF κ B inhibitor α (I κ B α), and induction of matrix metalloproteinases and cytokines via serine/threonine-protein phosphatase 2A⁶⁴.

[H2] Accumulation of α_1 -antitrypsin in ER

[H3] Intracellular disposal mechanisms for misfolded α_1 -antitrypsin. The retention of some protein variants, such as the Z-type α_1 -antitrypsin in hepatocytes is the inciting event in the pathophysiology of A1ATD-associated liver disease (Figure 5)⁶⁵ and leads to apoptosis and oxidative stress of hepatocytes. In healthy cells, misfolded proteins accumulated in the ER are degraded by the ubiquitin proteasome system — a process called ER-associated protein degradation (ERAD) — or by macroautophagy. In A1ATD, the increased load of misfolded protein in the ER causes excessive activation of these cellular disposal mechanisms. Soluble Z-type α_1 -antitrypsin is monitored in the ER and diverted to the ERAD pathway, whereas Z-type α_1 -antitrypsin polymers are degraded by macroautophagy. Much of the work investigating handling of misfolded α_1 -antitrypsin has concentrated on the Null Hong Kong (NHK) variant of α_1 -antitrypsin. The ERAD pathway is the major pathway for degradation of NHK α_1 -antitrypsin owing to its inability to fold,⁶⁶ but even polymerogenic mutants of α_1 -antitrypsin are also targeted for degradation by the ERAD pathway, despite having near-native conformations^{66,67}. The Z variant of α_1 -antitrypsin folds more slowly than M α_1 -antitrypsin and can adopt an

intermediate conformation; both of these factors might contribute to the targeting of Z-type α_1 -antitrypsin to the ERAD pathway^{35, 68, 69}.

Glycoproteins (such as α_1 -antitrypsin) undergo cycles of N-glycan modification whilst within the ER. This mechanism acts as a timer to identify proteins failing to fold in an appropriate time. Abnormalities in the enzymes involved in this process might have a role in α_1 -antitrypsin misfolding in A1ATD. Experimental overexpression of ER mannosyl-oligosaccharide 1,2- α -mannosidase (ERManI), an enzyme that trims mannose residues from N-glycans, accelerates degradation of both NHK and Z-type α_1 -antitrypsin variants *in vitro*^{70, 71}, whereas inhibition of ERManI stabilises the variants⁷². Interestingly, a less-frequent allele variant of *MAN1B1* (encoding ERManI) that is associated with reduced ERManI expression has been reported more frequently than expected in children requiring transplantation for Z-type α_1 -antitrypsin associated liver disease, suggesting that protein glycosylation has a role in α_1 -antitrypsin accumulation in patients⁷³.

Whole organelles (for example, the ER) or large protein aggregates can be degraded by macroautophagy. This process involves engulfment of the structures by endomembranes that form autophagosomes, which fuse with the lysosome so that the contents are hydrolysed and degraded. Both *in vitro* and mouse models support a role for autophagy in the degradation of Z-type α_1 -antitrypsin⁶⁶. Treatment of mice used to model A1ATD with carbamazepine (an autophagy-enhancing drug) reduces the accumulation of Z-type α_1 -antitrypsin protein in the liver^{74, 75}. It is currently unknown whether macroautophagy shows selectivity for ER containing polymers of α_1 -antitrypsin, or if the turnover of ER in general is just increased.

[H3] Endoplasmic reticulum stress. When misfolded proteins accumulate within the ER and threaten to precipitate the cell is said to experience ER stress. ER stress triggers an unfolded proteins response (UPR) that reduces the influx of nascent proteins into the ER and simultaneously reprograms the cell to fold or dispose of the misfolded proteins more efficiently (Figure 6). This process involves the detection of ER stress by three ER stress sensors^{76, 77}.

The misfolding variants NHK and Saar α_1 -antitrypsin, which are truncated and unable to fold, can trigger the UPR if expressed even at low levels⁷⁸⁻⁸³. All newly synthesized proteins sequester chaperones (including 78 kDa glucose-regulated protein (also known as BiP)) to keep them in a competent state for subsequent folding or direct them to the ERAD pathway if folding is impaired. NHK and Saar variants are normally efficiently degraded by the ERAD pathway, but if allowed to accumulate will sequester large numbers of BiP, leading to ER stress. The mechanism by which ER stress sensors are activated is debated. One model suggests the sequestration of BiP by accumulated misfolded proteins reduces the level of free BiP, leading to activation of ER stress sensors.⁸⁴ An alternative model suggests that the ER stress sensors interact directly with stretches of misfolded protein⁸⁵.

Interestingly, the dramatic accumulation of polymeric α_1 -antitrypsin does not activate the UPR in most circumstances, despite ER stress^{82, 83, 86-90}. As α_1 -antitrypsin polymers are generally thought to be relatively well-folded structures, they might not present misfolded stretches of amino acids and so fail to activate the ER stress sensors. However, the accumulation of polymers does seem to sensitize the cell to second insults that can cause ER stress^{83, 86-88}. The mechanism for this sensitization is not well understood, but might involve altered protein mobility in the ER lumen, either owing to local alterations in viscosity or on the degree of ER interconnectivity⁸³.

Accumulation of α_1 -antitrypsin polymers can impact on a variety of intracellular signalling pathways leading to the transcriptional upregulation of proinflammatory genes, including increased basal and LPS-induced expression of *IL6* and *CXCL8* in monocytes derived from patients with Z-type A1ATD, versus non- α_1 -antitrypsin deficient individuals. Upregulation of proinflammatory genes is associated with intracellular accumulation of Z-type α_1 -antitrypsin polymers in monocytes⁹¹. The accumulation of polymerogenic α_1 -antitrypsin triggers nuclear factor κ B (NF- κ B) signalling, which has been termed the 'ER overload response'. Little is known about this response, but leakage of calcium from the ER might have a role in NF- κ B activation; chelation of cytosolic calcium has been shown to limit the activation of NF- κ B⁸⁸. In contrast, primary bronchial epithelial cells expressing low levels of Z-type α_1 -antitrypsin without polymer formation, also showed enhanced basal NF- κ B signalling⁸⁹. This indicates that increased NF- κ B

signalling is not restricted to activation of the ER overload response. A possible alternative mechanism might involve the increased activity of ADAM17. Primary bronchial epithelial cells isolated from individuals with PI*ZZ show hyperactive extracellular signal-regulated kinase (ERK) signalling, which depends on the activity of ADAM17. Moreover, increased ADAM17 activity has been reported on the surface of neutrophils of patients with A1ATD.

[H2] Extracellular α_1 -antitrypsin polymers

Polymers of α_1 -antitrypsin can also be detected in the blood⁹², bronchoalveolar lavage fluid and lung tissue of patients with A1ATD^{93,94}. It is unclear if most polymers are formed by the polymerization of secreted Z-type α_1 -antitrypsin proteins in the extracellular space, because they can also be secreted at low levels by from dying cells⁹⁵. However, most extracellular polymers are of hepatic origin, as the circulating levels of these polymers falls to undetectable levels four days after liver transplantation⁹². α_1 -antitrypsin can also be synthesised locally by airway epithelial cells, but at levels too low to allow the formation of intracellular polymers⁸⁹.

Extracellular polymers of α_1 -antitrypsin have pro-inflammatory effects. For example, they are chemotactic and stimulatory for neutrophils and likely contribute to both pulmonary inflammation and the increased incidence of vasculitis or panniculitis seen in individuals with the PI*ZZ genotype, following the deposition of these polymers in other tissues⁹⁶.

[H1] Diagnosis, screening and prevention

α_1 -antitrypsin deficiency is a widely under-diagnosed condition. The estimate of the mean interval between the development of the first symptom of disease (usually dyspnoea (shortness of breath)) and initial diagnosis has not changed over the past 20 years and ranges from 5.6–8.3 years^{3,97}. Similarly, the number of healthcare providers that patients with A1ATD see before the diagnosis is initially made has not lessened over time². This delay in diagnosis has been associated with adverse psychosocial effects³ and delays the implementation of disease management and the identification of family

members at risk of A1ATD. Thus, better recognition of this disorder by healthcare providers is urgently needed.

Under diagnosis of A1ATD is supported by three lines of evidence. Firstly, in all countries where diagnosis of A1ATD has been examined, only a small minority of the actual number of individuals with A1ATD have been diagnosed with this disorder⁹⁸. Secondly, few physicians comply with the American Thoracic Society and European Respiratory Society (ATS/ERS) guidelines that state that all patients with COPD should be assessed for A1ATD⁹⁹. Thirdly, patients with A1ATD frequently experience long delays and see many healthcare providers between their first symptom and diagnosis of A1ATD.

