α_1-antitrypsin deficiency

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ABSTRACT

α_1-antitrypsin deficiency is an inherited disorder caused by mutations in *SERPINA1* leading to liver and lung disease. It is not a rare disorder; however, it is frequently underdiagnosed or misdiagnosed. The normal α_1-antitrypsin protein is a serine proteinase inhibitor that primarily targets neutrophil elastase; however, it can also inhibit other proteases and displays immuno-modulatory and anti-inflammatory properties. Over 150 *SERPINA1* alleles have been described. The most frequent disease-associated mutations include the S and Z alleles which lead to expression of aberrantly folded α_1-antitrypsin proteins by hepatocytes, leading to low levels of α_1-antitrypsin in the circulation. The liver disease is a 'gain-of function' effect due to accumulation of misfolded α_1-antitrypsin within the endoplasmic reticulum (ER) of hepatocytes. Currently there is no cure for severe liver disease. The lung disease occurs predominately in adults, and can be evident as early as the 3rd to 4th decade of life. Its hallmark is loss-of-function of the lungs' antiprotease protective screen but is also characterised by pro-inflammatory ER stress-related effects. α_1-antitrypsin deficiency is a genetic cause of COPD and *SERPINA1* MZ heterozygosity is a known risk factor for COPD in smokers. Treatment of the lung manifestations includes many standard therapies for COPD in addition to 'augmentation therapy' with human plasma-derived, purified α_1-antitrypsin. New therapies targeting misfolded α_1-antitrypsin proteins and novel strategies that attempt to correct the underlying genetic mutation are under development. Effective modalities and timely diagnosis can enable personalised medical care and greatly enhance the quality of life of people with α_1-antitrypsin deficiency.

[H1] Introduction

α_1-antitrypsin is a serine proteinase inhibitor and acute phase protein produced principally by the liver but also by neutrophils, monocytes and airway epithelial cells. Its primary target protease is neutrophil elastase; however, it can inhibit other proteases and also has anti-inflammatory and immuno-modulatory properties. α_1-antitrypsin deficiency (OMIM, 0107400), was first described in 1963[1], and is an autosomal co-dominant disorder caused by mutations in the *SERPINA1* gene (previously called the 'protease inhibitor' or PI locus) pre-disposing to liver and lung disease in affected individuals (Figure 1). Over 150 *SERPINA1* alleles have been described. The normal allele is referred to 'M'. The most frequent and best studied disease-associated *SERPINA1* mutations, including the so-called S and Z alleles, lead to expression of aberrantly folded α_1-antitrypsin proteins and lower than normal circulating levels of α_1-antitrypsin. The liver disease in children and adults is associated with gain-of function effects due to accumulation of misfolded α_1-antitrypsin protein within the endoplasmic reticulum (ER) of hepatocytes. Lung disease in adults can manifest as early as the 3rd decade of life and occurs mainly due to loss-of-function characterised by an inadequate antiprotease protective screen in the lung. Circulating and intrapulmonary polymers of misfolded α_1-antitrypsin, in particular the 'Z' form, as well as gain-of-function ER stress-related effects in monocytes and neutrophils also play roles in the inflammatory manifestations of the lung disease. There is no current cure for severe liver disease other than liver transplantation. The lung disease shares many characteristics of cigarette smoke-induced emphysema but is different in pathology being more panlobular rather than centrilobular, and most commonly has an initial basal rather than apical distribution. It also has different patterns of gene expression. α_1-antitrypsin deficiency is a genetic cause of COPD, being responsible for 1-2% COPD cases. Moreover, *SERPINA1* MZ heterozygosity (PI*MZ) is a risk factor for COPD in smokers. α_1-antitrypsin deficient individuals with lung disease receive many standard therapies for chronic obstructive pulmonary disease (COPD) in addition to augmentation therapy with human plasma-derived, purified α_1-antitrypsin. New therapies that target misfolding of mutant α_1-antitrypsin or attempt to correct the underlying genetic mutation are being developed. α_1-

antitrypsin deficiency is not a rare disorder; however, it is frequently underdiagnosed or misdiagnosed as asthma, COPD, or cryptogenic liver disease, amongst others. The timely identification of α_1-antitrypsin deficient individuals can enhance their quality of life by enabling personalised medical care.

In this primer article, we summarize the epidemiology of α_1-antitrypsin deficiency, present the pathobiology of lung and liver disease, and discuss current research in the field. We also consider existing treatment options and developments that might further improve the outlook for α_1-antitrypsin deficient individuals.

[H1] Epidemiology

α_1-antitrypsin deficiency is relatively common but widely and persistently under-recognized[2, 3]. This section considers the world-wide prevalence of α_1-antitrypsin deficiency, evidence that it is under-recognized, and the reasons for under-recognition.

Although most prevalent in Scandinavia, North America, and Iberia, α_1-antitrypsin deficiency occurs world-wide. In their review of 514 published cohorts of α_1-antitrypsin deficient individuals reported from 69 countries in 11 geographic regions of the world, de Serres *et al*. observed that α_1-antitrypsin deficiency affects individuals in virtually all racial subgroups studied[4]. In aggregate, the estimated worldwide prevalence of PI*MS and PI*MZ heterozygotes is 116 million and that of PI*ZZ, PI*SZ, and PI*SS individuals is 3.4 million. The prevalence of α_1-antitrypsin deficiency has been estimated based on two detection strategies – population-based screening and case-finding, also called targeted detection. Of the many population-based screening studies to assess the prevalence of α_1-antitrypsin deficiency (Table 1[4]), the largest two were performed in newborn infants in Sweden (N = 200,000 newborns)[4] and Oregon (N = 107,038)[5]. In Sweden, the prevalence of PI*ZZ individuals was 1/1639 and in Oregon, the prevalence was 1/5097. Estimates suggest that of the approximately 320 million people in the United States approximately 100,000 have severe α_1-antitrypsin deficiency[6].

Table 2[7] summarizes the results of targeted detection studies that have also assessed the prevalence of α_1-antitrypsin deficiency among individuals with various

suggestive clinical features. Prevalence estimates of severe α_1-antitrypsin deficiency among individuals with COPD range from 0 to 12% with a mean value in the reported studies of 3.6%.

That α_1-antitrypsin deficiency is widely under-recognized is supported by three lines of evidence: First, in all countries where the issue has been examined, only a small minority of expected individuals with α_1-antitrypsin deficiency have been recognized clinically[8]. Second, few physicians comply with guidelines to test all COPD patients for α_1-antitrypsin deficiency. Third, individuals with α_1-antitrypsin deficiency commonly experience long delays between their first symptom and first diagnosis of α_1-antitrypsin deficiency and may see many healthcare providers before the diagnosis is first rendered. Estimates of the mean interval between first symptom (usually dyspnoea) and initial diagnosis range from 5.6 – 8.3 years[3, 9]. Diagnostic delay intervals remain as long in studies from 2013 as they were in the earliest study in 1995, suggesting little improvement in detection pace over nearly two decades despite the publication of many guidelines[10] which recommend that all COPD patients should be tested for α_1-antitrypsin deficiency. Similarly, the number of healthcare providers that affected individuals see before the diagnosis is first made has not lessened over time[2]. In addition to delaying any management interventions for the affected individual (e.g., smoking cessation, consideration of augmentation therapy) and identification of family members at risk, the need to see multiple healthcare providers before initial diagnosis and the associated diagnostic delay have been associated with adverse psychosocial effects[3]. In the context that establishing a diagnosis of α_1-antitrypsin deficiency can directly affect both the patient's clinical management and can identify potential risk among the patient's family members, continuing under-recognition of α_1-antitrypsin deficiency provides a world-wide call to action for enhanced detection by healthcare providers.

[H1] Mechanisms/pathophysiology

Misfolding of mutant forms of α_1-antitrypsin within the endoplasmic reticulum (ER) of α_1-antitrypsin-producing cells can lead to toxic loss-of-function and gain-of-function effects. Loss-of-function effects primarily affect the lungs, whereas gain-of-

function effects contribute to both lung and liver manifestations of the disorder through two principal mechanisms: the perturbation of homeostasis within the lumen of the ER and the production of polymers of Z α_1-antitrypsin within the circulation, the lumen of the lung or within tissues that can cause chemotaxis and/or activation of inflammatory cells[11].

[H2] Genetic basis of disease

α_1-antitrypsin is encoded by the *SERPINA1* gene on the long arm of chromosome 14 at 14q32.1. The gene is comprised 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 cornea[12]. The hepatocyte *SERPINA1* promoter is located within exon 1C, upstream of the hepatocyte transcription start site[12, 13]. Alternative promoter regions are located upstream of exon 1A and before exon 1B; these control *SERPINA1* expression in monocytes and macrophages[12, 14]. Thus different transcripts are produced due to the different transcription initiation sites, however alternative splicing of non-coding exons (1A, 1B and 1C) can also occur in a stimulus- and cell-type specific manner[12, 15, 16]. Proinflammatory cytokines in particular IL-6 and leukaemia-inhibitory factor, and essentially the acute phase mediator oncostatin M, contribute to tissue-specific α_1-antitrypsin expression[17-21]. Recently a specific qPCR test has been developed to quantify the expression of *SERPINA1* transcripts, with the aim of better understanding regulatory mechanisms controlling *SERPINA1* expression[22].

The *SERPINA1* gene is highly polymorphic and mutations in α_1-antitrypsin cause an hereditary co-dominant autosomal disorder, characterized by reduced serum levels of α_1-antitrypsin and high risk of developing emphysema at an early age. Pathological α_1-antitrypsin variants are either ‘deficient' or ‘null’. Deficient variants occur as a result of a point mutation that causes retention of the α_1-antitrypsin protein within hepatocytes and other α_1-antitrypsin-producing cells, and low levels of α_1-antitrypsin in plasma. There is no detectable α_1-antitrypsin in serum of individuals with null mutations which generally occur due to a premature stop codon. The most common severely deficient variant is Z α_1-antitrypsin (Glu342Lys, rs28929474), whose frequency spans 2–5% in Caucasians of

European descent. The hypothesis of a recent and single origin of the *PI* Z mutation is consistent with different publications. Microsatellite genotyping of the *SERPINA1* gene in populations with different historical backgrounds showed a common genotype variation[23] and analysis of non-recombinant SNPs revealed that the age of the *PI* Z mutation was 2902 years (SD±1983) in Latvia and 2362 years (SD±1614) in Sweden[24]. Moreover, evidence of some degree of founder effect of the Z mutation has been revealed by archaeological data on the settlement in Courland of people from Sweden and the island of Gotland after the seventh century[25]. Besides the Z mutation, at least 40 other deficient variants, often referred to as 'rare', have been identified over the last few decades; the molecular mechanism by which these mutations can cause disease vary and they can be prognostic for either liver and lung diseases. Similarly, up to 34 Null alleles have been characterized to date[26] (Table 3[27-63], reports a list of pathological mutations which cause α_1-antitrypsin deficiency).

[H2] α_1-antitrypsin deficiency in the lung

[H3] Biochemical characteristics of α_1-antitrypsin deficiency.

The α_1-antitrypsin protein is a 394 residue, 52kDa glycoprotein that is synthesised by hepatocytes, but is also produced by lung and gut epithelial cells, neutrophils and alveolar macrophages. It is the major circulating antiprotease but its key function is regulation of the proteolytic effects of neutrophil elastase within the lung. The inhibitor uses the characteristic serpin inhibitory mechanism in which elastase docks with, and cleaves the exposed reactive loop of α_1-antitrypsin. The covalently-bound enzyme is then translocated from the upper to the lower pole of α_1-antitrypsin as the cleaved reactive loop inserts into β-sheet A. This movement distorts the catalytic triad and irreversibly inhibits the activity of the enzyme[64]. The Z mutant of α_1-antitrypsin is retained within the ER of hepatocytes as ordered polymers that become sequestered in the Periodic Acid Schiff-positive, diastase-resistant inclusions[53, 65]. This same process underlies the severe plasma deficiency and intra-hepatic inclusions of three other mutants of α_1-antitrypsin: Siiyama (Ser53Phe)[66], Mmalton (ΔPhe52)[67] and King's (His334Asp)[53]. Polymerisation also underlies the deficiency of the mild S (Glu264Val), I (Arg39Cys), Queen's (Lys154Asn) and Baghdad (Ala336Pro) alleles of α_1-antitrypsin[40, 68-70]. However the rate

of polymer formation, which is proportional to the destabilising effect of the mutation on the protein[71], is much slower and explains the absence of liver disease and the association with only mild plasma deficiency.

