REVIEW ARTICLE

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Alpha$_1$-Antitrypsin Deficiency

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Alpha$_1$-antitrypsin (AAT) deficiency is one of the most common genetic diseases. Most persons carry two copies of the wild-type M allele of *SERPINA1*, which encodes AAT, and have normal circulating levels of the protein. Ninety-five percent of severe cases of AAT deficiency result from the homozygous substitution of a single amino acid, Glu342Lys (the Z allele), which is present in 1 in 25 persons of European descent (1 in 2000 persons of European descent are homozygotes). Mild AAT deficiency typically results from a different amino acid replacement, Glu264Val (the S allele), which is found in 1 in 4 persons in the Iberian peninsula. However, many other alleles have been described that have variable effects, such as a lack of protein production (null alleles), production of misfolded protein, or no effect on the level or function of circulating AAT (Table 1).

AAT is synthesized in the liver and secreted into the circulation, where its primary role is to protect lung tissue against attack by the enzyme neutrophil elastase. Point mutations can lead to retention of AAT in the liver, causing liver disease through a toxic "gain of function," whereas the lack of an important circulating proteinase inhibitor predisposes homozygotes with severe deficiency to early-onset emphysema ("loss of function"). The high frequency of genetic variants suggests that AAT mutations confer a selective advantage, perhaps by amplifying the inflammatory response to invasive respiratory and gastrointestinal infection.[2] We review the current understanding of the pathophysiology of AAT deficiency and discuss how this knowledge has led to new therapeutic strategies.

Pathophysiology and Clinical Features of Liver Disease

AAT is synthesized within hepatocytes, intestinal and pulmonary alveolar cells, neutrophils, macrophages, and the cornea. The liver produces approximately 34 mg of AAT per kilogram of body weight per day, leading to a plasma level of 0.9 to 1.75 mg per milliliter, with a half-life of 3 to 5 days. AAT levels can rise by 100% in persons with a normal proteinase inhibitor (PI) genotype (MM) during the acute-phase response, but the increase is markedly attenuated in persons with severe deficiency alleles. AAT is synthesized within the endoplasmic reticulum and secreted through the Golgi apparatus. Severe deficiency alleles — such as the common Z (Glu342Lys) allele and the rare S$_{iiyama}$ (Ser53Phe), M$_{malton}$ (ΔPhe52), and King's (His334Asp) alleles — do not affect synthesis but cause approximately 70% of the mutant AAT to be degraded in the endoplasmic reticulum within hepatocytes,[3] 15% to be secreted, and 15% to form ordered polymers.[4,5] These polymers are partially degraded by autophagy[6,7] and a small amount is secreted,[8,9] but a proportion persists within the endoplasmic reticulum as inclusions that are positive on periodic acid–Schiff staining and resistant to diastase; such inclusions are the