[H2] Diagnostic tests

Owing to the heterogeneous clinical presentation, the diagnosis of A1ATD is predominantly based on the results of laboratory testing, although clinical features of α_1 -antitrypsin deficiency may be useful for selecting individuals for testing (Figure 7). The diagnostic test are well established throughout the world and involve the quantification of either plasma or serum α_1 -antitrypsin levels typically performed using a nephelometer, in combination with either genotyping or PI typing¹⁰⁰. Genotyping usually starts off with the detection of the most common disease-associated alleles (Z and S) using genotype-based allele specific amplification by qPCR. This genotyping can be performed using DNA isolated from dried blood spots, whole blood and saliva and results from this diagnostic test dictate what subsequent diagnostic tests are performed (for example, Figure 7)¹⁰¹. Reflex testing, meaning, follow up testing for identification of alleles other than S or Z, is usually performed by protease inhibitor typing using isoelectric focusing of serum or plasma. Each phenotype of A1ATD displays a characteristic banding pattern on isoelectric focusing that can be compared to known reference samples¹⁰⁰. Although S and Z alleles are present in >95% of all patients with A1ATD, the remaining 5% have rare deficiency alleles, such as those associated with reduced, dysfunctional or absent plasma α_1 -antitrypsin levels¹⁰². These rare alleles are not detected by routine diagnostic methods,

but require a combination of protease inhibitor typing and next generation sequencing of *SERPINA1*¹⁰².

[H2] Patient selection for A1ATD diagnostic testing

Three approaches to select individuals for diagnosis testing of A1ATD exist. First, the diagnostic testing of individuals with symptoms or signs consistent with A1ATD, such as early onset, primarily lower lobe emphysema. In the past, this paradigm led to under diagnosis and late diagnosis of A1ATD. Second, predisposition testing of those who might be high-risk for A1ATD, such as asymptomatic individuals carrying a genetic mutation in *SERPINA1* and who have low levels α_1 -antitrypsin levels and a family member with A1ATD. Development of symptoms is likely for these patients, but it is not certain. Third, targeted detection in patients with a clinical reason to suspect A1ATD. Including, all patients with conditions associated with increased prevalence of A1ATD, such as COPD, poorly responsive asthma, cryptogenic liver disease, granulomatosis with polyangiitis, bronchiectasis of unknown aetiology and panniculitis, in addition to first degree relatives of patients with A1ATD. Targeted detection is similar to diagnostic testing but applies the ATS/ERS guidelines for the diagnosis and management of patients with A1ATD and has been shown to substantially increase the rates of diagnosis¹⁰³. The use of the ATS/ERS criteria for targeted detection has been shown to enrich the detection of A1ATD; the allele frequency for the Z allele was over fourfold higher in the targeted population, compared with an unselected sample of the general population¹⁰³. Guidelines regarding the potential benefits of targeted detection versus screening should be revisited particularly in lieu of the increased understanding of the pathogenesis of A1ATD-associated-disease.

[H2] Screening

Screening guidelines for A1ATD are dynamic and rapidly evolving. The ATS/ERS guidelines do not recommend population-wide neonatal screening for A1ATD⁹⁹ (the testing of groups without known risk factors). This is based on evidence from a Swedish study¹⁰⁴, which showed that although neonatal screening reduced parental smoking rates following detection of a genetic mutation in *SERPINA1*, there was an increased incidence

of parental distress and a negative impact on the mother–child relationship¹⁰⁴. Also, the ATS/ERS guidelines do not generally recommend universal screening of adolescents aged <11 years, but suggest that screening should be discussed with individuals in areas with a high prevalence of A1ATD or if parental smoking rates are high, providing that adequate genetic counselling is given. Recommendations for adults are similar to those for adolescents⁹⁹. The ATS/ERS guidelines⁹⁹ recommend testing high-risk groups, including all individuals appropriate for targeted detection.. This screening approach has been shown to increase the detection of α A1ATD.

The 2014 Global Initiative for Chronic Obstructive Lung Disease (COPD) recommendations¹⁰⁵ quote the World Health Organization¹⁰⁶, who recommend that COPD patients from areas with a particularly high prevalence of α_1 -antitrypsin deficiency should be tested for A1ATD. Also, they noted that individuals with COPD with α_1 -antitrypsin deficiency present with panlobular, lower lobe emphysema (compared to centrilobular apical distribution in COPD) at a younger age (<45 years), compared to patients with COPD and suggest that family members at risk of α_1 -antitrypsin deficiency should be identified. The Global Initiative for COPD recommendations are not dissimilar from those that led to substantial under diagnosis of α A1ATD for the past 50 years.

[H2] A1ATD registries

Data from rare disease patient registries increases awareness and knowledge of rare diseases, and is important for supporting both clinical and epidemiological research and monitoring of orphan drugs and the use of off-label medications¹⁰⁷. Moreover, these registries are important for patients and their families; they provide a beneficial effect on health and social services planning and the ability to improve quality of care, quality of life and patient survival. According to the European Organization for Rare Diseases, the ideal patient registry should be disease-centred, be able to be used in combination with other data sets, contain minimum set of common data elements, be sustainable through the encouragement of public-private partnerships, should include data from various

sources (patients and healthcare professionals) and link with corresponding biobank data., Currently, no A1ATD registries exist that meets these criteria.

The first prospective registry for patients with A1ATD was the National Heart, Lung and Blood Institute (NHLBI) Registry which enrolled 1,129 patients with severe A1ATD between 1989-1992 and followed them until 1996¹⁰⁸. This Registry collected information on patient demographics, medical history, measurements of pulmonary function and other laboratory evaluations at baseline and at 6-month or yearly intervals during follow-up. Data generated from this registry has produced some of the pivotal findings on the natural history of A1ATD, patient mortality and problems associated with a delayed diagnosis. Also, the effects of α_1 -antitrypsin augmentation therapy for patients was examined, although it was recognized that these results needed to be viewed with circumspection as results were not generated from a randomized clinical trial.

The current Alpha-1 Foundation Research Registry for patients in the US commenced enrolment in 1997 and enrolled patients with mildly-deficient genotypes between 1997-2002¹⁰⁹. This registry is a contact registry, allowing appropriate patients with A1ATD to receive invitations to participate in clinical trials, although plans are to enlarge the remit of this registry. An Alpha One International Registry was also founded in 1997, which aims to establish an international database of patients with A1ATD to collect data on demographics, to promote and coordinate basic and clinical research into α_1 -antitrypsin deficiency, to collect, assess and disseminate information regarding different aspects of α_1 -antitrypsin deficiency and also, to raise support and awareness of α_1 -antitrypsin deficiency. The Alpha One International Registry now includes almost twenty European and non-European countries¹¹⁰. There is only one inclusion criterion for the international registry, in that the patients must have a PI*ZZ, PI*SZ or other severely deficient phenotype. Some patients (for example patients in certain national registries) received annual follow up to collect information allowing the documentation of disease characteristics, treatments, in addition to the smoking habits and lung and liver function of patients. There are also other large non-affiliated registries for α_1 -antitrypsin deficiency^{103, 111}.

[H2] Prevention

There are compelling reasons to identify patients with A1ATD early, for example to allow patients access to specific therapies and improve opportunities to avoid the environmental triggers of lung disease, including by the avoidance of personal and passive cigarette smoking¹¹²⁻¹¹⁴.

Personal cigarette smoking is associated with a considerable reduction in the life span of patients with A1ATD¹¹⁵ and one study has shown that never-smokers with A1ATD might have normal life spans¹¹⁵. Importantly, patients with A1ATD who have developed COPD do so following exposure to a much lower number of pack-years of cigarette smoking, compared with individuals with COPD in the absence of A1ATD.

The early identification of α_1 -antitrypsin deficiency in neonates, adolescents and adults is associated with a reduction in the number of patients electing to start smoking with lower addiction rates as a consequence, and also, an increase in smoking cessation rates^{116, 117}. For these reasons, counselling on the avoidance or cessation of smoking should be the number one focus for physicians and health care providers following the identification of α_1 -antitrypsin deficient patients of any age.

In addition to exposure to cigarette smoke, some occupational exposures such as mineral dust and certain fumes are associated with increased impairments in lung function and symptoms of respiratory disease in patients with A1ATD¹¹⁸.

Modifiable risk factors for liver disease are less well understood, but obesity is known to increase the risk. In addition, males are more at risk of liver disease, compared to females¹¹⁹. Current recommendations for patients with A1ATD include vaccinations for hepatitis A and B and moderate alcohol consumption and a healthy diet in individuals with PI*ZZ¹¹².

[H2] Monitoring A1ATD-associated complications

[H3] Monitoring lung complications. Spirometry (lung function tests) typically seen in patients with A1ATD who have emphysema include a reduced forced expiratory volume in 1 second (FEV₁), reduced FEV₁/forced vital capacity ratio, air trapping (raised residual volume/total lung capacity ratio) and a low diffusion capacity. A partial reversibility of the airflow obstruction (as defined by an increase of 12% or 200 ml in FEV₁ after a

bronchodilator) is common in individuals with COPD secondary to A1ATD. Beyond this CT is a good parameter for lung diagnosis and has been used in various A1ATD augmentation therapy trials.

[H3] Monitoring liver complications. Many authorities, such as the American Association for the Study of Liver Disease, suggest regular monitoring (at least once per year) of patients with A1ATD for the development of liver disease, by a physician familiar with liver disease and associated complications¹²⁰. Monitoring should include history and physical examination sensitive for liver disease, such as focusing on the detection of splenomegaly, and laboratory assessment of white blood cell count, platelet count, levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, albumin, bilirubin and assessment of the international normalized ratio. Progressive liver disease is often accompanied by granulocytopenia, thrombocytopenia and elevated liver enzymes and bilirubin and coagulopathy. As with the detection of other liver diseases, a baseline liver ultrasonography is often considered useful and is recommended every 6 months for patients at increased risk (>2% per year) hepatocellular carcinoma, in guidelines issued by the American Association for the Study of Liver Disease (AASLD)¹²¹. Although data for the risk of hepatocellular carcinoma in α_1 -antitrypsin deficiency is lacking, the AASLD guidelines should be applied to patients with A1ATD with evidence of cirrhosis, portal hypertension or persistently large elevations of liver tests.