The original description of polymers of Z α_1-antitrypsin described a linkage between the reactive centre loop and β-sheet A[65] (Figure 2i). However, alternative linkages 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[72]) and a trimer of α_1-antitrypsin (linkage by strands 1C, 4B and 5B)[73] (Figure 2ii and 2iii respectively). The biophysical characteristics of polymers of α_1-antitrypsin formed by refolding from guanidine gave support to the β-hairpin linkage[74]. The cause of the controversy became clear with development of a monoclonal antibody (termed '2C1') that recognises the pathological polymers from hepatocytes of individuals with α_1-antitrypsin deficiency[53]. This antibody recognises an epitope on polymers formed by heating monomeric α_1-antitrypsin that is not present in polymers formed by refolding from guanidine and urea[75]. This is due to the fact that polymers form by different loop-sheet linkages in response to heat rather than urea or guanidine[75]. NMR studies followed the polymerisation of Queens (Lys154Asn) α_1-antitrypsin under physiological conditions or in urea. Intermediate formation under physiological conditions was associated with highly native-like behaviour with changes in a few key motifs[40]. Global changes were observed in urea consistent with more widespread unfolding, in keeping with data from hydrogen-deuterium exchange[76]. Consequently, different polymeric linkages can be accessed by different chaotrophic conditions with the application of heat to monomeric α_1-antitrypsin recapitulating the features of polymers associated with disease[77]. Recent work using small-angle X-ray scattering (SAXS) suggested that the trimer, tetramer, and pentamer of Z α_1-antitrypsin all form ring-like structures in keeping with C-terminal domain-swap mechanism of polymerization (Figure 2 right)[78]. However, ring structures are only rarely seen in inclusions from the livers of individuals with Z α_1-antitrypsin deficiency[65].

[H3] Pathological consequences of α_1-antitrypsin loss-of-function.

There is a plethora of loss-of-function effects that contribute to the pathophysiology of α_1-antitrypsin deficiency lung disease. Events directly related to unopposed elastase activity include cleavage of coagulation factors, complement, immunoglobulins, and cell surface receptors such as CXCR1[79-82] (Figure 3). Antimicrobial peptides[83], elastin[84], collagen[85], fibronectin[86] and proteoglycan[87] have also been reported to be cleaved by elastase. Some of the gene expression changes that occur in cells responding to elastase include increased matrix metalloprotease and cathepsin expression mediated via elastase-induced activation of TACE- and Meprin-mediated EGFR signalling[88-91]. Other significant outcomes that occur directly or indirectly due to the decreased antiprotease protective screen in the lung are goblet cell hyperplasia, increased mucus secretion and impaired mucociliary clearance. Inactivation of tissue inhibitors of metalloproteases[92], secretory leucoprotease inhibitor[83], elafin[93] and cystatin C[94] can also occur. α_1-antitrypsin can inhibit caspase-3 and its loss can promote apoptosis in lung endothelial cells[95]. Lack of sufficient α_1-antitrypsin is also responsible for decreased responsiveness to LPS in monocytes and decreased efficiency of neutrophil killing due to unopposed extracellular serine protease activity cleaving CXCR1 and CD14[82, 96]. More recently, data have emerged indicating that LTB4 production, and associated BLT1 membrane receptor expression[97] are increased, as are TNF-α mediated peripheral blood neutrophil apoptosis[98] and p38 and IκBα phosphorylation and matrix metalloproteinase and cytokine induction via PP2A[99]. These events contribute to inflammation and an enhanced rate of neutrophil reactive oxygen species production. Likewise lower than normal FcγRIIIb membrane expression and increased chemotaxis in response to IL-8 and soluble immune complexes[100] that occur in α_1-antitrypsin deficient neutrophils, together with degranulation of tertiary and secondary granules further exaggerate reactive oxygen species production[101].

[H2] Endoplasmic reticulum homeostasis

[H3] Intracellular disposal mechanisms for misfolded α_1-antitrypsin.

The inciting event in the pathophysiology of α_1-antitrypsin deficiency-related liver disease is the retention of the mutant Z protein within the hepatocyte during biogenesis (Figure 4)[102]. This can lead to cellular apoptosis and redox injury. Normally

proteins accumulated within the ER are degraded by the proteasome or by macro-autophagy. In α_1-antitrypsin deficiency, in order to cope with the increased load of misfolded protein within the ER, cellular disposal mechanisms are also more potently activated than normal. Soluble Z α_1-antitrypsin proteins are monitored within the ER and diverted to the ubiquitin-proteosome ER associated degradation (ERAD) pathway whereas polymerised Z α_1-antitrypsin is degraded by the process of autophagy. Much of the work investigating handling of misfolded α_1-antitrypsin has concentrated on the Null Hong Kong (NHK) variant. For degradation by the proteasome, misfolded proteins must be identified, returned to the cytoplasm and tagged with ubiquitin. ERAD is the major pathway for disposal of NHK α_1-antitrypsin owing to its inability to fold[103-106], but even polymerogenic mutants of α_1-antitrypsin can be degraded by ERAD despite having near-native conformations[107, 108].

Glycoproteins undergo cycles of N-glycan modification whilst within the ER. This acts as a timer to identify proteins failing to fold in an appropriate time. ER-α-1,2-mannosidase I (ERManI) trims mannose residues from N-glycans and its overexpression accelerates degradation of both NHK and Z α_1-antitrypsin[103, 109], while inhibition of ERManI with kifunensine stabilises both mutants[110]. An enzymatically inactive paralogue of ERManI called EDEM interacts with misfolded glycoproteins to enhance their degradation[111-113]. Interestingly, a minor allele of *MAN1B1* (encoding ERManI) associated with reduced protein expression has been reported more frequently than expected in children requiring transplantation for Z α_1-antitrypsin associated liver disease[114].

Z α_1-antitrypsin folds more slowly than M α_1-antitrypsin and can adopt a non-native intermediate conformation, both of which might contribute to its targeting for ERAD[53, 75, 115]. When α_1-antitrypsin emerges from the ER into the cytosol it is tagged with ubiquitin by the E3 ligases Hrd1 and gp78 and their associated E2 ligases, UBE2j1 and UBE2g2[116-118].

Whole organelles or large protein aggregates can be destroyed through engulfment by endomembranes that form into autophagosomes. These fuse with the lysosome so that the contents are hydrolysed. Mouse and cell models support a role for the autophagy in the degradation of Z α_1-antitrypsin[108, 115, 119, 120] and treatment of mice

with carbamazepine, a drug that can enhance autophagy, reduces accumulation of Z α_1-antitrypsin in the liver[119, 121]. It remains controversial, however, whether autophagy shows selectivity for ER containing polymers of α_1-antitrypsin or if this simply reflects turnover of the entire organelle.

[H3] Endoplasmic reticulum stress.

When misfolded proteins accumulate within the ER and threaten to fall out of solution, the cell is said to experience 'ER stress'. This triggers an 'unfolded proteins response' (UPR) that reduces the influx of nascent proteins into the ER whilst reprogramming the cell to fold or dispose of these proteins more efficiently. This process involves the detection of ER stress by three transmembrane sensors, PERK, IRE1 and ATF6 (Figure 5), and has been reviewed extensively elsewhere[122, 123]. The misfolding variants NHK and Saar α_1-antitrypsin trigger the UPR if expressed even at low levels[33, 105, 106, 124-126]. Both of these variants are truncated and so unable to fold. They are normally degraded efficiently by ERAD, but if allowed to accumulate will sequester large numbers of chaperones, including BiP, and thus lead to ER stress. The precise mechanism by which ER stress sensors are activated remains a matter for debate. One model suggests that it is the sequestration of BiP by misfolded proteins that provides the signal[127]. Normally, BiP binds to and inhibits the ER stress sensors, but when misfolded proteins accumulate within the ER the level of free BiP falls leading to activation of the sensors. An alternative model suggests that the sensors interact directly with stretches of misfolded protein[128]. In both models, however, it is the exposure of normally buried residues of the client protein that constitutes the signal that is sensed by the cell. Curiously, the dramatic accumulation of polymeric α_1-antitrypsin fails to activate the UPR in most circumstances[125, 126, 129-133]. Since α_1-antitrypsin polymers are thought to be relatively well-folded structures, they may not present misfolded stretches of amino acids and so fail to trigger the ER stress sensors. However, the accumulation of polymers does appear to sensitize the cell to second insults that cause ER stress[126, 129-131]. The mechanism for this sensitization remains to be fully worked out, but appears to involve altered protein mobility within the ER lumen, either owing to local effects on viscosity or on the degree of ER interconnectivity[126].

These events can impact on a variety of intracellular signalling pathways leading to transcriptional upregulation of proinflammatory gene expression. For example, basal and LPS-induced IL-6 and IL-8 expression are increased in monocytes from Z α_1-antitrypsin deficient versus non-α_1-antitrypsin deficient individuals; this phenomenon is due to intracellular accumulation of Z α_1-antitrypsin[134]. Despite the lack of a robust UPR, the accumulation of polymerogenic α_1-antitrypsin triggers signalling by nuclear factor κB (NF-κB), which has been termed the 'ER overload response' (EOR). Little is known about this response although chelation of cytosolic calcium appears to limit the activation of NF-κB, suggesting it might involve increased calcium leak from a distended ER. However, in primary bronchial epithelial cells (PBECs) Z α_1-antitrypsin is expressed at low levels that fail to form polymers and yet these cells show enhanced basal NF-κB signalling[132]. This indicates that NF-κB signalling is not synonymous with EOR activation. A possible alternative mechanism by which mutants of α_1-antitrypsin can activate NF-κB signalling appears to involve increased activity of ADAM17. PBECs isolated from individuals homozygous for Z α_1-antitrypsin show hyperactive ERK signalling and this is dependent upon ADAM17. Moreover, increased ADAM17 activity has been reported on the surface of neutrophils from α_1-antitrypsin deficient individuals[98].

[H2] Contribution of extracellular Z α_1-antitrypsin polymers

Polymers of α_1-antitrypsin can be detected in the blood[135], bronchoalveolar lavage fluid and lung tissue of affected individuals[136, 137]. It is unclear if secreted Z α_1-antitrypsin polymerises in the extracellular space or if circulating polymers originate from dying cells. However, most polymers are of hepatic origin since following liver transplantation, the circulating levels fall to undetectable within four days[135]. However, α_1-antitrypsin can by synthesised locally by airway epithelial cells, albeit at levels too low to allow polymerization within the cell[132]. The importance of extracellular polymers relates to their pro-inflammatory effects. They are chemotactic and stimulatory for neutrophils and so are likely to contribute to pulmonary inflammation, and their

deposition in other tissues may explain the increased incidence of vasculitis or panniculitis seen in PI*ZZ individuals[138] .

[H2] Clinical manifestations

The Z allele of α_1-antitrypsin causes the protein to misfold and form ordered polymers that are retained within the endoplasmic reticulum of hepatocytes as Periodic Acid Schiff-positive, diastase-resistant inclusions. These inclusions form *in utero*[139] and 73% of PI*ZZ children have raised serum aminotransferases in the first year of life. However, this typically resolves and only remains abnormal in 15% of individuals by 12 years of age. Similarly serum bilirubin is elevated in 11% of Z α_1-antitrypsin homozygote infants in the first few months of life but falls to normal by 6 months of age. Ten percent of PI*ZZ infants develop jaundice as a result of cholestasis and 6% develop clinically evident liver disease in the absence of jaundice. The clinical symptoms typically resolve by the second year of life but 15% of children with cholestatic jaundice progress to cirrhosis[5, 140]. The risk of death from liver disease in Z α_1-antitrypsin homozygote children is 2-3%[141, 142]. All adults with Z α_1-antitrypsin deficiency have slowly progressive hepatic damage that is only apparent as a minor degree of portal fibrosis and no clinical symptoms. However, one a post-mortem study showed that 50% of Z α_1-antitrypsin deficiency individuals develop cirrhosis and occasionally with hepatocellular carcinoma[143]. Risk factors for cirrhosis include male gender and obesity but not alcohol or viral hepatitis[144]. The predilection for hepatocellular carcinoma in PI*ZZ individuals is higher than that attributable to cirrhosis alone.