Table 1. Key Alleles Associated with Alpha$_1$-Antitrypsin (AAT) Deficiency.*

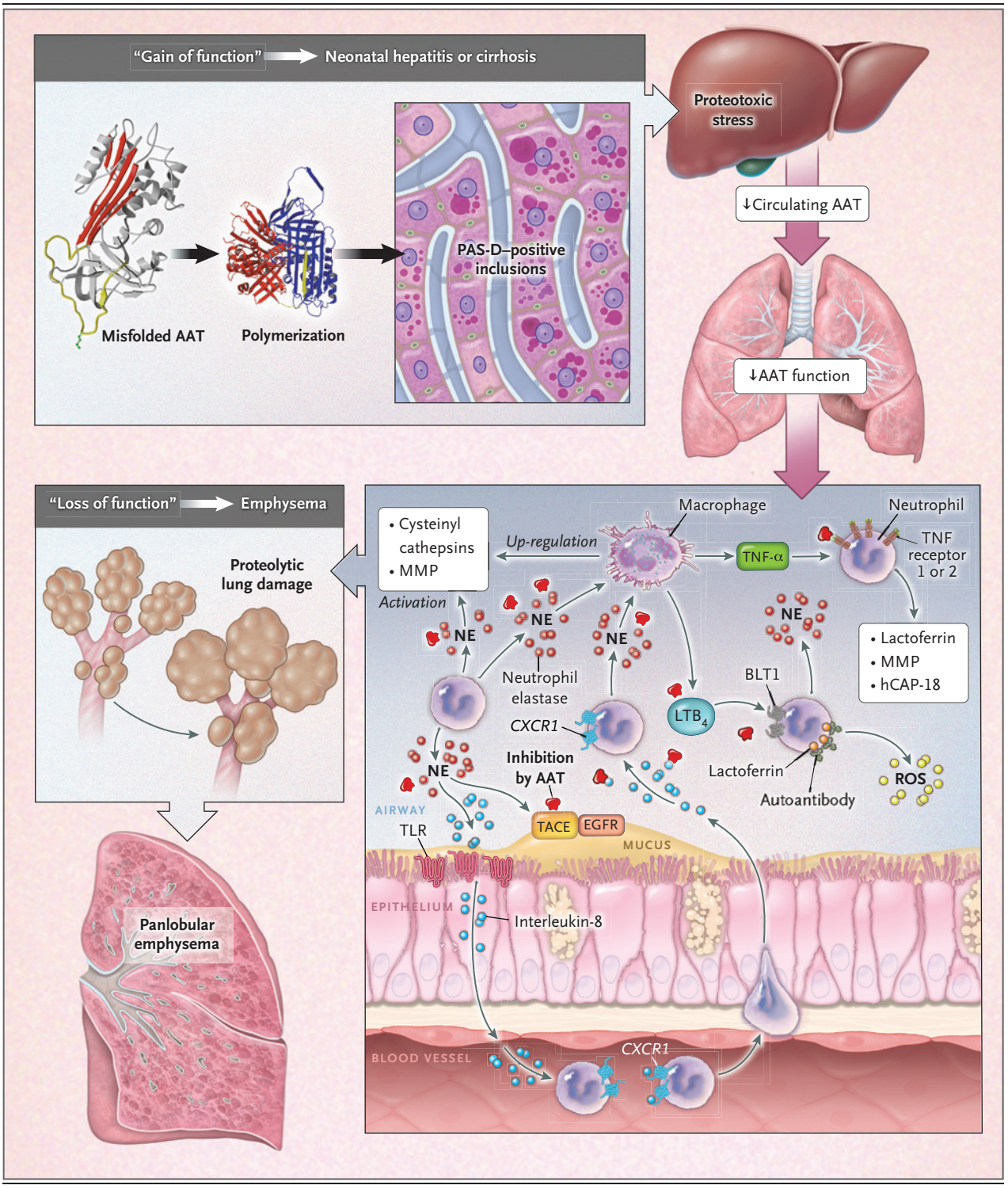
Variant†	Mutation and rs Number‡	Molecular Basis of Disease	Clinical Features	Epidemiology
Deficiency alleles				
F	Arg223Cys	Reduced association rate constant with neutrophil elastase; slow formation of polymers	Coinheritance with the Z deficiency allele may confer a predisposition to emphysema	Case report
I	Arg39Cys rs28931570	Protein misfolding; can form heteropolymers; reduced serum protein	No clear disease association	Disease reported only in compound heterozygotes
Iners	Gly349Arg	Type II mutant (i.e., normal secretion but functionally deficient)	Not known	Case report
King's	Hisp334Asp	Rapid polymerization in hepatocyte endoplasmic reticulum; delayed secretion	Neonatal jaundice; presumed high risk of emphysema in homozygote or compound Z heterozygote	Case report
M$_{malton}$	Δ52Phe (M2 variant) rs775982338	Intracellular degradation and polymerization; low serum level	Well-established association with liver disease and emphysema in homozygotes	Most common rare deficiency allele in Sardinia; seen sporadically in the United Kingdom and Canada
M$_{mineral\ springs}$	Gly67Glu rs28931568	Abnormal posttranslational biosynthesis but no polymerization; low serum level	Emphysema in homozygotes	Unusual; described in an Afro-Caribbean person in the United States
M$_{procida}$	Leu41Pro rs28931569	Unstable protein structure leading to intracellular degradation; reduced catalytic activity of circulating protein	High risk of emphysema in homozygotes	Case report
Pittsburgh	Met358Arg rs121912713	Function altered to an antithrombin	Fatal bleeding disorder	Case report
Queen's	Lys154Asn	Polymer formation	Compound heterozygote with Z	Case report
S	Glu264Val rs17580	Protein misfolding and reduced secretion; can form heteropolymers with Z AAT	Emphysema seen in SZ heterozygotes but less severe than in ZZ; cirrhosis reported in SZ heterozygotes	Most common deficiency variant; carrier frequency: 1 in 5 persons in southern Europe, 1 in 30 in the United States, 1 in 23 among persons of European descent in Australia, 1 in 26 among those of European descent in New Zealand; rare or nonexistent in Asia, Africa, and Aboriginal Australians
S$_{iiyama}$	Ser53Phe rs55819880	Intracellular degradation and polymerization; low serum level	Liver disease and emphysema in homozygotes	Rare, but most common deficiency allele in Japan