[H1] Management

[H2] Lung disease

The 2011 Global Initiative for COPD (GOLD) strategy can identify patients with A1ATD at increased risk of poorer outcomes from lung disease, specifically focusing on mortality, lung function decline and exacerbations¹²². In the Swedish registry of individuals with PI*ZZ, respiratory symptoms were the most common presenting feature A1ATD (in 43% of patients) and respiratory disease was the most common cause of death (55% of patients)¹⁰. The presence of lung disease is dependent on the smoking

history of the patient; in never-smokers 28% of patients fulfilled spirometric criterion for COPD, which rose to 72% in ex-smokers.

The rationale for the treatment of α_1 -antitrypsin deficiency-associated lung disease is to increase the levels of α_1 -antitrypsin in the lung towards levels seen in healthy individuals (that is, augmentation therapy), thus reversing the lack of inhibition of neutrophil elastase and other proteases, which, when uninhibited, can cause emphysema. Initially, augmentation therapy was performed by the intravenous delivery of plasma-purified α_1 -antitrypsin to patients¹²³, which resulted in increased levels of α_1 -antitrypsin and increased inhibition of neutrophil elastase both in serum and on the pulmonary epithelial surface. Data from a number of clinical trials have shown a clinical benefit of the use of augmentation therapy for the treatment of A1ATD (Table 1). Augmentation therapy is usually commenced with evidence of lung disease (usually equating to a decreased FEV1) and is administered once per week as intravenous infusions.

Concerns about the purity of human plasma-derived purified α_1 -antitrypsin and the transmissibility of infection has led to the evaluation of transgenic and recombinant sources of α_1 -antitrypsin. Recombinant α_1 -antitrypsin has been successfully produced in bacteria, yeast and transgenic sheep (engineered to produce α_1 -antitrypsin in their milk), but; these recombinant proteins have lack of, or abnormal glycosylation, which causes altered renal clearance and a short half-life following intravenous administration¹²⁴. Clinical trials investigating the use of an inhaled, recombinant form of α_1 -antitrypsin that has an appropriate pulmonary half-life are currently underway. Also, aerosolization of plasma-purified α_1 -antitrypsin and recombinant α_1 -antitrypsin is effective at delivery to both the alveolar surface and alveolar interstitium, but whether the amount of α_1 -antitrypsin is of sufficient quantity for clinical efficacy has not yet been evaluated^{125, 126}.

Other treatments for A1ATD-associated lung disease include therapeutics commonly used for the management of COPD, including short-acting and long-acting beta adrenergic receptor agonists, muscarinic receptor antagonists (anticholinergics), in addition to influenza and pneumonia vaccination

Currently, there is no specific treatment for α_1 -antitrypsin liver disease and treatments for progressive liver injury are primarily supportive and focus on preventing malnutrition, rickets, and managing the complications of portal hypertension such as ascites or variceal bleeding. Patients with A1ATD-associated cirrhosis can remain stable with compensated disease and minimal signs and symptoms for years ¹²⁷. In these patients, the detection of cirrhosis with portal hypertension is critical, so the patient can be cautioned against the development of splenic injury from playing contact sports, advised to abstain from alcohol, undergo surveillance for variceal bleeding, and cautioned to avoid the use of NSAIDs. Consumption of NSAIDs in patients with portal hypertension can result in life-threatening bleeding, even in well-compensated patients. There are no data regarding alcohol consumption in individuals with PI*ZZ who have no evidence of liver injury. AASLD guidelines for adults with hepatitis C without evidence of liver injury suggest that up to three alcoholic drinks per week may be safe.

For life-threatening, progressive liver failure or uncompensated cirrhosis, liver transplantation is the only therapeutic option. In the United States, patients at the highest need for liver transplant are classified by empirically derived severity scores for both children and adults, which are correlated with an increasing risk of mortality without transplant. The early evaluation at a transplant centre is recommended for patients with signs or symptoms of deteriorating liver function, although in the United States neither early addition to the transplant wait list or the time spent on the list influence the severity scores. In other countries, wait lists and liver transplantation is highly variable and is often influenced by time to referral, waiting and centre-specific factors. Many transplant centres have reported excellent liver transplant outcomes for α_1 -antitrypsin deficiency and outcomes are often better in α_1 -antitrypsin deficient patients, compared to those with other liver diseases; 1-year survival rates are 73% in adults and 87% children, dropping to 60% and 83%, respectively at 5 years ¹²⁸. In addition to cadaver-based donor transplantation, living donor liver transplants in infant (using a donated left lateral segment of the liver) and adult (using a split liver donation) patients with A1ATD are also successful, including surgeries performed using a donor liver from a patient with PI*MZ¹²⁹. Complications of liver transplantation include hepatic artery thrombosis, portal vein thrombosis, severe graft dysfunction, sepsis, intraoperative death,

lymphoproliferative disease, chronic rejection, biliary complications. Death from liver transplantation occur primarily in the first six months , most often due to hemorrhage, infection , or graft failure ~ 5-10%.

[H1] Quality of life

[H2] Life expectancy

A1ATD can both shorten survival^{108, 115, 130-133} and can compromise patients quality of life (QOL)(Table 2)¹³⁴.

A1ATD is associated with considerable morbidity and mortality¹³⁵. The median age at death for smokers with severe A1ATD is estimated at 40 years¹¹⁵ and in a separate study, the cumulative survival up to age 50 was 52%¹³². In the largest available longitudinal study from the National Heart, Lung and Blood Institute Registry of Individuals with A1ATD¹⁰⁸, the mortality rate was approximately 3% per year for patients with α_1 -antitrypsin deficiency (in which most of the subjects were either current (8%) or ex-smokers (72%)) .

FEV₁ is a major correlate of mortality in α_1 -antitrypsin deficiency and patients entering the National Heart, Lung and Blood Institute Registry with an FEV₁>50% had a normal expected survival, whereas patients with a baseline FEV₁<15% had a 3-year mortality rate of 36%¹³³. In a Danish Registry of 347 patients, the median survival of patients with a FEV₁<25% was 6.3 years but increased for patients with FEV₁>25% (10.5 years) and FEV₁>50% (14.2 years)¹³⁰. FEV₁¹³⁶ and thoracic CT densitometry¹³⁷, are both important predictors of patient survival, with a more rapid deterioration associated with the patient being a current smoker, aged between 30–44 years, of male sex, a predicted FEV₁ between 35–60% and a history of asthmatic features, chronic bronchitis and episodes of pneumonia^{136, 138}.

COPD is less prevalent and patient survival is longer in never-smokers with A1ATD. For example, the median age at death of never smokers with COPD and A1ATD has been estimated as 65 years, versus 40 years for patients with COPD and A1ATD who smoked¹¹⁵. Also, never-smoking individuals with PI*ZZ who were asymptomatic had a normal lifespan (odds ratio for death = 0.7 compared with age- and

gender-matched peers)¹³¹. Other factors contributing to prognosis of patients with A1ATD include the initial reason that prompted diagnosis of A1ATD, for example, the standardized mortality ratio for patients with A1ATD is highest (5.0) in patients who are diagnosed following the development of liver symptoms¹³¹.

The most frequent cause of death among patients with A1ATD is COPD or sequelae¹³³, including malignancy (not HCC), diverticulitis, sepsis and/or infection and trauma. In the NHLBI Registry, emphysema accounted for 72% of deaths and cirrhosis for 10% in all patients with A1ATD¹³³, whereas among PI*ZZ never smokers, emphysema accounted for fewer deaths (45%) but liver disease for more (28%)¹³¹.

[H2] Associated complications and quality of life

A1ATD also contributes to substantial morbidity and impaired QOL. As with patients with COPD, patients with A1ATD-associated COPD experience depression (25%), clinically relevant anxiety (36%), dyspnoea and impaired health-related QOL¹³⁹. The number of patients with anxiety and depression was similar among patients with COPD with or without A1ATD, but dyspnoea was more common in patients with A1ATD (assessed using the Modified Medical Research Council Dyspnea Scale) and so was their health-related QOL (based on the St. George's Respiratory Questionnaire (SGRQ)). In a series of 1062 individuals with severe A1ATD¹³⁴, those >59 years of age experienced fewer exacerbations and had better QOL scores (SGRQ and the Short Form (36) Health Survey) than younger individuals.

Healthcare-related QOL improved in patients with A1ATD receiving augmentation therapy following participation in a disease management program, consisting of directed patient self-education (with a comprehensive reference guide describing COPD and A1ATD) and organized supervision (monthly telephone conversations with A1ATD program coordinators, supervising participants' understanding of their long-term treatment plans)¹⁴⁰. Participation in disease management programs was also associated with a 1-year improvement in medication adherence, enhanced compliance with supplemental oxygen and reductions in the use of some healthcare resources (though not overall hospitalization rates)¹⁴⁰. However, although augmentation therapy alone has not been shown to improve health-related

quality of life measures, despite slowing the progression of emphysema and exacerbations^{141, 142}.