Emphysema associated with Z α_1-antitrypsin deficiency is typically panlobular and affects the bases of the lungs. Individuals present with breathlessness with cor pulmonale and polycythaemia occurring late in the disease[10]. Lung function tests are typical for emphysema with a reduced forced expiratory volume in 1 second (FEV_1), reduced FEV_1/forced vital capacity ratio, gas trapping (raised residual volume/total lung capacity ratio), and a low gas-transfer factor. Partial reversibility of airflow obstruction (as defined by an increase of 12% and 200 ml in FEV_1 after a bronchodilator) is common in individuals with chronic obstructive pulmonary disease secondary to α_1-antitrypsin deficiency. All the PI*ZZ 35-year-olds followed up in the Swedish birth cohort had

normal liver and lung function but smoking frequency was significantly lower among individuals with α_1-antitrypsin deficiency than in the controls[145]. There was evidence to suggest that ever smokers had abnormal scans and lung function[146].

PI*ZZ α_1-antitrypsin deficiency is also associated with an increased prevalence of asthma[147], panniculitis[148] and granulomatosis with polyangiitis[149]. The underlying disease mechanisms are not known but it is possible that the pro-inflammatory polymers and deficiency of an important antiproteinase contribute to GPA and panniculitis.

[H2] Gene modifiers, gene-by-environment interactions

A recent genome wide association study tightly linked circulating α_1-antitrypsin levels in a general population sample to the *SERPINA* gene cluster[150], and the detrimental role of smoke exposure on the clinical phenotype of α_1-antitrypsin deficiency has been recently demonstrated[151]. Nevertheless, the wide spectrum of clinical phenotypes associated with α_1-antitrypsin deficiency could be caused by interactions between genetic factors other than *SERPINA1,* and environmental determinants other than smoking alone. Indeed, single studies in recent years have identified potential genetic modifiers of COPD phenotypes in individuals with severe α_1-antitrypsin deficiency. Variations in MMP1/ MMP3 and TNFα have been associated with gas transfer and chronic bronchitis, respectively, in α_1-antitrypsin deficiency[152, 153]; polymorphisms in IL-10, the cholinergic nicotine receptor alpha 3 (CHRNA3) and iron regulatory binding protein 2 (IREB2) were associated with FEV_1 and/or FEV_1/FVC in PI*ZZ individuals[154, 155]. Between PI*ZZ individuals there can be a significant variability in the expression of the lung disease i.e., ranging from asymptomatic to severe emphysema. This occurs as a result of genetic predisposition and environmental factors. For example, an interplay between cigarette smoke induced oxidative stress and Z α_1-antitrypsin protein polymerization can impact on cellular inflammation and cytokine expression[156]. Regarding the role of the environment, few data are available however outdoor air pollution can worsen respiratory status and predict lung function decline in PI*ZZ individuals[157, 158]. In another study a statistically significant interaction ($p<0.0001$) was observed between the PI*MZ genotype and high levels of exposure to vapours, gas, dusts and fumes (VGDF) on annual change in FEF25–75%. A similar statistically significant interaction ($p=0.03$) was

observed between the PI*MZ genotype and high-level VGDF exposure on annual change in FEV_1/FVC. Overall, larger annual declines in lung function in association with outdoor particulate matter ≤10 μm were observed in PI*MZ carriers than in PI*MM carriers, and VGDF-associated FEF25-75% decline was observed only in ever smoking PI*MZ individuals[159]. Unlike smoking[160], environmental or passive tobacco smoke exposure is not a risk factor for PI*MZ individuals[159].

[H1] Diagnosis, screening and prevention

While clinical features of α_1-antitrypsin deficiency may be useful for selecting individuals for testing, the spectrum of disease manifestations is exceptionally variable and the diagnosis is largely a laboratory diagnosis and is well established in many laboratories throughout the world. The diagnosis requires either a plasma or serum α_1-antitrypsin level typically performed using a nephelometer and either genotyping or Protease Inhibitor (PI) typing[161]. Presently, most laboratories begin testing by using genotype-based allele specific amplification of the most common deficiency alleles, Z and S. One such testing algorithm is shown in Figure 6 but there is a series of alternative schemes that are used[162]. Genotyping may be performed using DNA from dried blood spots, whole blood and saliva. Reflex testing for risk alleles is usually performed by PI typing using isoelectric focusing of serum or plasma at a pH of 4-5. While S and Z alleles are present in greater than 95% of all α_1-antitrypsin deficient individuals, approximately 5% of deficient individuals of various populations studied will have rare deficiency alleles, including alleles associated with reduced, dysfunctional or no plasma α_1-antitrypsin. These rare alleles are not detected by routine methods and in order to identify them a combination of PI typing and next generation sequencing of the α_1-antitrypsin gene is used[163].

Printed and online educational materials have been created in several languages by organizations such as the Alpha-1 Foundation and are available at www.alpha1-foundation.org. These education materials assure that appropriate information is available for helping to determine the risk and benefit of genetic testing and interpret the results of genetic testing for α_1-antitrypsin deficiency for physicians and patients.

[H2] Population screening, predispositional testing and targeted detection programmes

α_1-antitrypsin deficiency remains underdiagnosed[164]. There are three approaches to diagnosis of α_1-antitrypsin deficiency: 1) diagnostic testing of individuals with symptoms/signs consistent with α_1-antitrypsin-related disease; 2) predispositional testing of individuals who may be at high-risk of having α_1-antitrypsin deficiency, and 3) targeted detection in patients with a clinical reason to suspect α_1-antitrypsin deficiency. In the past, diagnostic testing in α_1-antitrypsin deficiency meant testing of individuals with early onset, primarily lower lobe, emphysema. This paradigm has led to under diagnosis and late diagnosis and is no longer acceptable. Predispositional testing involves follow-up of asymptomatic subjects in whom a gene mutation has been identified, usually family members with low α_1-antitrypsin levels. While development of disease related to α_1-antitrypsin deficiency is likely in the future for these individuals, it is not certain and awaits further developments in our understanding of the natural history of α_1-antitrypsin deficiency. Regarding targeted detection, whilst this is similar to diagnostic testing, the method applies the ATS/ERS guidelines and increases diagnosis rates significantly. These guidelines do not recommend neonatal screening [10] (i.e. testing groups without known risk factors for α_1-antitrypsin deficiency) and point to a Swedish study[165] which showed that while neonatal screening reduced smoking rates following detection, there was an increased incidence of parental distress with a negative impact on the mother–child relationship. Screening guidelines are evolving and appear to be quite dynamic and the potential benefits of screening versus targeted detection should be revisited particularly in the light of increased understanding of the pathogenesis of α_1-antitrypsin deficiency-related disease and the experience with other new screening programmes such as those for cystic fibrosis. The ATS/ERS guidelines do not generally recommend testing in adolescents aged <11 years, but suggest that testing should be discussed with individuals in areas with a high prevalence of α_1-antitrypsin deficiency or if smoking rates are high, providing that adequate counselling is given. Recommendations for adults are similar to those for adolescents. The 2014 Global Initiative for Chronic Obstructive Lung Disease (COPD) recommendations[166] quote the World Health Organization[167], who

recommend that COPD patients from areas with a particularly high prevalence of α_1-antitrypsin deficiency should be tested for α_1-antitrypsin deficiency. They also noted that compared to other forms of COPD, typical patients with α_1-antitrypsin deficiency tend to present at a younger age (<45 years) with lower lobe emphysema and suggest that family members can be identified. These recommendations are not that different from those which have led to significant under diagnosis of the condition for the past 50 years. The ATS/ERS guidelines[10] recommend testing high-risk groups, such as: all people with COPD; all nonresponsive asthmatic adults/adolescents; all people with cryptogenic cirrhosis/liver disease; people with granulomatosis with polyangiitis; bronchiectasis of unknown aetiology; panniculitis; and first-degree relatives of patients with α_1-antitrypsin deficiency. This increases detection of α_1-antitrypsin deficiency. Any targeted detection program must be linked to robust laboratory diagnostics[168]. Measurement of α_1-antitrypsin levels alone will not differentiate between the various genetic subtypes of α_1-antitrypsin deficiency and should be accompanied by either phenotyping or genotyping, both of which have potential problems which can be solved by evaluation in conjunction with levels and resort to gene sequencing as required[162]. Data from the Irish National Targeted Detection Programme has shown that targeted detection based on the ATS/ERS criteria enriches the detection of α_1-antitrypsin deficiency; the allele frequency for Z was over four-fold higher in the targeted population compared to an unselected sample of the general population[168].

[H2] Alpha-1 registries and awareness of α_1-antitrypsin deficiency in the medical community and beyond

In 2012, the National Organization for Rare Disorders (NORD), the European Organization for Rare Diseases (EURORDIS) and the Canadian Organization for Rare Disorders (CORD) recognized that Rare Disease Patient Registries "constitute key instruments for increasing knowledge on rare diseases, supporting fundamental clinical and epidemiological research, and post-marketing surveillance of orphan drugs and treatments used off-label"[169]. They also stressed the importance for patients and their families; the positive effect on health and social services planning and the ability to improve quality of care, quality of life and survival of patients. The earliest prospective

registry for people with α_1-antitrypsin deficiency was the National Heart, Lung and Blood Institute (NHLBI) Registry which enrolled 1129 individuals with severe α_1-antitrypsin deficiency from 1989-1992 and followed them until 1996[170]. This Registry collected demographic information, medical history, pulmonary function measurements, and other laboratory evaluations at baseline and at 6-month or yearly intervals during follow-up. The resulting dataset has produced some of the pivotal findings on the natural history of α_1-antitrypsin deficiency, on mortality, on the problems associated with delayed diagnosis. This analysis also revealed effects of α_1-antitrypsin augmentation therapy within the registrants whilst recognising that the results needed to be viewed with circumspection because the registry was not a randomized trial. The current Alpha-1 Foundation Research Registry began enrolment in 1997 with enrolment of mildly deficient genotypes in 2002[171]. This is essentially a contact registry with sufficient data to stratify study invitations to appropriate α_1-antitrypsin deficiency affected individuals although plans are to enlarge this remit. In 1997, the Alpha One International Registry (AIR) was founded to establish an international database of patients and their demographic details; to promote basic and clinical research into α_1-antitrypsin deficiency and to coordinate the activity; to collect, assess and disseminate information concerning all aspects of α_1-antitrypsin deficiency; and to encourage support and awareness of α_1-antitrypsin deficiency. AIR now includes almost twenty European and non-European countries[172]. The sole inclusion criterion for the registry is the presence of phenotype PI*ZZ, PI*SZ or other severely deficient variants. Some i.e. those in certain national registries, but not all patients are followed up annually and information collected to document characteristics of the disease, treatment, smoking habits and lung and liver function. There are also other large non-affiliated registries. The ideal registry, according to EURORDIS, should be disease-centred, demonstrate interoperability and harmonization, utilize a minimum set of common data elements, be linked with corresponding biobank data, include data directly reported by patients and data reported by healthcare professionals, and should encourage public-private partnerships to ensure sustainability. No present α_1-antitrypsin deficiency registry meets these criteria.

[H2] Prevention of morbidity and death in α_1-antitrypsin deficient individuals

There are compelling reasons to identify individuals with α_1-antitrypsin deficiency early. Among these reasons are access to specific therapies and opportunities to avoid environmental triggers of lung disease through avoidance of personal and passive cigarette smoking[173-175]. It has been long recognized that personal cigarette smoking is associated with a significant life span reduction in α_1-antitrypsin deficient individuals[176]. Importantly, α_1-antitrypsin deficient individuals develop COPD following exposure to a much lower number of pack-years of cigarette smoking than usual COPD individuals. Studies based on the Swedish population demonstrate that never smokers may have normal life spans. Occupational exposures such as mineral dust exposure and fumes are also associated with increased lung function impairment and symptoms of respiratory disease in α_1-antitrypsin deficiency individuals[177].

Early identification of α_1-antitrypsin deficient adolescents and adults is associated with reduction of the number electing to start smoking and increase in smoking cessation rates[178, 179]. In addition, screening programs that identify α_1-antitrypsin individuals at birth or during adolescence could substantially reduce the frequency of cigarette smoking since cigarette addiction is highest in those who start smoking when young. In this context, avoidance and smoking cessation counselling should be the number one focus for physicians and health care providers following the identification of α_1-antitrypsin deficient individuals of any age.

While environmental risk factors for obstructive lung disease are well established**,** modifiable risk factors for liver disease are less understood but are reported to include obesity and male gender[143]. Vaccination for hepatitis A and B are currently recommended for α_1-antitrypsin deficient individuals. Furthermore, moderate alcohol consumption and good nutritional behaviours may reduce the risk of liver disease in those homozygous for the Z allele[173].

[H1] Management

[H2] Lung disease

The rationale for the treatment of α_1-antitrypsin deficiency-related lung disease is to increase lung levels of α_1-antitrypsin towards normal, thus inhibiting neutrophil

elastase and other proteases, which, uninhibited, can cause emphysema. In 1987, plasma-purified α_1-antitrypsin at a dose of 60mg/kg once weekly was safely delivered intravenously to patients with α_1-antitrypsin deficiency to achieve plasma levels exceeding a protective threshold of 11 μM[180]. This target concentration was derived from α_1-antitrypsin deficient PI*SZ individuals, who if they refrain from smoking, rarely develop pulmonary disease. Increased levels of α_1-antitrypsin and increased anti-elastase capacity both in serum and on the pulmonary epithelial surface were shown following intravenous α_1-antitrypsin administration in these studies. Later studies looked at larger doses over longer time intervals. While these early studies illustrated biochemical efficacy, there remained a need to demonstrate clinical benefit. There were a number of observational studies suggesting benefit of α_1-antitrypsin augmentation therapy[181-184]; the earliest controlled study evaluated an untreated Danish group of α_1-antitrypsin deficient ex-smokers against a comparable German cohort who received augmentation therapy[170]. This study showed a small but significant reduction with α_1-antitrypsin augmentation in the annual rate of FEV_1 decline (21 mL/year) in those with a moderately reduced FEV_1 (31%–65%). Comparable results were noted within the NHLBI registry, and this latter data set also illustrated a mortality benefit with augmentation not identified in previous work[185]. In 1999, Dirksen *et al.* conducted the first randomized controlled trial and assessed chest CT changes in those receiving α_1-antitrypsin augmentation therapy compared to those receiving placebo[186]. This study showed no significant difference (P=0.07), but provided enough information to develop a power statistic which showed that a significant protection against CT determined loss of lung tissue with augmentation therapy could be detected in a placebo-controlled trial over a period of 3 years with 130 patients. A corresponding correction of the FEV_1 slope would require 550 patients over a 24-month period, a study population almost impossible to obtain. This was a significant breakthrough in the field, acknowledged by the regulatory authorities. Consequently; spirometry was considered a secondary efficacy end point in the study of augmentation therapy. The second randomized trial, EXAcerbations and Computed Tomography scan as Lung End points (EXACTLE), followed[187]. This multicentre, randomized, placebo-controlled, double-blind, exploratory trial utilized CT densitometry and exacerbations to assess the effect of weekly intravenous α_1-antitrypsin augmentation over an

approximately 2-year period This study illustrated that CT was a sensitive and effective measure of emphysema progression. A number of statistical analyses were utilized in this study, with P-values ranging from 0.049 to 0.084, but all suggested at least a trend toward efficacy of augmentation therapy in reducing loss of lung density by α_1-antitrypsin augmentation. It was acknowledged, however, that this study was underpowered. Following this, a larger multicentre, multinational, randomized controlled trial (RAPID) was conducted[188]. This study randomized PI*ZZ α_1-antitrypsin deficiency patients to receive α_1-antitrypsin augmentation therapy intravenously 60 mg/kg weekly or placebo over 2 years, measuring CT scan lung density at regular study intervals. One hundred and eighty subjects were evaluated over the 2-year period followed with an extension study (RAPID Extension) in which all study participants received active drug. The weight of evidence from RAPID and RAPID extension supported efficacy of augmentation therapy. Similar rates of lung density decline were observed in Early-Start and Delayed-Start groups during the Extension study and the reduction in absolute change in lung density decline was statistically significant when subjects switched from placebo to α_1-antitrypsin. There was a consistent treatment effect irrespective of when treatment was started, but lung density loss in the first two years on placebo was irreversible – suggesting early treatment may be more beneficial. Neither RAPID nor EXACTLE showed an effect of augmentation therapy on the number of exacerbations or quality of life.

Concerns about product purity and transmissibility of infection from human plasma-derived α_1-antitrypsin have led to evaluation of transgenic and recombinant sources of α_1-antitrypsin. Recombinant α_1-antitrypsin was successfully produced in bacteria and yeast as well as in transgenic sheep that were engineered to produce α_1-antitrypsin in their milk. A major disadvantage to these recombinant protein forms of α_1-antitrypsin was lack of glycosylation or abnormal glycosylation with altered renal clearance and short half-life following intravenous administration. An inhaled product with an appropriate half-life on the pulmonary epithelial surface has been investigated. Aerosolization of plasma-purified α_1-antitrypsin (Prolastin) and recombinant α_1-antitrypsin n were effective at delivery to the alveolar surface and alveolar interstitium

but whether in sufficient quantity for clinical efficacy remains to be evaluated[189, 190] (Table 4[170, 181-186, 188] lists the various treatments).

[H2] Liver disease

Liver disease associated with α_1-antitrypsin deficiency is highly variable. The risk of life threatening liver disease in children is about 3-5%, although many children may have self-limited neonatal cholestasis or mild serum aminotransferase elevations[5, 191]. Liver disease is uncommon in young and middle aged adults but increases with increasing age. The lifetime risk of cirrhosis in PI*ZZ individuals may be as high as 50%[192]. Given the unpredictability of disease progression, many authorities suggest regular monitoring for liver disease, on at least an annual basis, by a physician familiar with liver disease and its complications[192]. Monitoring should include history and physical examination sensitive for liver disease, such as a focus on the detection of splenomegaly, and laboratory exam including WBC, platelet count, AST, ALT, alkaline phosphatase, albumin, bilirubin and INR. Granulocytopenia, thrombocytopenia, climbing enzymes and bilirubin, and coagulopathy often accompany progressive liver injury. As in many liver diseases, a baseline liver ultrasound is often considered useful. American Association for the Study of Liver Diseases (AASLD) guidelines for the detection of hepatocellular carcinoma (HCC) recommend a liver ultrasound every 6 months for individuals at >2%/year risk of HCC[193]. Although data for the magnitude of HCC risk in α_1-antitrypsin deficiency is lacking, this is often interpreted to apply to α_1-antitrypsin individuals with evidence of cirrhosis, portal hypertension or persistently large elevations of liver tests.

There is no specific treatment for α_1-antitrypsin liver disease. Current treatment for progressive liver injury is primarily supportive with attention to the prevention of malnutrition, rickets, or managing the complications of portal hypertension such as ascites or variceal bleeding. It is not uncommon for children or adults with α_1-antitrypsin deficiency-associated cirrhosis to remain stable and compensated, with minimal signs and symptoms for years to decades. In this situation, the recognition of the presence of cirrhosis with portal hypertension is critical, even of the patient is minimally symptomatic, so they can be cautioned against splenic injury from contact sports, advised

to abstain from alcohol, undergo surveillance for variceal bleeding, and cautioned to avoid non-steroidal anti-inflammatory drugs (NSAIDS). Consumption of NSAIDs in the presence of portal hypertension can result in life-threatening bleeding even in well-compensated individuals. There are no data regarding alcohol consumption in PI*ZZ individuals 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.

If progressive liver failure or uncompensated cirrhosis is present and becomes life-threatening, then liver transplantation is considered. In the U.S., cadaveric organs are allocated by empirically derived severity scores for both children and adults, which are correlated with increasing risk of mortality without transplant. Early evaluation at a transplant centre is recommended for patients with signs or symptoms of deterioration, although early listing and time on the list do not influence the severity scores in the U.S. Listing and transplantation in other countries is highly variable, and is often influenced by referral, waiting and centre-specific factors. Many centres have reported excellent liver transplant outcomes for α_1-antitrypsin deficiency, often better than the median benchmark outcomes for other liver diseases. Living related liver transplants in infants (left lateral segment) and adults (split liver) are also reported as successful, including successful anecdotes when one of the donors is heterozygous, PI*MZ.

[H2] Emerging therapies

Many new approaches are currently being examined for potential value in the treatment of α_1-antitrypsin deficiency. Extensive studies have been published using *in vitro* analyses of molecular structure, and more than ten different compounds have been shown to block liver injury in the PiZ mouse model of α_1-antitrypsin liver disease, although none is yet approved for human use[119, 194, 195]. Regarding therapies that target the liver injury cascade at the point of synthesis, several applications of RNA inhibition technology are being examined to prevent mutant Z protein synthesis, and thereby to prevent accumulation and liver injury. In the PiZ mouse model, these methods have been shown to eliminate liver injury and to return the liver to wild type health[196]. Two different Phase I human trials of siRNA inhibition of mutant Z protein synthesis as liver disease

therapy are now underway in Australia and Europe. The major caveat associated with an α_1-antitrypsin-directed siRNA approach is that there would be no α_1-antitrypsin production thus presenting its own management issues which may be supplemented by transfection for instance with the normal gene and/or augmentation therapy in order to protect the lung.

Extensive studies have also examined methods to accelerate the intracellular degradation of mutant Z protein as a treatment for the liver. Several successful cell culture and mouse experiments have shown that enhanced autophagic degradation reduces the burden of mutant Z protein in the liver and reduces liver injury[119, 194, 195]. Sirolimus, carbamazepine, and the bile acid norUDCA, plus a genetic approach to augment expression of key autophagy regulators, have all been shown to reduce mutant Z protein accumulation within cells via enhanced autophagy and to reduce liver cell injury in a model system. However, excessively high doses of all of these agents were required to show an effect. A human trial of low dose carbamazepine in PI*ZZ patients with cirrhosis is currently underway, although results to date are inconclusive.

There has been longstanding interest in chemical chaperone approaches to improve proper folding and to augment secretion of Z $\alpha_1\alpha_1$-antitrypsin, instead of intrahepatic protein retention. Such an approach might treat the lung and the liver, as well. The primary barrier to this approach is the sheer mass of α_1-antitrypsin protein synthesized, which is up to 2g/d in an adult. If a 1:1 binding stoichiometry is needed as part of the mechanism, then a huge mass of drug would need to be delivered to the ER of the hepatocytes. Still, studies in cell culture have shown that several compounds promote the secretion of α_1-antitrypsin, and one, 4-phenyl butyrate (4PBA), was effective in the mouse model[197]. A pilot human trial was conducted, but no effect on secretion was detected, likely due to the inability of peak drug levels to reach the therapeutic range documented in the mouse[198]. Strategies designed *in silico* or cell free systems for therapeutic disruption of mutant Z protein polymerization, likely an event distal to the protein retention signal, have also been examined in a number of studies[195, 199]. These approaches aim to modulate the conformational behaviour of α_1-antitrypsin by targeting it directly to rescue folding, stabilize functional conformers and limit the population of polymerogenic intermediates[200-206]. However, many of the compounds examined have not

had the predicted effect when examined in cell culture and there have been chemical hurdles to creating medicinal molecules for trials in animal models. Other problems associated with some of these peptide-based strategies are that reactive loop analogues tend to generate complexes with α_1-antitrypsin that are inactive as antiproteases; nonetheless, these still have potential to treat the gain-of-function effects in the liver. Since both loss- and gain-of-function in α_1-antitrypsin deficiency are driven by protein misfolding and aberrant conformational change, addressing this behaviour may counter both pathogenic cascades at source. An approach to target the proteostasis network has identified the histone deacetylase 7 inhibitor suberoylanilide hydroxamic acid (SAHA), as an agent capable of restoring Z α_1-antitrypsin secretion from epithelial cells[207].