[H1] Outlook

The reasons why the clinical presentation of disease in individuals with Pi*ZZ is so variable, remain unclear, but this might, in part, be due to the contributions of genetic modifiers, such as *MAN1B1*⁷³. The use of iPSCs from patients with A1ATD and severe liver disease^{143, 144}, which show delayed clearance of Z-type α_1 -antitrypsin and have a more prominent accumulation of inclusions when differentiated into hepatocytes, combined with whole genome analysis would allow characterization of these differences and is likely to clarify the effect of genetic modifiers and help to identify patients more likely to develop liver disease.

[H2] Augmentation therapy

Augmentation therapy with purified α_1 -antitrypsin is not universally accepted for the prevention of emphysema in patients with A1ATD, although recent trials using surrogate endpoints, such as CT lung density measurements, for lung protection have been encouraging^{141, 142, 145}. On balance, most evidence supports the efficacy of augmentation therapy in slowing the progression of emphysema in patients with A1ATD. Augmentation therapy has some downsides, namely, that the treatment is expensive and requires repeated, lifelong, intravenous infusions. Moreover, the estimate of the level of plasma α_1 -antitrypsin required to protect against emphysema (11 μ M) is arbitrary and was based on the not fully proven hypothesis that PI*SZ patients who do not smoke do not have an increased risk for COPD. The optimal dose of augmentation therapy has yet to be determined, as patients receiving augmentation therapy lose some of the immunomodulatory effects of α_1 -antitrypsin just before they receive their next infusion. Also, one study conducted in 2015 (RAPID study) suggested that higher doses of α_1 -antitrypsin resulted in less lung density decline, observed using CT, compared with lower doses. Also, the full mechanism of the protective action of augmentation therapy is currently unknown; if the effect of α_1 -antitrypsin is solely mediated by correction of the

protease-antiprotease balance, or if the beneficial effects are evident primarily due to modification of inflammation requires further research.

[H2] Emerging therapies

Many new approaches are currently being examined for their use in the treatment of A1ATD. Extensive studies have been published using *in vitro* analyses of molecular structure of α_1 -antitrypsin and >10 different compounds have been shown to block liver injury in the PiZ mouse model (transgenic mice expressing the human Z-type α_1 -antitrypsin) of α_1 -antitrypsin liver disease, although none have been approved for human use^{74, 146, 147}.

[H3] *SERPINA1* silencing. Therapies that target the synthesis of Z-type α_1 -antitrypsin, aiming to prevent the accumulation of these proteins and subsequent liver injury are currently being examined through the application of RNA inhibition technology. Preclinical data obtained from the PiZ mouse model showed the complete reversal of the liver injury following the delivery of *SERPINA1* small interfering RNAs (siRNA)¹⁴⁸. Alnylam are undertaking a Phase I clinical trial examining the safety of siRNA-mediated inhibition of the Z-type α_1 -antitrypsin synthesis as a therapy for liver disease. One of the major caveats associated with the use of *SERPINA1* siRNA, is that this would prevent all α_1 -antitrypsin production, which could cause further lung damage. To counteract this, patients receiving α_1 -antitrypsin siRNA will require supplementation using, for example, transfection with the normal gene (using, for example, viral vector-mediated gene therapy) and/or augmentation therapy.

[H3] Intracellular degradation of Z-type α_1 -antitrypsin. Methods to accelerate the intracellular degradation of Z-type α_1 -antitrypsin as a treatment for liver disease have also been assessed. Data from *in vitro* and mouse model experiments have shown that enhanced macroautophagy can reduce the burden of Z-type α_1 -antitrypsin in the liver and also reduce liver injury^{74, 146, 147}. Drugs that enhance autophagy, such as sirolimus (also known as rapamycin), carbamazepine, and the bile acid 24-norursodeoxycholic acid

(norUDCA), in addition to a genetic approach used to augment expression of key autophagy regulators, have all been shown to reduce Z-type α_1 -antitrypsin accumulation in hepatocytes and to reduce liver cell injury in a mouse model system^{74, 146}. However, excessively high doses of all of these agents were required to show an effect. A phase II clinical trial assessing low dose carbamazepine in patients with PI*ZZ-associated cirrhosis is currently underway, although results to date have been inconclusive (NCT01379469).

[H3] Improving α_1 -antitrypsin folding. Other studies have focused on the use of chemical chaperone approaches to improve correct folding of α_1 -antitrypsin and to augment the secretion of Z α_1 -antitrypsin instead of retention in the liver (Figure 5). These approaches might be able to treat both the lung and the liver, however; the primary barrier to this is the large volume α_1 -antitrypsin protein that is synthesized per day. If a 1:1 binding stoichiometry is needed for efficacy of these drugs a large concentration of drug would need to be delivered to hepatocyte ER, which might prove clinically difficult. Nonetheless, *in vitro* studies have shown that several compounds promote the secretion of Z α_1 -antitrypsin and one of these compounds, 4-phenylbutyrate (4PBA), was also effective in a mouse model¹⁴⁹. Following on from these studies, a pilot trial was conducted in patients, which showed no effect on α_1 -antitrypsin secretion¹⁵⁰. Results from this trial are likely explained due to the inability of drug levels to reach the therapeutic range in patients, as those documented in the mouse model¹⁵⁰.

Protein folding systems found within different cellular compartments are highly intertwined¹⁵¹ and data from some studies suggest that targeting maladaptive protein folding responses in the cytosol can improve the folding of substrates in the ER, including Z α_1 -antitrypsin¹⁵². One study has identified the histone deacetylase 7 inhibitor suberoylanilide hydroxamic acid as molecule that can restore Z α_1 -antitrypsin secretion from epithelial cells¹⁵³.

[H3] Inhibiting α_1 -antitrypsin polymerization. Strategies designed *in silico* or using cell free systems for the therapeutic disruption of Z-type α_1 -antitrypsin polymerization,

(likely an event separate to the protein retention signal), have also been examined^{147, 154}. These largely peptide-based approaches aim to modulate the conformational behaviour of α_1 -antitrypsin by targeting it directly to fold correctly, stabilize functional conformers and limit the population of polymerogenic intermediates¹⁵⁵⁻¹⁶¹. However, many of these compounds had an aberrant effect following examination *in vitro* and there have been difficulties into creating medicinal molecules for use in animal models. Moreover, compounds sharing a similar structure to the reactive loop of α_1 -antitrypsin (reactive loop analogues) can generate complexes with α_1 -antitrypsin that do not show antiprotease activity. Nonetheless, these molecules could still have potential for the treatment of α_1 -antitrypsin accumulation in hepatocytes.

[H3] M-type α_1 -antitrypsin synthesis. Finally, several studies that have progressed to evaluation in clinical trials, have evaluated strategies to synthesize M-type α_1 -antitrypsin in tissues outside the liver, which might then lead to an increase in serum levels to protect patients from lung, but which would not change the risk of liver injury^{162, 163}. However, to date, these studies have only been able to generate less than 5% of the serum M-type α_1 -antitrypsin level thought to be needed for a therapeutic benefit in humans using an adeno-associated vector carrying *SERPINA1*¹⁶³. Various gene therapy approaches have been designed to increase the levels of circulating α_1 -antitrypsin with one having reached Phase II testing¹⁶³⁻¹⁶⁸. Two of these approaches involve haematopoietic stem cell therapy, coupled with delivery of lentiviral vector containing *SERPINA1*^{169, 170} or intrapleural administration of a replication-deficient adeno-associated viral vector containing the *SERPINA1* transgene¹⁷¹.

Other future potential treatments include the use of induced pluripotent stem cells^{143, 172, 173} generated from skin fibroblasts harvested from individuals with PI*ZZ, then reprogrammed to form hepatocyte-like cells that still possess the A1ATD phenotype¹⁴³. Using the CRISPR-CAS9 system to correct the Z mutation in these cells¹⁷² could generate PI*MM cells, that share the characteristics of those found in healthy individuals. Theoretically, these cells could be used for autologous grafting without the risk of immune rejection. *In vitro* reports using this technique are still limited and no clinical

trials have been commenced, however; this approach is still promising and might be a long term answer to the treatment of both lung and liver disease manifestations of this α_1 -antitrypsin deficiency.

[H2] Other disorders

α_1 -antitrypsin is one member of a large family of serine protease inhibitors, known as serpins. Other members of this protease family are mutated in human diseases and so it is likely that data obtained from the study of α_1 -antitrypsin will have wider biomedical application. For example, mutant forms of the neuron-specific serpin (neuroserpin) also undergoes polymerization and formation of inclusion bodies in the same way as α_1 -antitrypsin, but accumulation of mutant neuroserpin leads to neurodegeneration and early onset dementia¹⁷⁴. The development of therapeutics that prevent the polymerization of α_1 -antitrypsin will also rapidly lead to the development of therapies for other serpinopathies that are primarily associated with accumulation of polymers. Similarly, the development of small molecules designed to mimic the anti-inflammatory effects of α_1 -antitrypsin would be applicable for use in other disorders, since α_1 -antitrypsin augmentation therapy has been shown to be beneficial in other diseases, including cystic fibrosis^{175, 176}.

Finally, the mechanism by which accumulation of polymers of α_1 -antitrypsin in the lumen of the ER can trigger downstream signalling is unknown, but it has been proposed that the ER overload seen in this condition, might also mediate cellular responses to enveloped viruses. The study of α_1 -antitrypsin is highly important and could improve knowledge of other more prevalent conditions^{177, 178}.