Finally, several studies, including human trials, have examined strategies to synthesize normal α_1-antitrypsin in tissues outside the liver, which might increase serum levels to protect the lung, but which would not change the risk of liver injury[208, 209]. To date, these studies have only been able to generate less than 5% of the serum M α_1-antitrypsin level thought to be needed for therapeutic benefit. Several gene repair technologies are also being investigated. For the lung disease, various gene therapy approaches designed to increase circulating α_1-antitrypsin levels with one having reached Phase II testing[209-214]. Two of these approaches involve haematopoietic stem cell therapy coupled with lentiviral α_1-antitrypsin cDNA gene therapy[215, 216] and intrapleural administration of a replication-deficient adeno-associated virus expressing α_1-antitrypsin[217]. α_1-antitrypsin deficiency has been at the forefront of the application of induced pluripotent stem cell (iPSC) technology[218-220] with skin fibroblasts from PI*ZZ individuals having been induced to form hepatocyte-like cells that recapitulated the disease phenotype[219]. This technology coupled with the recently developed CRISPR method of gene editing to correct the Z mutation[218] could generate 'corrected' PI*MM cells; theoretically, these cells could be used for autologous grafting without immune rejection. No human trials have yet begun and *in vitro* reports are still limited. However, the promise of this approach, which might be a long term answer to both lung and liver disease manifestations of this disorder is exciting (Box 1).

[H1] Quality of life

α_1-antitrypsin deficiency can both shorten survival[170, 176, 221-224] and can compromise affected individuals' quality of life (QOL)(Box 2)[225]. This section reviews the prognosis of α_1-antitrypsin deficiency, the impact of α_1-antitrypsin deficiency on QOL, and factors that affect these.

α_1-antitrypsin deficiency is associated with significant morbidity and mortality[226]. In a 1978 series, the median age at death for smokers with severe deficiency of α_1-antitrypsin was 40 years[176] and in a 1988 series of 124 patients[223], the cumulative survival to age 50 was 52%. In the largest available longitudinal study, the National Heart, Lung and Blood Institute (NHLBI) Registry of Individuals with α_1-antitrypsin deficiency[170] (in which 80% of subjects were current [8%] or ex-smokers [72%]), the mortality rate was ~3% per year.

In keeping with prognosis in COPD in general and on the importance of cigarette smoking as a driver of morbidity and mortality, FEV_1 is a major correlate of mortality in α_1-antitrypsin deficiency; individuals entering the NHLBI Registry with an FEV_1>50% experienced a normal expected survival whereas those with baseline FEV_1<15% experienced a 36% 3-year mortality rate. In the Danish Registry of 347 patients, median survival for patients with FEV_1<25% was 6.3 years, and increased to 10.5 and 14.2 years for those with FEV_1>25% and 50%, respectively [221]. Further regarding FEV_1[227] and thoracic computed tomography densitometry[228], these are important predictors of survival, with more rapid deterioration being associated with current smoking, age between 30 to 44 years, male sex, FEV_1 between 35 to 60% predicted, asthmatic features, chronic bronchitis and previous episodes of pneumonia[227, 229].

Among never smokers with α_1-antitrypsin deficiency, COPD is less prevalent and survival is longer. For example, Larsson [176] reported that the median age at death of never smokers was 65 years versus 40 years for smokers. On the basis of follow-up data from 568 individuals in the Swedish Registry, Tanash *et al*. reported that PI*ZZ never-smoking individuals ascertained as asymptomatic non-index cases experienced a normal lifespan (odds ratio for death = 0.7 compared with age- and gender-matched peers)[222]. In addition to smoking and lung function, the method by which individuals are ascertained as having α_1-antitrypsin deficiency conditions prognosis in α_1-antitrypsin deficiency; the

standardized mortality ratio is highest (5.0) for who come to attention because of liver symptoms[222].

The most frequent cause of death among individuals with α_1-antitrypsin deficiency is COPD or sequelae. In the NHLBI Registry, emphysema accounted for 72% of deaths and cirrhosis for 10%[224], whereas among PI*ZZ never smokers, emphysema accounted for fewer deaths (45%) but liver disease for more (28%)[222].

α_1-antitrypsin deficiency also contributes to substantial morbidity and impaired QOL. As with usual COPD, individuals with α_1-antitrypsin deficiency-associated COPD experience depression, anxiety, dyspnea, and impaired health-related QOL. A comparison of these symptoms in patients with usual COPD versus α_1-antitrypsin deficiency-associated COPD showed that a quarter of α_1-antitrypsin deficient individuals reported symptoms of depression and 36% reported anxiety that was deemed clinically important[230]. While the degree of anxiety and depression was similar among α_1-antitrypsin deficient versus α_1-antitrypsin-replete COPD patients, those with α_1-antitrypsin deficiency reported higher degrees of dyspnea (using the Modified Medical Research Council Dyspnea Scale) and worse health-related QOL (based on the St. George's Respiratory Questionnaire [SGRQ]). In a series of 1062 individuals with severe deficiency of α_1-antitrypsin[225], those older than 59 years experienced fewer exacerbations and had better QOL scores (SGRQ and SF-36) than younger individuals. Though available randomized controlled trials have shown that augmentation therapy tends to slow emphysema progression[186, 188], no convincing effect of augmentation therapy on exacerbation or health-related quality of life measures has been observed to date. That said the 2011 Global Initiative for Chronic Obstructive Lung Disease (GOLD) strategy performs well in identifying α_1-antitrypsin patients with increased risk of poorer outcomes, specifically mortality, lung function decline and exacerbations[231].

On the other hand, participation in a disease management program consisting of directed patient self-education (i.e., with a comprehensive reference guide describing COPD and α_1-antitrypsin deficiency) and organized supervision (i.e., through monthly telephone conversations with α_1-antitrypsin deficiency program coordinators supervising participants' understanding of long-term treatment plans) by 878 α_1-antitrypsin deficient

individuals receiving augmentation therapy was associated with 1-year improvements in medication use, enhanced compliance with supplemental oxygen, reductions in some measures of healthcare resource utilization (though not overall hospitalization rates), and selected improvements in healthcare-related QOL measures[232].

[H1] Outlook

It remains unclear why the clinical presentation of patients homozygous for Z α_1-antitrypsin is so variable. In the Swedish registry of PiZZ individuals, respiratory disease was the most common cause of death (55%) while only a minority died of liver disease (13%)[146]. Overall, respiratory symptoms were the most common presentation (43%) while liver disease was the presentation in only 7%. In never-smokers 28% of individuals fulfilled the spirometric criterion for COPD, which rose to 72% in exsmokers. Nevertheless, α_1-antitrypsin deficiency is the most common genetic cause for paediatric liver transplantation. Moreover, when patients with PiZZ-related lung disease in one British centre were screened for liver disease, 17.5% were found to have severe fibrosis on liver biopsy[233]. Moreover as discussed, this variability may reflect the contributions of gene modifiers such as *MAN1B1*[114]. The ability to model these genetic differences using patient-derived iPSCs is beginning to address this[219, 234]. When differentiated into hepatocyte-like cells, iPSCs from individuals who had developed severe liver disease show delayed clearance of Z α_1-antitrypsin and more prominent accumulation of inclusions. When combined with whole genome analysis, characterization of these differences is likely to clarify the effect of genetic modifiers. It is also possible that similar techniques could help personalize medical care by identifying those likely to develop liver disease.

Augmentation with α_1-antitrypsin is not yet universally accepted to prevent emphysema, although recent trials using surrogate endpoints for lung protection have been encouraging[185, 186, 188]. Although no one study is definitive, the weight of evidence clearly supports the efficacy of augmentation therapy in slowing the progression of emphysema in α_1-antitrypsin deficient individuals. This therapy is expensive and requires repeated, lifelong, intravenous infusions. The level of 11μM as the normal α_1-antitrypsin level is arbitrary and based on the not fully proven hypothesis that SZ individuals who do

not smoke do not have an increased risk for COPD/emphysema. There are a series of studies which suggest that in α_1-antitrypsin deficient patients receiving augmentation therapy, when their α_1-antitrypsin levels are at their nadir (just below the next infusion), that some of the immune-modulatory effects of α_1-antitrypsin may be lost or lessened. The RAPID study also suggested that higher doses resulted in less CT lung density decline. Thus future trials should look at higher dosages and/or more sustained elevated levels of α_1-antitrypsin. What also remains to be shown is whether the protection afforded by augmentation therapy is mediated solely by correction of the protease-antiprotease balance or whether the beneficial effects are evident primarily due to modification of inflammation. Moreover, since the contribution of circulating polymers to the inflammation associated with the PI*ZZ genotype is unknown, it is impossible to predict if simple augmentation therapy can ever be successful without suppression of the endogenous protein.

Other potential therapies that may supersede augmentation therapy are already on the horizon. In addition to the gene therapy, iPSC and gene editing approaches that have been discussed as yet it remains unclear whether these strategies can produce sufficient quantities of α_1-antitrypsin in an active form to render augmentation unnecessary. Regarding targeting proteostasis, it is now appreciated that protein folding within different compartments of the cell is far more intertwined than previously believed[235]. Recent studies have suggested that targeting maladaptive protein folding responses in the cytosol can improve the folding of substrates within the ER including that of Z α_1-antitrypsin[236].

α_1-antitrypsin is only one member of a larger family of serine protease inhibitors (serpins). Many other members of this family are mutated in human disease and so it is likely that lessons learned from the study of α_1-antitrypsin will have wider application (Box 3). For example, the neuron specific neuroserpin undergoes polymerization and formation of inclusion bodies in a manner precisely mimicking α_1-antitrypsin, but neuroserpin accumulation leads to neurodegeneration and early onset dementia[237]. When agents are developed that prevent polymerization of α_1-antitrypsin, they will lead rapidly to therapies for this and other serpinopathies where accumulation is the primary problem. Similarly, small molecules developed to mimic the anti-inflammatory effects of α_1-

antitrypsin would have much wider applicability since α_1-antitrypsin augmentation therapy appears to be beneficial in other disorders including cystic fibrosis[238, 239].

There is more for the cell biologist to learn from α_1-antitrypsin. The fact that different mutants of this one protein can induce either selective ER stress or ER overload makes it a versatile tool with which to probe ER dysfunction. The mechanism by which luminal accumulation of polymers can trigger downstream signaling is unknown, but it has been proposed that the ER overload may also mediate cellular responses to enveloped viruses and so, once again, the study of α_1-antitrypsin could shed light on other more prevalent conditions[240, 241].

Box 1. Emerging Therapies

Liver directed

- siRNA targeting the α_1-antitrypsin mRNA
- Autophagy regulators
- Methods to enhance proteostasis
- Approaches to refold +/or inhibit polymerisation of mutant α_1-antitrypsin

Lung directed

- Inhaled α_1-antitrypsin
- Hematopeoietic stem cells + lentiviral α_1-antitrypsin gene delivery
- Intramuscular and intrapleural AAV-mediated delivery of α_1-antitrypsin gene therapy
- CRISPR-mediated correction of the Z α_1-antitrypsin mutation in iPSCs

Box 2. Factors affecting symptoms and QOL in α_1-antitrypsin deficient individuals with COPD[230]

Symptom	Comment
Depression	Decreased in those in a stable relationship rather than single
Anxiety	Increased in those who are younger and less educated
Dyspnea	Worse if single, and compared to non-α_1-antitrypsin deficient individuals with COPD
Impaired QOL	Poorer compared to non-α_1-antitrypsin deficient individuals with COPD, but less severe above 59 years of age

Box 3. Other disorders caused by ER overload for which α_1-antitrypsin deficiency represents a good model

Early onset dementia and neurodegeneration resulting from neuroserpin accumulation[237]
Thrombosis caused by antithrombin deficiency[242, 243]
Angioedema associated with mutations in C1-inhibitor[244, 245]
Emphysema due to loss of circulating α_1-antichymotrypsin[246, 247]

Figures

[Editor's note to peer reviewers: We would welcome any specific comments you may have on how figures could be improved or any suggestions for new figures that would enhance the manuscript. Please note that all figures will be re-drawn by the *Nature Reviews* art team following peer review. As such, we kindly request that you focus your attention on the content of the figures rather than their overall appearance.]

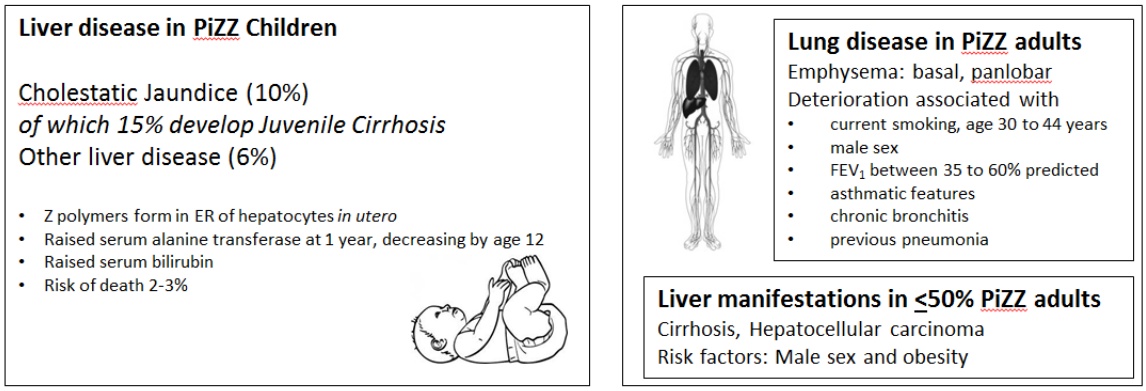
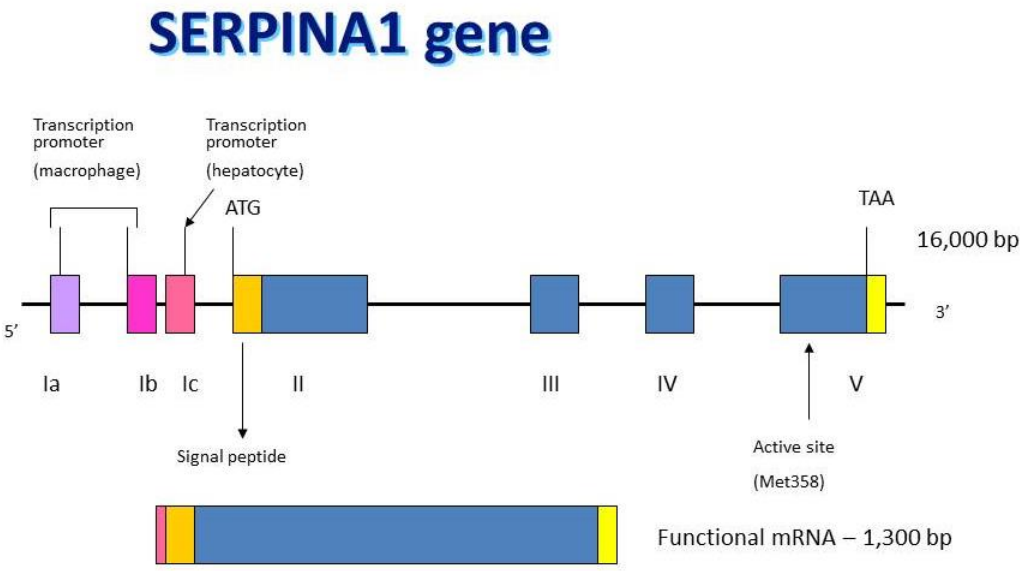
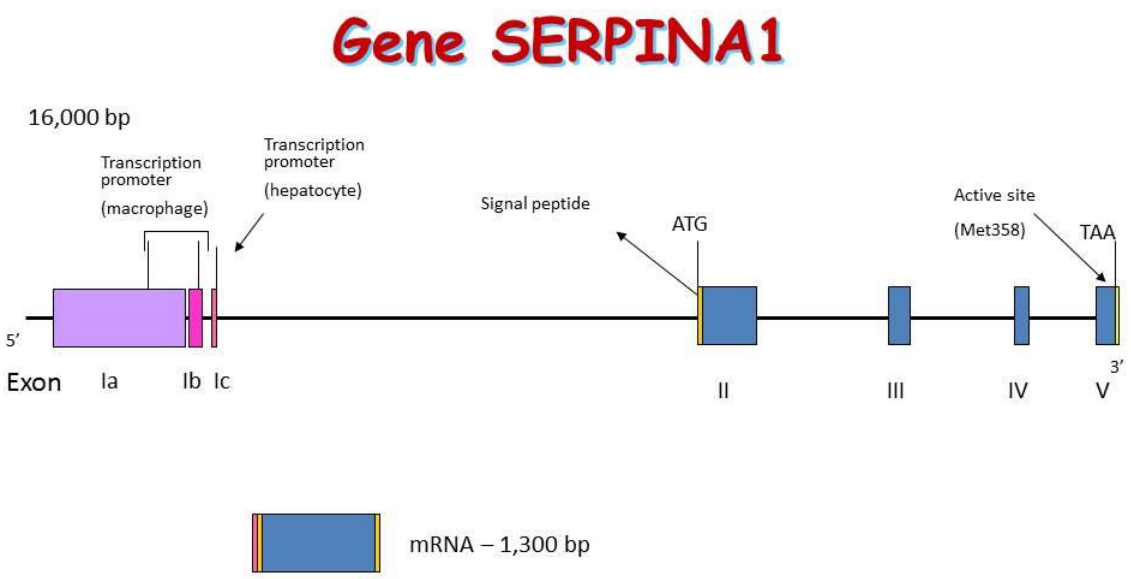


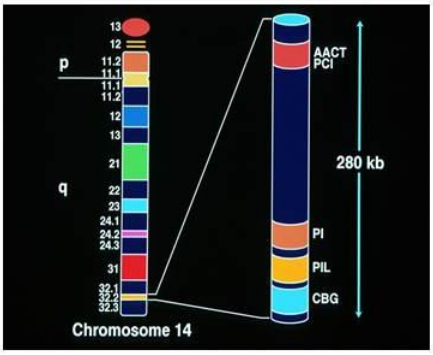
Figure 1. The natural history of α_1-antitrypsin deficiency and/or Figure showing lung and liver manifestations.

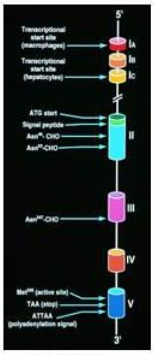
Please remove all text, and redraw as suggested by the reviewer as "one life timeline with different % along the life of an affected individual. This will summarize % of getting liver and pulmonary diseases along life, leaving a % asymptomatic (like all the % cited at the beginning of page 13)." See Fig. 1 in Huntington Disease Primer and/or Fig 3 in Menopause Disease Primer as examples.

Figure 2A: SERPINA1 gene/promoter structure and location of various mutations to be based on these diagrams

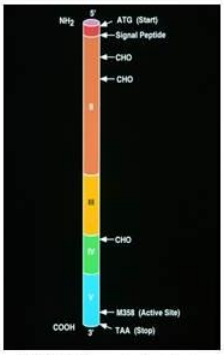




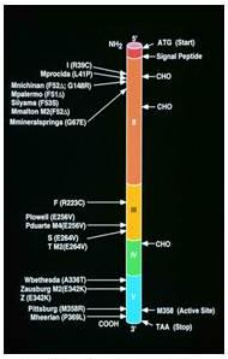




SERPINA1 Gene



SERPINA1 Gene Coding Region



Location of various mutations

1

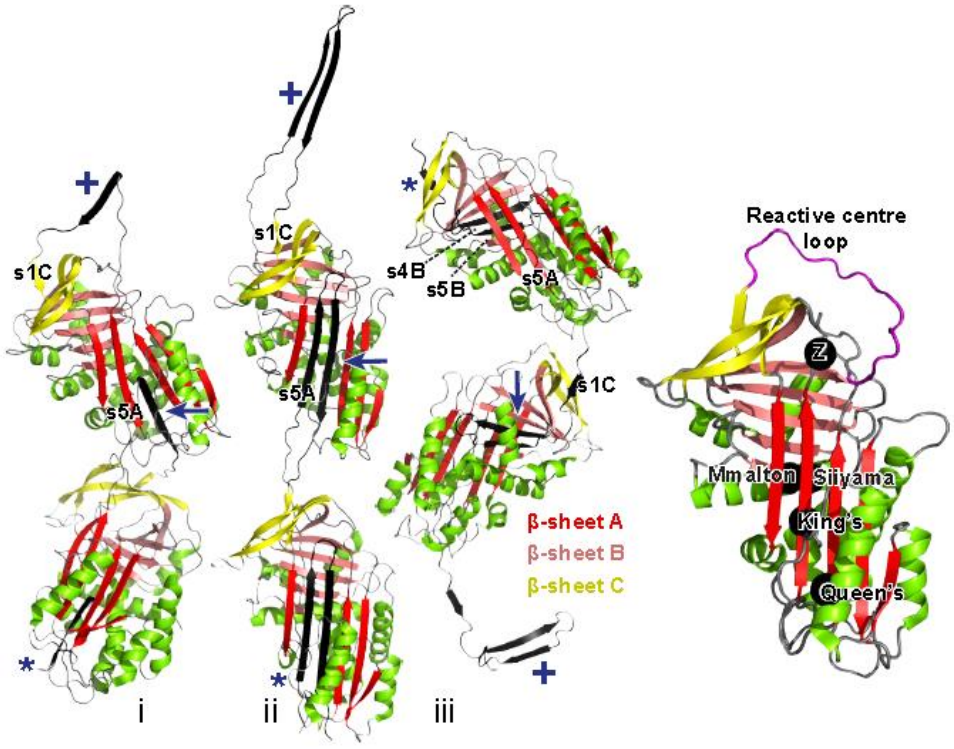


Figure 2B. Left. Proposed models of serpin polymerisation (key linkage motifs highlighted in black): i. Reactive centre loop- β-sheet A linkage, ii, linkage by a β-hairpin of the reactive centre loop and strand 5A and iii, linkage with strands 1C, 4B and 5B. Right. Structure of monomeric α_1-antitrypsin with the position of key mutations shown in black. "The intermolecular domain swap that forms the basis of the dimer is indicated by an arrow; '+' denotes the donor region, and '*' the acceptor region, that mediate interactions with adjacent subunits in the polymer chain Figures generated with PyMol by Dr James Irving, UCL, UK.

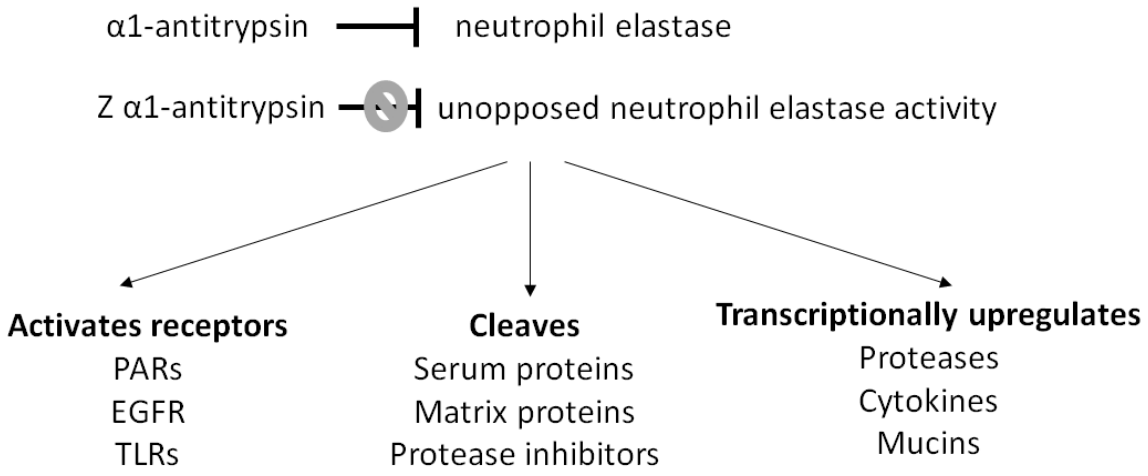


Figure 3. Intrapulmonary consequences of unopposed neutrophil elastase activity. Neutrophil elastase is normally inhibited by α1-antitrypsin. However, in the α1-antitrypsin deficient lung, unopposed elastase activity can activate cell surface receptors, cleave proteins and transcriptionally upregulates expression of classes of genes.

Please expand this figure to include…..