Acknowledgements

Funding for research in C.M.G's group is gratefully acknowledged from The Health Research Board, Science Foundation Ireland, The Irish Research Council, The National Children's Research Centre, The European Respiratory Society, Horizon 2020, Cystic Fibrosis Foundation Therapeutics, and the Alpha One Foundation USA. The authors would like to thank Dr James Irving, UCL, UK for preparing Figure 2.

Author contributions

Introduction (C.M.G.); Epidemiology (C.M.G. and J.K.S.); Mechanisms/pathophysiology (C.M.G., D.A.L., I.F., S.J.M., and J.H.T); Diagnosis, screening and prevention (C.M.G., M.L.B., J.K.S. and N.G.M.); Management (C.M.G., J.H.T., and N.G.M.); Quality of life (C.M.G. and J.K.S.); Outlook (C.M.G., S.J.M., and N.G.M.); Overview of Primer (C.M.G.).

Competing interests

C.M.G. has received research grants from the Alpha-1 Foundation and received an honorarium for educational materials from Vertex Pharmaceuticals. J.H.T. has served as a consultant for Alnylam Pharmaceuticals, Arrowhead Research, Proteostasis Therapeutics, Isis Pharmaceuticals (now Ionis Pharmaceuticals), Editas Medicine, Genkyotex, GLG Pharma, INSERM, Intellia Therapeutics, Retrophin, RxCelerate, Velgene. He also received honoraria for speaking from the Alpha-1 Foundation and the Cystic Fibrosis Foundation and research grants or support from the Alpha-1 Foundation, National Institutes of Health USA, Alnylam Pharmaceuticals, Arrowhead Research and the Cardinal Glennon Children's Foundation . M.L.B. has received research support for clinical trials from Baxalta, Kamada and Grifols, is an owner of GeneAidyx, a genetic diagnostic company, and holds patents for AAT gene therapy and compounds to modify AAT secretion. D.A.L. has received research funding from GlaxoSmithKline to develop small molecule therapies for antitrypsin deficiency. He was also chair of the GlaxoSmithKline Respiratory Therapy Area Board between 2012–2015. J.K.S. has served as a consultant to Kamada, Grifols, Arrowhead Research, CSL Behring, Baxalta, Pfizer and Boehringer-Ingelheim. He is a member of the board of directors of the Alpha-

1 Foundation, and the Medical and Scientific Advisory Council for both the COPD Foundation and the Alpha-1 Foundation. N.G.M. has served as a consultant for Chiesi and Bayer and received honoraria for speaking from Chiesi, Grifols and CSL Behring. He also received grants or research support from Chiesi, Grifols, Vertex, and the Alpha-1 Foundation. S.J.M. and I.F. declare no competing interests.

Figure legends

Figure 1 | Clinical manifestations of PI*ZZ. About 15% of infants with PI*ZZ genotype have or develop clinical relevant liver disease. The clinical symptoms of jaundice typically resolve by the second year of life but can progress to cirrhosis in 15% of children who had jaundice at birth^{5, 179}. The risk of death from liver disease in children with PI*ZZ is between 2-3%^{180, 181}. Serum aminotransferases and bilirubin levels (markers for liver dysfunction) are raised in 70% and 11% of infants, respectively, in their first year of life but the levels typically go down. Raised aminotransferases remain abnormal in 15% of patients by 12 years of age and serum bilirubin levels fall to within the normal range by 6 months of age. Some adults with Z-type A1ATD have slowly progressive hepatic damage that is asymptomatic and is only apparent as a minor degree of portal fibrosis. A1ATD-associated cirrhosis can present in adults of any age. 13% of patients with PI*ZZ die from liver disease¹⁰. Lung dysfunction develops between the third and fourth decade of life. Emphysema associated with Z-type A1ATD is typically panlobular and affects the bases of the lungs. Patients initially present with breathlessness and cor pulmonale (enlargement of the right side of the heart owing to lung disease) and polycythaemia (increased red blood cell levels in the blood can occur late in the disease⁹⁹). PI*ZZ A1ATD is also associated with an increased prevalence of asthma¹⁸², panniculitis¹⁸³ and granulomatosis with polyangiitis¹⁸⁴. The underlying disease mechanisms are currently unknown but pro-inflammatory polymers of Z α_1 -antitrypsin and deficiency of M-type α_1 antitrypsin might contribute to both granulomatosis with polyangiitis and panniculitis.

Figure 2 | *SERPINA* and α_1 -antitrypsin. A | *SERPINA* gene, which encodes α_1 -antitrypsin, is located on the long arm of chromosome 14 at 14q32.1. The gene consists of four coding exons (II, III, IV, and V), three untranslated exons (Ia, Ib, and Ic) and six introns. Distinct promoters and transcription start-sites in the 5'-untranslated region (5'-UTR) have been identified for hepatocytes and extra-hepatic tissues such as monocytes/macrophages and the cornea²¹. The hepatocyte *SERPINA* promoter is located within exon 1C, upstream of the hepatocyte transcription start site^{21, 185}. Alternative promoter regions are located upstream of exon 1A and before exon 1B; these control *SERPINA* expression in monocytes and macrophages^{21, 186}. The most common A1ATD-associated mutation are depicted. B| The α_1 -antitrypsin protein is a 394 residue, 52 kDa glycoprotein that is synthesised by hepatocytes, lung and gut epithelial cells, neutrophils and alveolar macrophages. Structure of monomeric α_1 -antitrypsin with the position of key mutations shown in black α_1 -antitrypsin contains a 'reactive loop', which contains a neutrophil elastase binding site. Neutrophil elastase docks with, then cleaves the reactive loop of α_1 -antitrypsin, which causes the translocation of elastase from the upper to the lower pole of α_1 -antitrypsin, and irreversibly inhibits the activity of the enzyme³³.

Figure 3. Proposed models of serpin polymerisation (key linkage motifs highlighted in black): i. Reactive centre loop- β -sheet A linkage, ii, linkage by a β -hairpin of the reactive centre loop and strand 5A and iii, linkage with strands 1C, 4B and 5B. Figure generated with PyMol by Dr James Irving, UCL, UK.

Figure 4. Intrapulmonary consequences of unopposed neutrophil elastase activity. Neutrophil elastase is normally inhibited by α_1 -antitrypsin. However, in the lung of A1ATD patients, unopposed neutrophil elastase activity can activate cell surface receptors and transcriptionally upregulates expression of classes of genes.

Figure 5 | Accumulation of misfolded α_1 -antitrypsin in hepatocytes. Misfolding of α_1 -antitrypsin leads to α_1 -antitrypsin accumulation in the ER, which either gets degraded via the ERAD pathway or macroautophagy. When the capacity of these processes is exceeded, α_1 -antitrypsin polymers accumulates which, depending on the level of

accumulation, results in oxidative stress and cell death, or proliferation. The final outcome is liver failure. Potential interventions are depicted in red boxes.

Figure 6. Fates of α_1 -antitrypsin within the endoplasmic reticulum.

The nascent α_1 -antitrypsin protein is translated and enters the endoplasmic reticulum (ER) where it undergoes N-linked glycosylation. Glucose residues are trimmed from α_1 -antitrypsin by the action of glucosidases I (GS1) and glucosidases II (GS2), which promotes interaction of the nascent protein with the lectin chaperones calnexin (CNX) and calreticulin (CRT). This allows the folding of the protein by protein disulphide-isomerase A3 (ERp57), following which, it is deglycosylated by GS2²⁴⁸. If folding of the α_1 -antitrypsin protein is correct, it is exported from the ER and trafficked to the Golgi apparatus. Conversely, misfolded α_1 -antitrypsin protein (for example, NHK or Z isoforms) is reglycosylated by UDP-glucose:glycoprotein glucosyltransferase 1 (UGGT1), which allows the reinteraction of misfolded α_1 -antitrypsin with CNX and CRT and refolding by ERp57. This cycle can persist, but misfolded α_1 -antitrypsin eventually undergoes demannosylation by ER α -mannosidase I (ERManI and EDEM). Further demannosylation leads to the interaction of α_1 -antitrypsin with protein OS-9 (OS-9) and endoplasmin (GRP94) and delivery to the E3 ubiquitin-protein ligase synoviolin (HRD1) complex (containing E3 ubiquitin-protein ligase AMFR (gp78)) for ER associated degradation (ERAD)¹¹⁶. Misfolded α_1 -antitrypsin (for example the NHK isoform) can also accumulate in the ER and is speculated to sequester BiP away from the ER stress sensors eukaryotic translation initiation factor 2- α kinase 3 (PERK), cyclic-AMP-dependent transcription factor ATF-6 α (ATF6) and serine/threonine-protein kinase/endoribonuclease IRE1 (IRE1), leading to activation of the unfolded protein response (UPR) and ER stress. By contrast, accumulation of Z α_1 -antitrypsin results in the formation of ordered polymers that lead to the activation of the ER overload response (EOR) and upregulation of proinflammatory cytokines. The underlying mechanisms of polymer-mediated activation of EOR are poorly understood, but appear to require calcium release of calcium from the ER lumen, NF- κ B signalling and a proinflammatory

response. Under some circumstances, polymers of α_1 -antitrypsin can be degraded by macroautophagy.