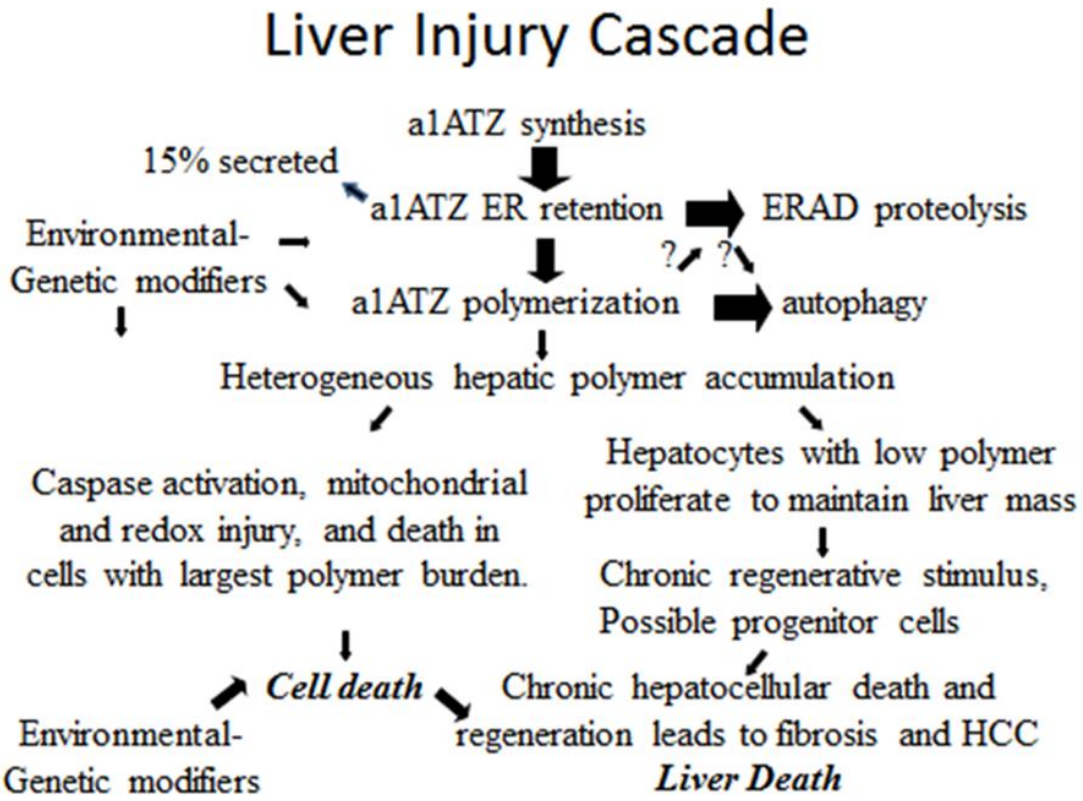


Figure 4. Liver injury cascade. The Z α_1-antitrypsin protein is synthesized and retained in the ER of hepatocytes rather than secreted. Most of the mutant proteins molecules are degraded by ERAD but some escape proteolysis, polymerise and form inclusions in the ER. Although autophagy is activated to degrade the polymers, some cells remain engorged with Z polymers. Cells with the most polymers undergo apoptosis and redox injury. Hepatocellular regeneration is stimulated but a chronic cycle of cell death and regeneration leads to fibrosis, HCC and end organ injury. These events are impacted upon by genetic and environmental modifiers.

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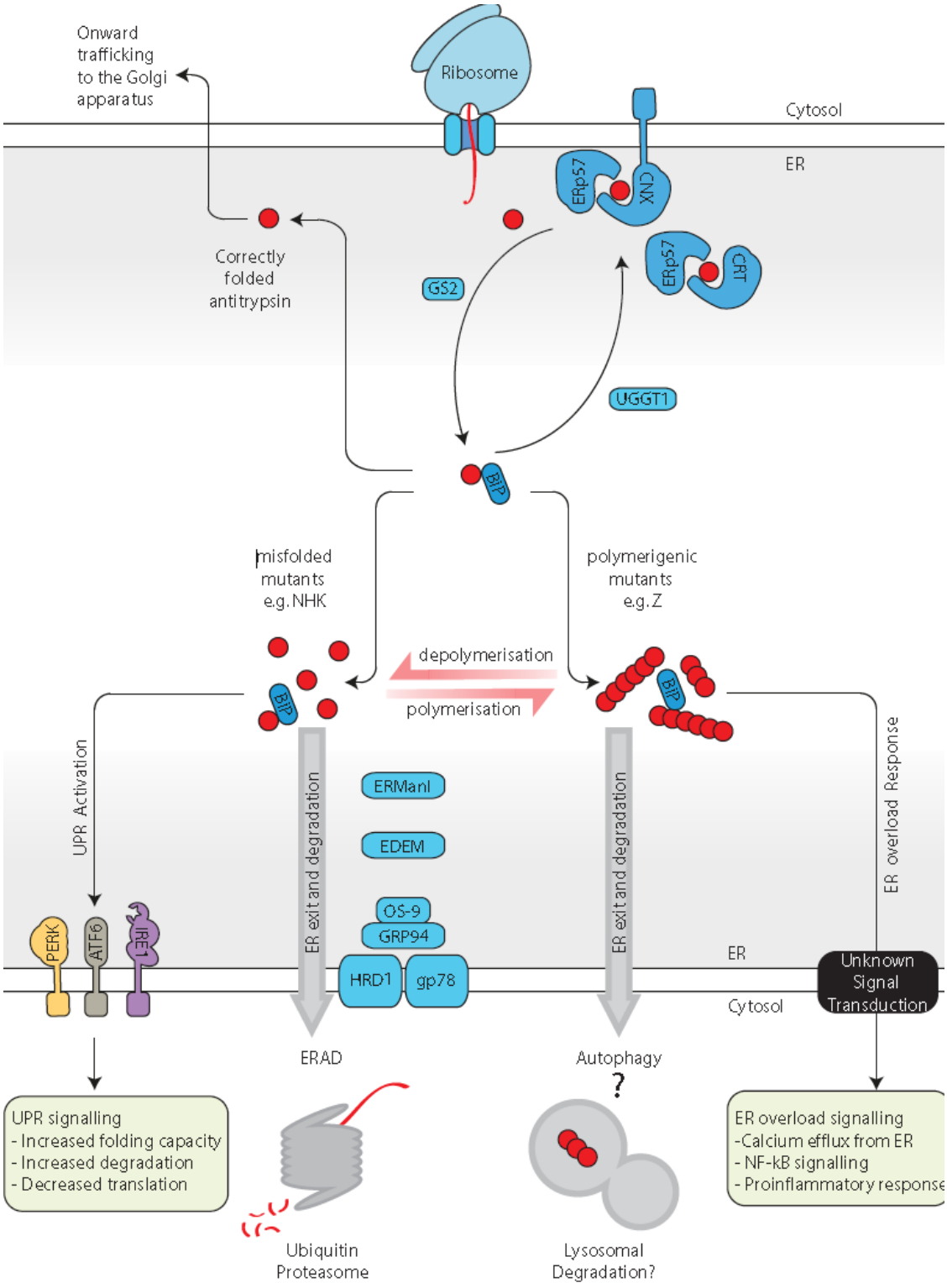

Figure 5. Fates of antitrypsin within the endoplasmic reticulum. The nascent α_1-antitrypsin protein is translated and enters into the endoplasmic reticulum (ER) where it is cotranslationally glycosylated. Exposed hydrophobic stretches are bound by the

HSP70 class chaperone BiP to prevent aggregation. Trimming of glucose residues of the N-linked glycan by glucosidases I (GS1) and GS2 regulate interaction with the lectin chaperones calnexin (CNX) and calreticulin (CRT), which lead to folding by ERp57[248]. Antitrypsin is thought to be maintained in a soluble monoglucosylated form by reglucosylation by UGGT1[249]. If correctly folding, the antitrypsin in packaged in to COPII coated vesicles for traffic to the Golgi apparatus. Misfolded antitrypsin (e.g. NHK) is eventually undergoes demannosylation by ER α-mannosidase I (ERManI) and exits the CNX cycle and interacts with EDEM. Further demannosylation eventually leads to interaction with the chaperones OS-9 and GRP94 and delivery to the HRD1 ubiquitin E3 ligase complex for ER associated degradation (ERAD)[116]. The E3 ligase gp78 has also been implicated in the degradation of antitrypsin. If misfolded antitrypsin (e.g. NHK) accumulates within the ER, it is thought to sequester BiP away from the ER stress sensors PERK, ATF6 and IRE1 leading to activation of the unfolded protein response (UPR). By contrast, if Z antitrypsin, which can also be degraded by ERAD, accumulates within the ER forms ordered polymers that appear inefficient at activating the UPR, perhaps owing to more limited interactions with BiP. The mechanism of this polymerisation remains controversial. The mechanisms by which polymers leads to activation of the ER overload response (EOR) are also poorly understood, but appear to require the release of calcium from the ER lumen. Under some circumstances, polymers within the ER can be degraded by autophagy.

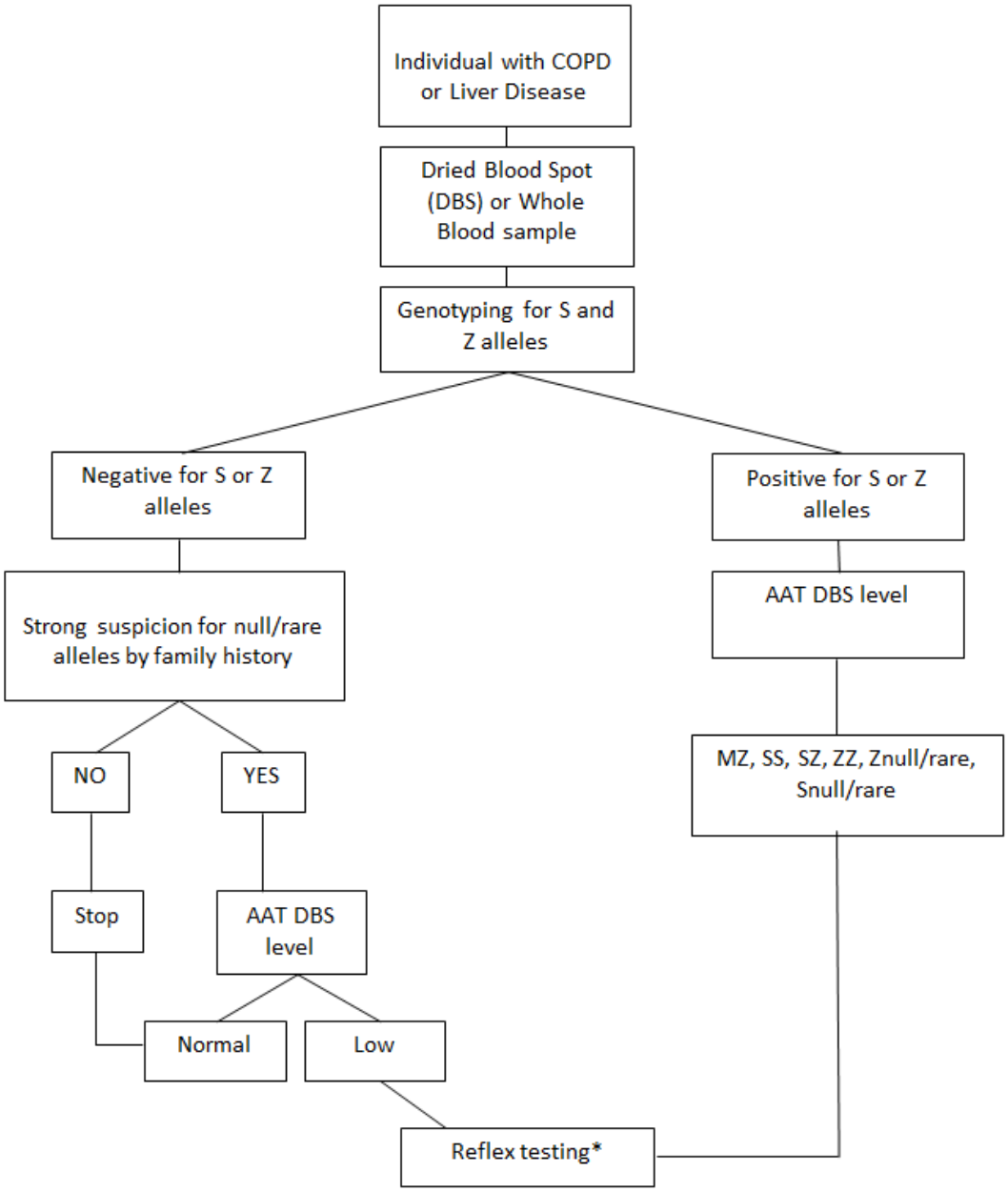

Figure 6. α_1-antitrypsin DNA Sequencing and/or PI typing testing algorithm.

(*)Protease Inhibitor Typing (by isoelectric focusing and/or DNA Sequencing).