Figure 7. One diagnostic algorithm for A1ATD. A1ATD diagnosis involves quantification of α_1 -antitrypsin levels, genotyping of *SERPINA1* and PI typing.

Table 1. α_1 -antitrypsin augmentation therapy observational studies and clinical trials

Clinical trial design	Lead author or group name	Year	Main outcome	Duration of follow up	Ref .
Multicenter, randomised	Chapman (RAPID Study)	2015	Slower rate of lung tissue loss assessed using CT	2-4 years	¹⁴²
Multicentre, randomised, placebo-controlled, double-blind	Dirksen EXACTLE Study)	2009	Trend towards slower rate of lung tissue loss assessed using CT	2-2.5 years	¹⁴¹
	Dirksen	1999	Trend towards slower rate of lung tissue loss assessed using CT and increased mortality	3 years	¹⁴⁵
Observational	Seersholm	1997	Reduction in FEV ₁ decline in cohort with FEV ₁ 31-65%	>1 year	¹⁸⁷
Observational	NHLBI Registry	1998	Slower rate of FEV ₁ decline in cohort with a baseline FEV ₁ between 35-49%	7.2 years	¹⁰⁸
Observational	Lieberman	2000	Reductions in the incidence of lung infections	1-10 years	¹⁸⁸
Observational	Wencker	2001	Slower rate of FEV ₁ decline	>12 months	¹⁸⁹
Observational	Tonelli	2009	Slower rate of FEV ₁ decline	3.5 years	¹⁹⁰

Table 2. Factors affecting symptoms and QOL in α_1 -antitrypsin deficient patients with COPD¹³⁹

Symptom	Comment
Depression	Decreased in those in a stable relationship rather than single
Anxiety	Increased in those who are younger and less educated
Dyspnoea	Worse if the patient is single compared with patients who were married or in a long-term relationship, and compared with non- α_1 -antitrypsin deficient patients with COPD
Impaired QOL	Poorer compared to non- α_1 -antitrypsin deficient patients with COPD, but less severe above 59 years of age

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Figure 1.

Liver disease in PiZZ Children

Cholestatic Jaundice (10%)
of which 15% develop Juvenile Cirrhosis
Other liver disease (6%)

- Z polymers form in ER of hepatocytes *in utero*
- Raised serum alanine transferase at 1 year, decreasing by age 12
- Raised serum bilirubin
- Risk of death 2-3%



Lung disease in PiZZ adults

Emphysema: basal, panlobar
Deterioration associated with

- current smoking, age 30 to 44 years
- male sex
- FEV₁ between 35 to 60% predicted

• asthmatic features

Liver manifestations in <50% PiZZ adults

- chronic bronchitis
- previous pneumonia

Cirrhosis, Hepatocellular carcinoma

Risk factors: Male sex and obesity

Figure 2.

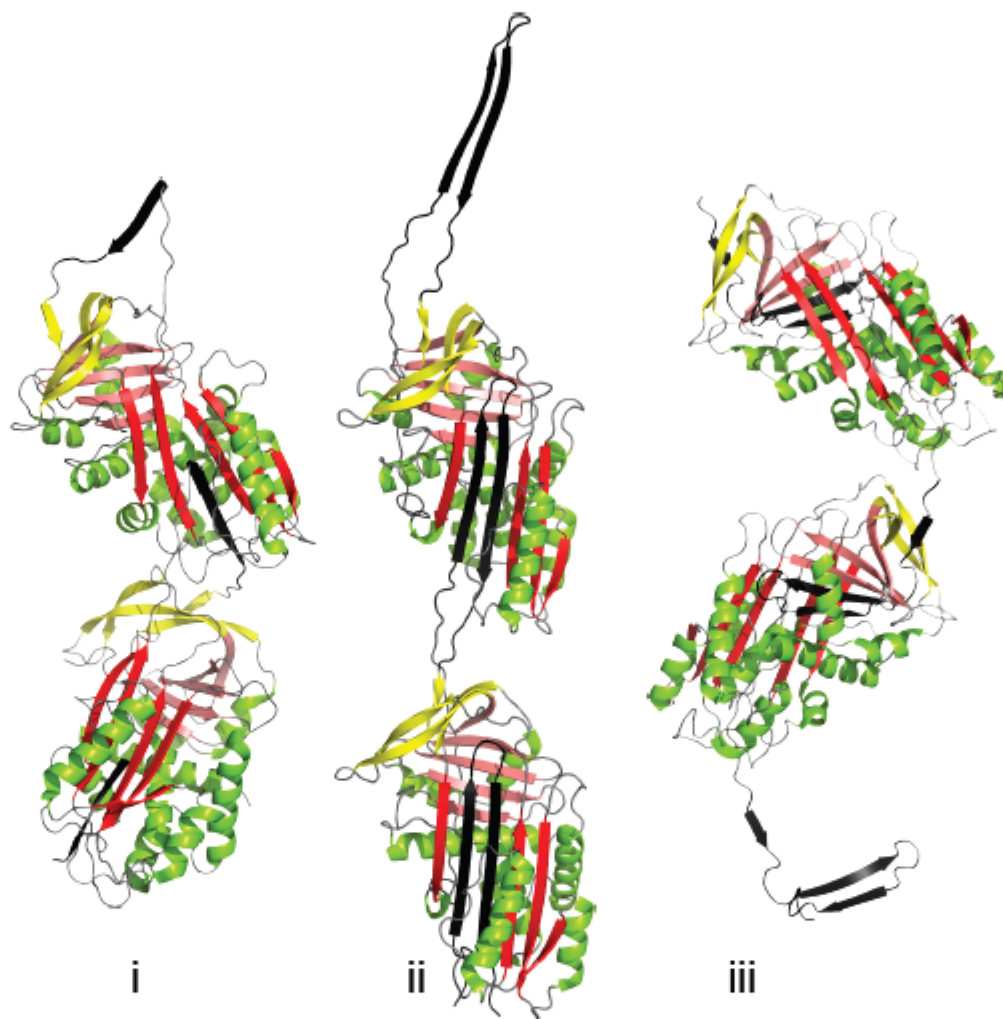


Figure 3.

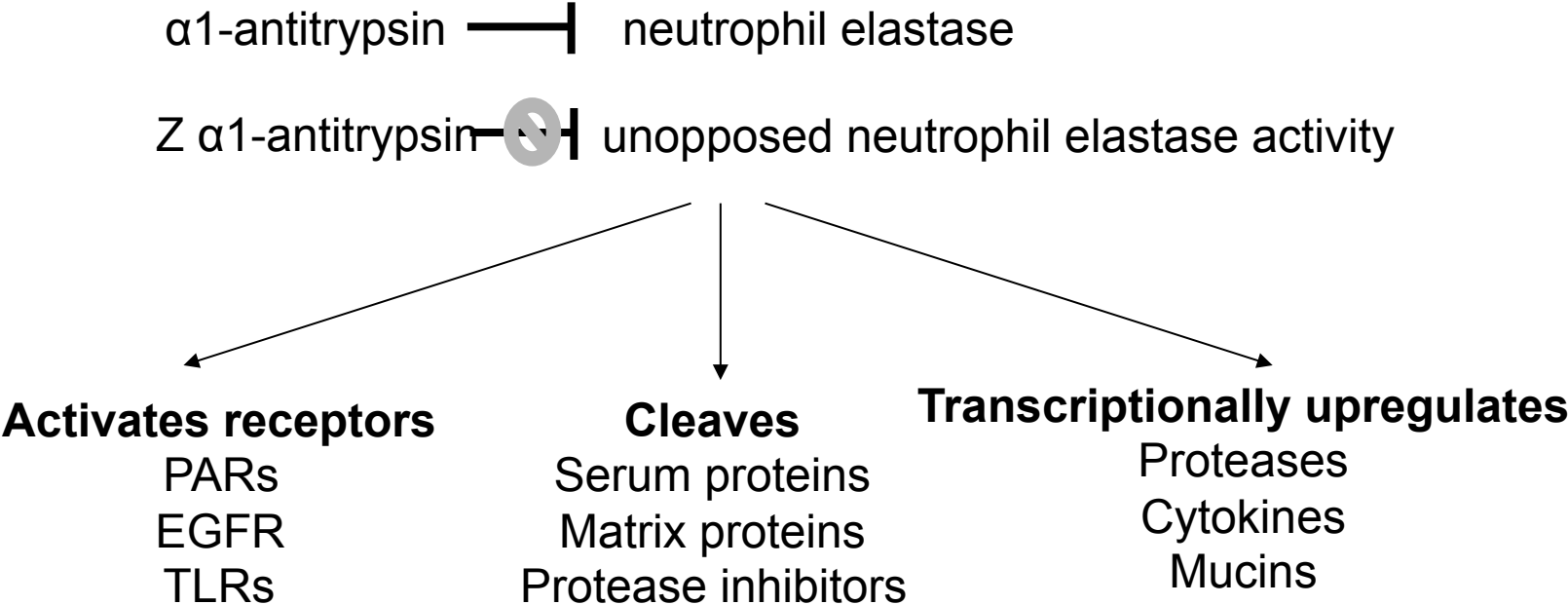


Figure 3.

α 1-antitrypsin —| neutrophil elastase

Z α 1-antitrypsin —| unopposed neutrophil elastase activity

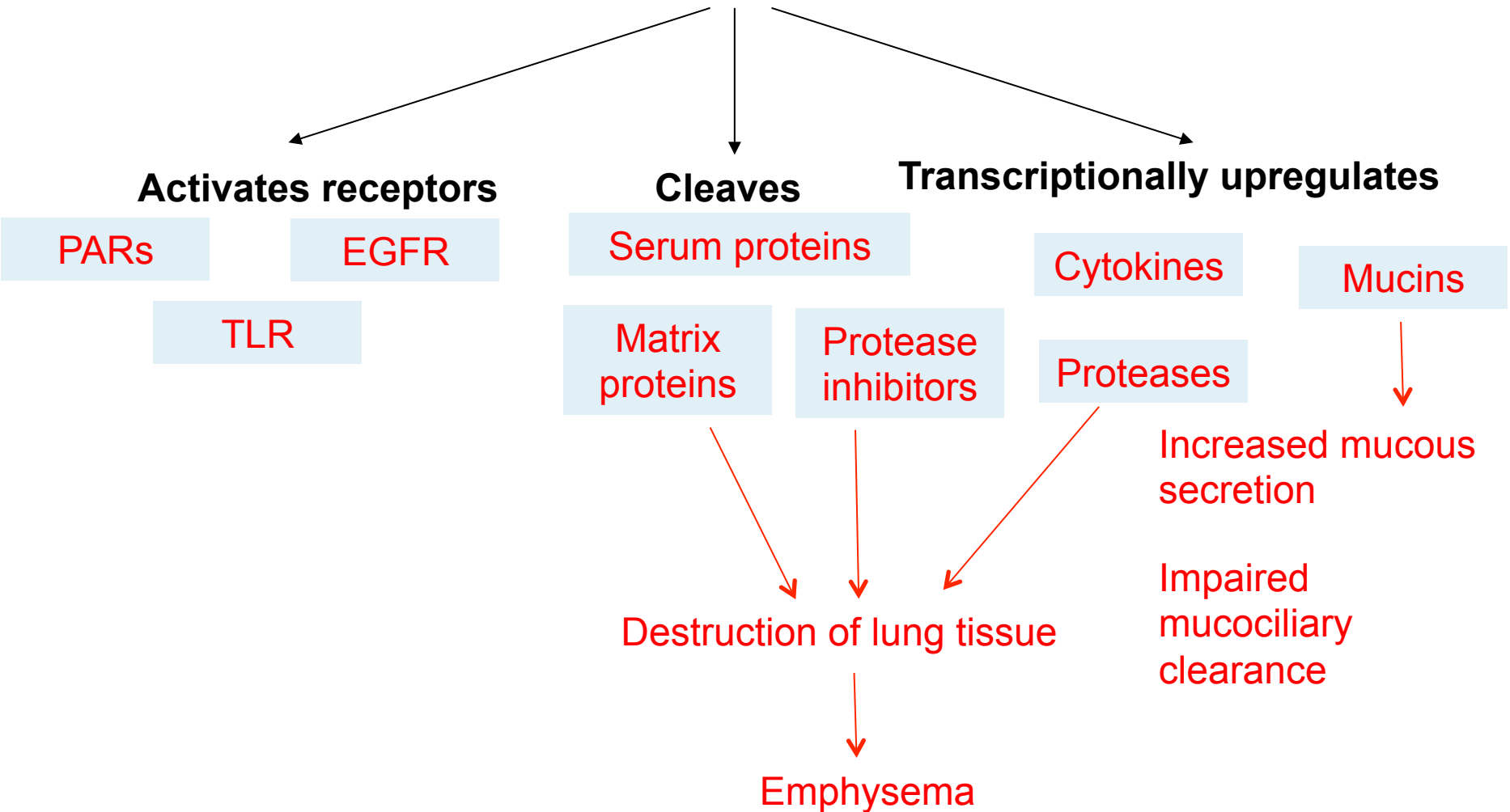


Figure 4. Similar content to this, but needs to be redrawn please

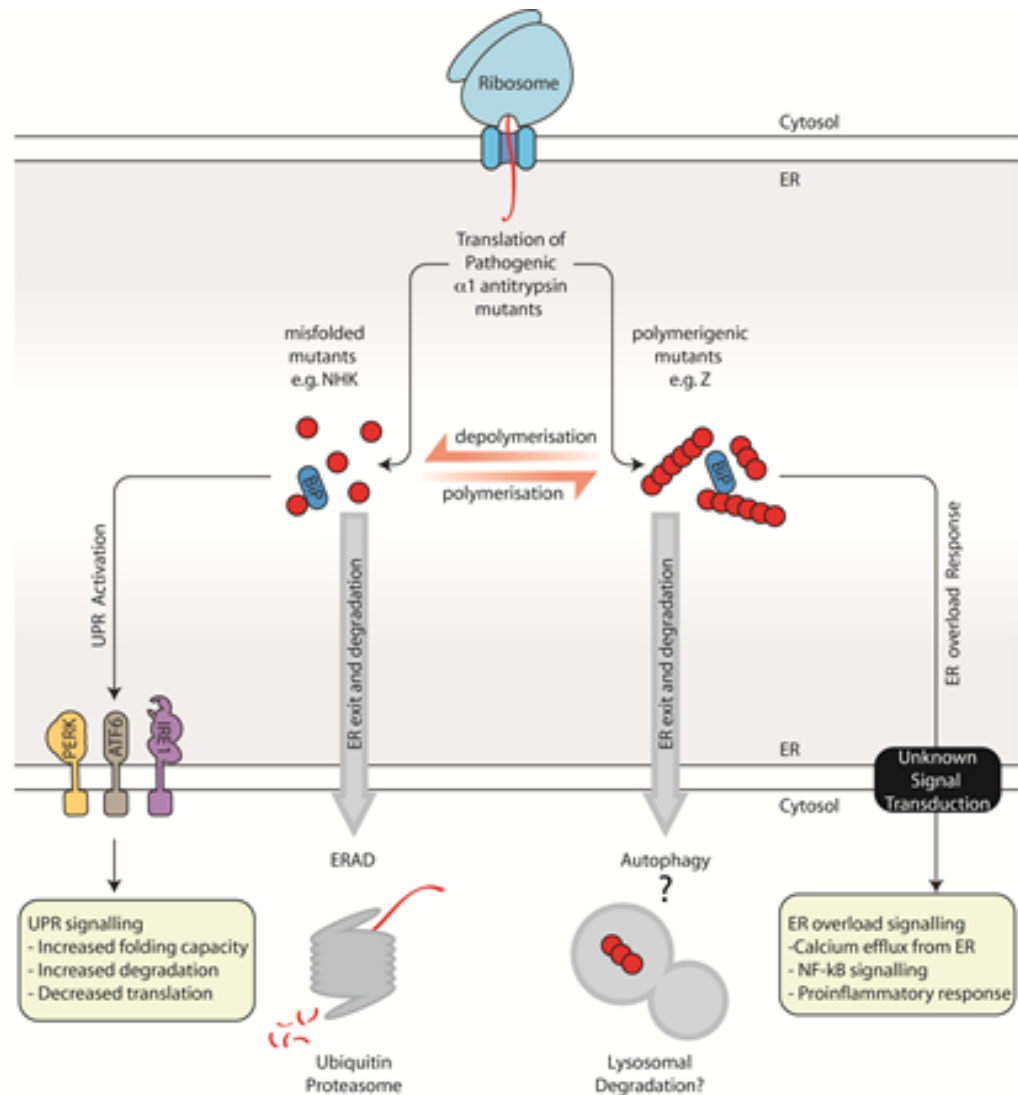


Figure 5.