Table 1. Prevalence of specific α_1-antitrypsin deficiency phenotypes in selected population screening studies (adapted from [4, 146])

Year	Location	Ref.	Subject Population	Number Screened	Prevalence of Selected α1-antitrypsin Genotyp			
					ZZ	SZ	MZ	SS
2011	Ireland	[168]	Electoral Register	1,100	0	0.18	4.18	0.1
2007	Poland	[250, 251]	Random sample	859	0	0	2.10	0.1
1972	Finland	[252]	College	664	0.15	-	5.12	-
1976	Sweden	[5]	Newborns	200,000	0.06	0.02	-	-
1979	Sweden	[253]	Military recruits	11,128	0.04	0.08	0.03	-
2002	Denmark	[254]	Random sample	9,187	0.07	0.11	4.90	0.1
1976	Netherlands	[255]	Population survey	1,474	0.07	0.07	2.24	0
1980	Netherlands	[256]	Newborns	95083	0.03	-	-	0.0
1988	Belgium	[257]	Newborns	10,329	0.06	0.12	0.97	0.0
1975	United Kingdom	[258]	Population survey	5,588	0.04	0.21	2.02	0.3
1973	New York	[259]	Population survey	500	0	0	3.6	0.2
1976	California	[260]	High school	1,841	0	0.27	1.85	0.0
1977	New York	[261]	Newborns	1,010	0	0	1.19	0.8
1977	Arizona	[262]	Population survey	2,944	0.07	0.20	3.0	
1978	Oregon	[263]	Newborns	107,038	0.02	0.01		-
1984	Minnesota	[264]	Blood donors	904	0	-	2.77	0.2
1989	Missouri	[6]	Blood donors	20,000	0.04	0.01	0.01	
1993	New York	[265]	Newborns	11,081	0.03	0.05	0.53	0.0
1978	Italy	[266]	Outpatients	202	0	0	1.98	0
2011	Italy	[267]	Town screening	817	0.12		5.6	0.1
1973	Spain	[268]	Population survey	576	-	-	1.04	-
2009	Madeira	[269]	Volunteers	200	0	1	4	3
2010	Cape Verde	[270]	Volunteers	202	0	0	0.5	1.4
1973	Zaire	[271]	Population survey	132	0	0	0	0
1977	Somalia	[272]	Newborns	347	-	0.03	0.0006	0.0
2011	Saudi Arabia	[273]	Volunteers	158	0	3.8	2.53	1.9
1977	Japan	[274]	Blood donors	856	0	0	0.23	0

Table 2. Results of targeted detection studies for α_1-antitrypsin deficiency. Adapted from[7, 168, 275].

Detection Strategy[ref]	Number of Patients	Prevalence of Specific AAT Phenotype (%, N)					
		PI*ZZ	**PI*SZ**	**PI*MZ**	**PI*SS**	**PI*MS**	**Other**
Targeted detection (Patients with COPD, emphysema, asthma, or bronchiectasis)[276]	1060 evaluable samples from 1156 (Germany)	0	0.2% (N = 3)	3.7 % (N = 39)	0.09 % (N = 1)	3.4 % (N = 36)	PI*M Null - 0.09 % (N =1)
Case-finding linked to an AATD awareness program[277]	2696 (Germany)	9.9% (N = 268)	2.0% (N = 53)	18.1% (N = 488)		3.6% (N = 97)	Rare phenotypes – 0.5% (N =13)
Case-finding (Patients with COPD)[278]	2137 (Spain)	0.37% (N = 8)	0.14% (N = 3)		0.14% (N =3)		
Case-finding (Emphysema without risk factors or of early-onset, spontaneous pneumothorax, cervical artery dissection, PAS positive bodies in liver, isolated transaminase elevation, ANCA positive, or low alpha-1 proteins on protein electrophoresis)[279]	285 specimens collected over 9 years (Italy)	12% (N = 26)	8% (N = 17)	62% (N = 131)		14% (N = 29)	PI*ZI 0.35 % (N = 1) PI*ZM$_{malton}$ – 0.35% (N = 1) PI*MM$_{malton}$ 2.1% (N = 6)
Case-finding (Targeted detection in COPD with education program and free testing)[280]	969 (Florida)	3.2% (N = 31)	0.4% (N = 4)	11% (N = 107)			
Case-finding (missing or reduced alpha-1 globulin band, early onset emphysema, familial cluster, first degree relative of subjects with ascertained AATD or MZ heterozygosity)[281]	1841 (Italy)	6.4% (118)	0.9% (17)				Null Null 0.4% (8) Z null 0.2% (4) Rare variants 0.2% (4)
Case-finding (individuals with abnormal PFTs)[282]	225	0		2.7% (N=6)		7.1% (N=16)	PIFF 0.4% (N=1)
Case-finding (Patients with advanced COPD admitted for carotid body surgery)[260]	965	1.9% (N= 18)	0.3% (N=3/965)	7.7% (N=74/965)	0.3% (N=3/965)	10.1 (N= 75/742)	
Case-finding[283]	29	0		1			
Case-finding (Physicians receiving results of pulmonary function tests showing fixed airflow obstruction were prompted in the electronic medical record to test for AATD)[8]	624 (baseline) vs. 979 (after implementing the electronic alert)	1/38 whose phenotype was checked after implementing the electronic alert	0	1/38	0	2/38	No difference in the rate of detecting AAT deficient patients (serum level < 100 mg/dl) before (8.9%) vs. after (5.3%) implementing the electronic alert
National targeted detection programme following ATS/ERS guidelines[168]	12,000 (Ireland)	1.83% (N=219)	1.38% (N=165)	13.81% (N=1657)	0.5% (N=60)	10.08% (N=1209)	Electronic red-flag on AAT <1.0g/l in 7 participating centres

Table 3. List of pathological mutations, other than Null, of SERPINA1 gene which cause α_1-antitrypsin deficiency. Mutation(s) column reports codon contig by fixing codon 1 as first translated codon after signal peptide. Mutations are named according to electrophoretic mobility and eponym, as reported in literature. Base allele and RefSNp (rs) numbers are reported if available. Minor allele frequencies (MAF) are inferred from consultation of http://www.ncbi.nlm.nih.gov/projects/SNP.

Mutation(s)	Name	Base allele	Rs	Intron/exon position	Minor allele frequency (MAF)	AAT protein	Ref
S -19TCG>L TTG	Zwrexham		Rs140814100	Exon 2, signal peptide	0.0001-0.0002	reduced	[27]
H 15CAC>D AAC	Ejohannesburg	M1(Val)	Rs138070585	Exon 2	0.0000-0.0001	reduced	[28]
D 19GAT> A GCT	Pyonago	M1(Val)		Exon 2	Single reports	reduced	[29]
R 39CGT>C TGC	I	M1(Val)	Rs28931570	Exon 2	0,001-0,0006	reduced	[30]
L 41CTG>P CCG	Mprocida	M1(Val)	Rs28931569	Exon 2	<0,00001	reduced	[31]
L 41CTG del8bp, ins22bp, del30bp> Ter70	Mvarallo			Exon 2	Single reports	absent	[32]
F 52TTC, del TT	Mpalermo	M1(Val)		Exon 2	Single reports	reduced	[33]
F 52TTC, del TTC	Mmalton	M2		Exon 2	Single reports	reduced	[34, 35]
F 52TTC, del TT and G 148GGG>R AGG	Mnichinan	M1(Val)		Exon 2	Single reports	reduced	[36]
F 53TTC>S TCC	Siiyama	M1(Val)	Rs55819880	Exon 2	Single reports	reduced	[37]
G 67GGG>E GAG	Mmineral spring	M1(Ala)	Rs28931568	Exon 2	Single reports	reduced	[38]
T 85AGG>M ATG	Zbristol		Rs199422213	Exon 2	0,0000-0,0002	Reduced , unglycosylated	[39]
G 148GGG> R AGG	V	M1(Ala)	Rs112030253	Exon 2	0,0006-0,001	Slightly reduced	[33]
K 154AAG> N	Queen's			Exon 2	Single reports	reduced	[40]
K 174AAG> E GAG	Flyon		Rs766034720	Exon 2	<0,00001	Slightly reduced	[41]
H 209CAC> N AAC	E	M4		Exon 3	Single report	reduced	[42]
V 210GTG> E GAG	M1pierre-benite		Rs746197812	Exon 3	<0,00001	reduced	[43]
R 223CGT> C TGT	F	M1(Val)	Rs28929470	Exon 3	0,001-0,003	Slightly reduced	[44, 63]
G 225GGC> RCGC	Pbrescia			Exon 3	Single report	reduced	[45]

N 256GAT> V GTT	Plowell/Pduarte	M1(Val)/M4	Rs121912714	EXON 3	0,0004-0,0006	reduced	[46-48]
N 256GAT> V GTT and P 391CCC> H CAC	Ybarcelona			Exon 3-Exon 5	Single report	reduced	[49]
K 259AAA> I ATA	Mpisa	M1(Val)		Exon 3	Single report	reduced	[50]
E 264GAA> V GTA	S	M1(Val)	Rs17580	Exon 3	0,019-0,03	Slightly reduced	[51]
E 264GAA> V GTA	T, Pnorth adams	M4		Exon 3	Single reports	Slightly reduced	[33, 42]
T 268ACC> I ATC	Nhartford city	M1(Val)	Rs28929470	Exon 3	<0,0001	reduced	[42, 55]
L 276CTG> P CCG	Nnagato	M2		Exon 3	Single report	reduced	[29]
S 330TCC> F TTc	Smunich	M1(Val)	Rs201788603	Exon 4	0,0002	Slightly reduced	[33]
g.16770, del26bp,insGG	Mwhitstable	M2		Intron 4	Single report	reduced	[52]
H 334CAT> N GAT	King			Exon 5	single report	reduced	[53]
K 335AAG> E GAG	Etokyo	M1(Val)	Rs200945035	Exon 5	0,0002-0,0006	reduced	[54]
A 336GCT> T ACT	Wbethesda	M1(Ala)	Rs1802959	Exon 5	<0,0001	reduced	[46]
N 341GAC> HCAC	Zlittle rock	S		Exon 5	Single report	reduced	[42]
E 342GAG> K AAG	Z	M1(Ala)	Rs28929474	Exon 5	0,004-0,012	reduced	[56]
E 342GAG> K AAG	Zaugsburg	M2	Rs28929474	Exon 5	Single report	reduced	[57, 58]
M 358ATG> R AGG	Pittsburg		Rs121912713	Exon 5	Single reports	dysfunctional	[59]
P 362CCC> H CAC	Psäo tomè			Exon 5	Single report	reduced	[60]
E 363GAG> K AAG	Xchristchurch		Rs121912712	Exon 5	0,0018	Slightly reduced	[61]
K 368AAA> E GAA	Etaurisano	M2		Exon 5	Single report	reduced	[50]
P 369CCC> S TCC	Mwurzburg	M1(Val)	Rs61761869	Exon 5	0,0002-0,0003	reduced	[62]
P 369CCC> L CTC	Mheerlen	M1(Ala)	Rs199422209	Exon 5	0,0000-0,0001	reduced	[62]
P 391CCC> H CAC	Yorzinuovi	M1(Val)		Exon 5	Single report	reduced	[50]

1
2

Table 4. α_1-antitrypsin augmentation therapy observational studies and clinical trials

Design	Reference	Year	Main outcome measures	Outline	Ref.
Randomised	Chapman (RAPID Study)	2015	Slower rate of lung tissue loss on CT	177 subjects 2-4 yr follow up	188
Randomised	Dirksen EXACTLE Study)	2009	Trend towards slower rate of lung tissue loss on CT	77 subjects 2-2.5 yr follow up	186
	Dirksen	1999	Trend towards slower rate of lung tissue loss on CT	56 subjects 3yr follow up	185
Observational	Seersholm	1997	Reduction in FEV_1 decline in cohort with FEV_1 31-65%	295 subjects >1 yr follow up	184
Observational	NHLBI Registry	1998	Reduction in FEV_1 decline in cohort with FEV_1 35-49%	1,129 subjects c.7.2 yrs	170
Observational	Lieberman	2000	Reductions in exacerbations	96 subjects 1-10 yrs	181
Observational	Wencker	2001	Slower rate of FEV_1 decline	96 subjects >12 months	182
Observational	Tonelli	2009	Slower rate of FEV_1 decline	164 subjects 41.7 months	183

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