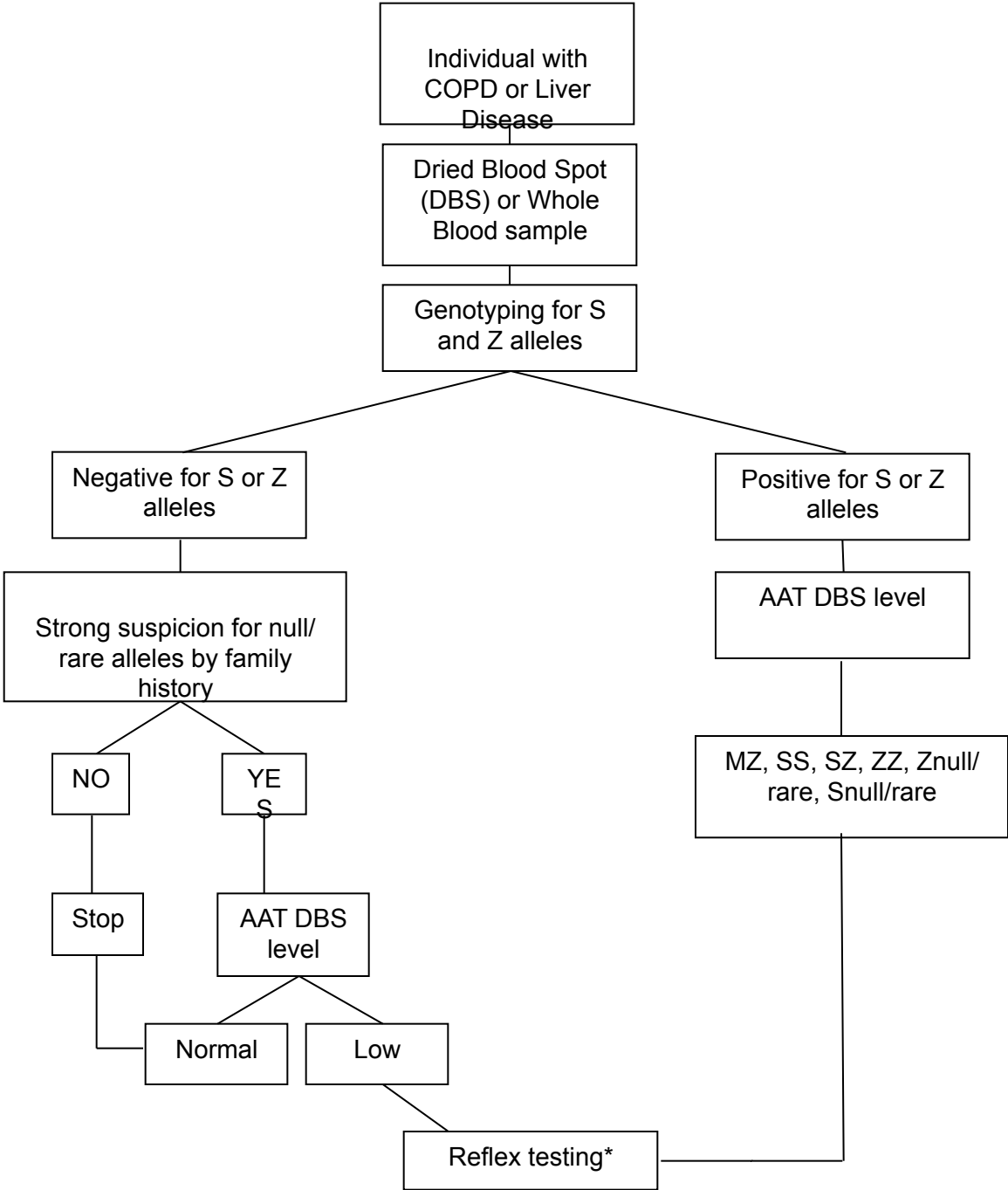
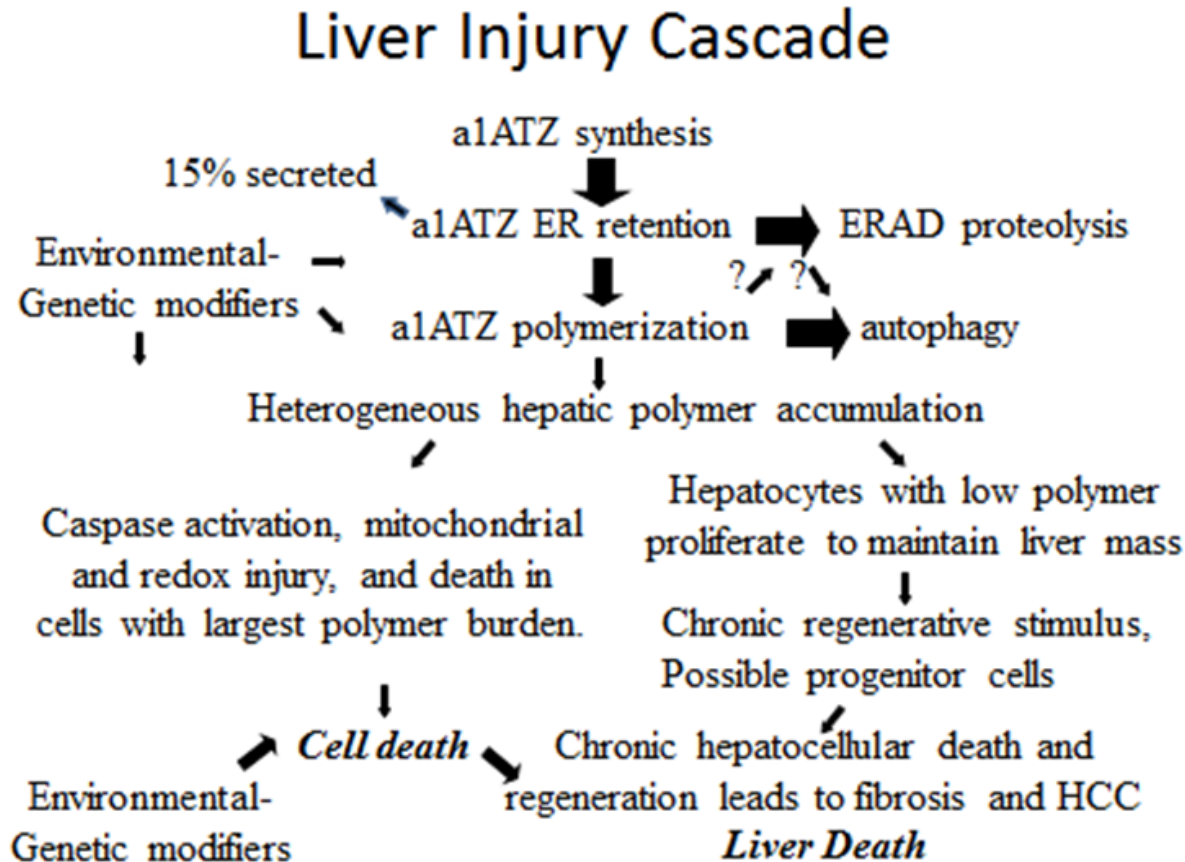


Figure 6. Can this be converted into a diagram, please



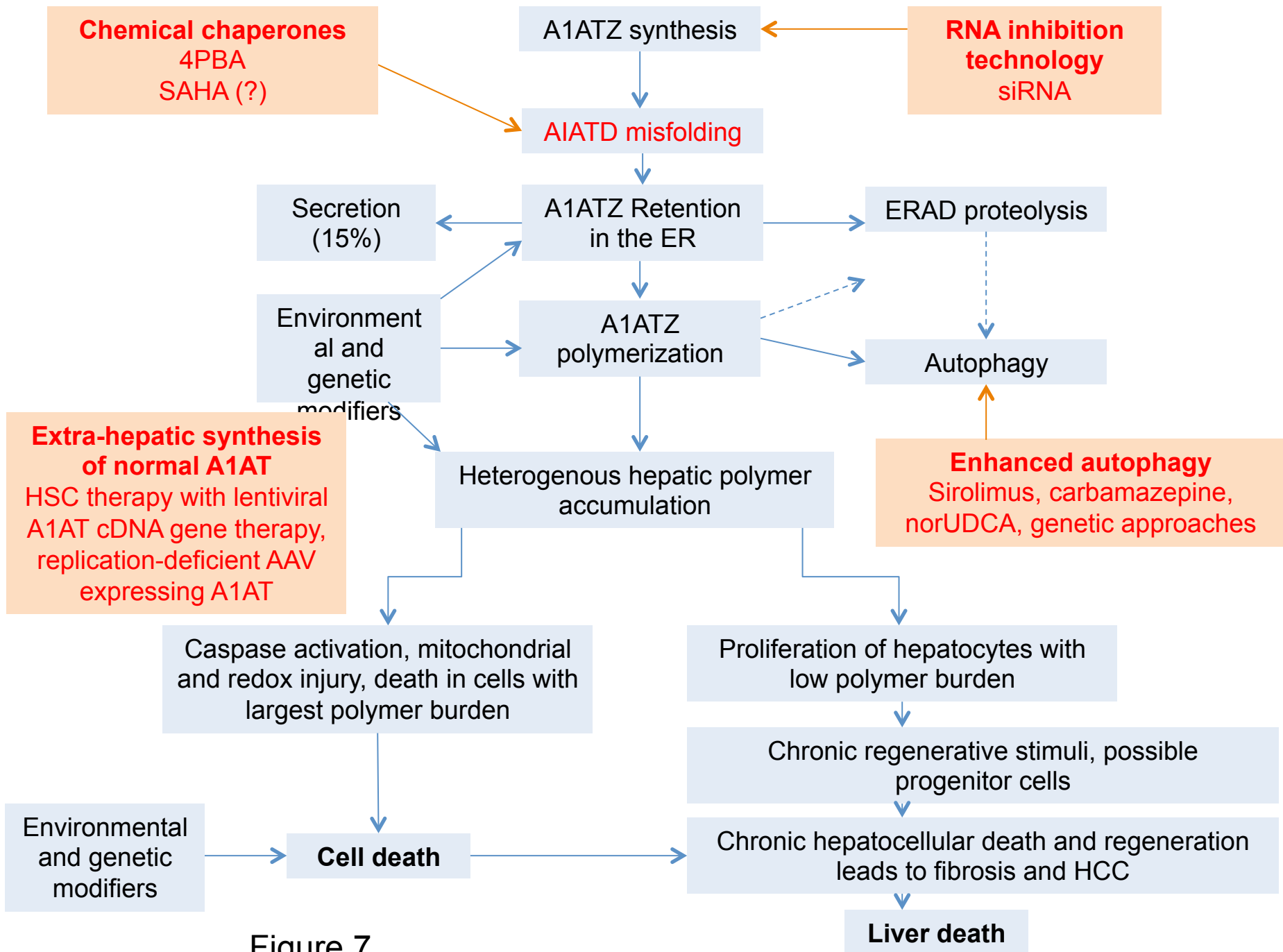


Figure 7.