Trento	Glu75Val	Nonclassical polymer formation	Emphysema in compound heterozygote with Z	Case report
Z	Glu342Lys rs28929474	Intracellular degradation and polymerization; low serum level	In homozygotes, well-established association with liver disease and emphysema; MZ heterozygotes may be more susceptible to airflow obstruction and chronic liver disease	Most common severe deficiency variant; carrier frequency: 1 in 27 persons in northern Europe, 1 in 83 in the United States, 1 in 75 persons of European descent in Australia, 1 in 46 persons of European descent in New Zealand; not seen in China, Japan, Korea, Malaysia, or northern and western Africa
Null (QO) alleles				
$QO_{bellingham}$	Lys217 stop codon rs199422211	No detectable AAT mRNA	High risk of emphysema in homozygotes and compound heterozygotes	Case report
QO_{bolton}	Δ1bpPro362, causing stop codon at 373	Truncated protein; intracellular degradation and no secreted protein	High risk of emphysema in homozygotes and compound heterozygotes	Case report
$QO_{granitefalls}$	Δ1bpTyr160, causing stop codon rs267606950	No detectable AAT mRNA	Severe emphysema reported in Z compound heterozygote	Case report
$QO_{hongkong}$	Δ2bpLeu318, causing stop codon at 334 rs1057519610	Truncated protein; intracellular aggregation (no polymerization), degradation, and no secreted protein	High risk of emphysema in homozygotes and compound heterozygotes	Case reports (persons of Chinese descent)

* The information is from Lomas et al.[1] AAT deficiency alleles with different functional effects are listed. The delta symbol denotes deletion, and mRNA messenger RNA.

† The single or initial letter in each name reflects the position of migration of the variant on isoelectric-focusing gels (e.g., A indicates the fastest migration, M moderate migration, and Z the slowest migration); the rest of the name represents the site of the first description (in the case of Iners, the site is unknown). Deficiency results from a range of effects on the protein: rapid polymerization of the Z, S_{iiyama}, M_{malton}, and King's variants; slower polymer formation of S, I, and Queen's AAT; a change in the inhibitory activity of Pittsburgh AAT; secretion of an inactive protein, Iners AAT; and some of the many null variants that result in no secreted protein.

‡ The rs number is the reference SNP cluster identification number.



histologic hallmark of the disease (Fig. 1). Milder deficiency alleles, such as the S (Glu-264Val), I (Arg39Cys), and Queen's (Lys154Asn) alleles, also form polymers of AAT but at a much slower rate, in keeping with only a mild plasma deficiency[1] (Table 1). The rate at which most AAT mutants form polymers correlates directly with alterations in the protein structure, as indicated by altered thermal stability.[10]

The key longitudinal, population-based co-

Figure 1 (facing page). Pathophysiology of Alpha$_1$-Antitrypsin (AAT) Deficiency.

Misfolded AAT forms ordered polymers that accumulate as hepatocyte inclusions, which are positive on periodic acid–Schiff staining with diastase digestion (PAS-D). This misfolded protein causes proteotoxic stress and a gain-of-function liver disease. A deficiency of AAT results in an excess of neutrophil elastase (NE), which in turn induces mucin production and secretion and increases expression of other proteases and inflammatory cytokines. AAT is not just an antiprotease but also a potent antiinflammatory agent, regulating neutrophil chemotaxis, degranulation, autoimmunity, and apoptosis through interactions with interleukin-8, leukotriene B_4 (LTB_4), and tumor necrosis factor α (TNF-α). AAT is inactivated by oxidation, proteolytic cleavage, and polymerization. Thus, with a deficiency of AAT, neutrophils are increased and protease activity is unopposed. Structural damage and susceptibility to infection occur, leading to tissue damage and emphysema. BLT1 denotes LTB_4 receptor 1, CXCR1 C-X-C motif chemokine receptor 1, EGFR epidermal growth factor receptor, hCAP-18 human cathelicidin antimicrobial peptide 18, MMP matrix metalloproteinase, ROS reactive oxygen species, TACE TNF-α converting enzyme, and TLR toll-like receptor.

hort study of AAT deficiency followed 127 persons with the PI ZZ genotype and 54 with the PI SZ genotype from birth to 45 years of age.[11,12] A total of 73% of infants with the PI ZZ genotype for AAT deficiency had an elevated serum alanine aminotransferase level in the first 12 months of life, but the level was abnormal in only 15% by 12 years of age. The serum bilirubin level was elevated in 11% of such infants in the first few months of life but was normal by 6 months of age. Cholestatic jaundice developed in 10% of infants with the PI ZZ genotype, and clinical features of liver disease without jaundice developed in 6%. The clinical symptoms usually resolved by the second year of life, but 15% of persons with cholestatic jaundice had progression to juvenile cirrhosis. The risk of death from liver disease among children with the PI ZZ genotype was 2 to 3%, but none of the survivors had clinical symptoms of liver disease at 12 years of age.[13] Persons in the cohort study who had the PI ZZ genotype and had a history of tobacco smoking had hyperinflation and emphysema at 37 to 39 years of age, whereas age-matched adults with the PI MM genotype who had never smoked did not have evidence of hyperinflation and emphysema.[14]

A cross-sectional biopsy study showed clinically significant liver fibrosis in 35% of adults with PI ZZ AAT deficiency, and a large European analysis that relied on a noninvasive assessment showed clinically significant liver fibrosis in 20 to 36% of such persons.[15,16] Risk factors for advanced fibrosis include male sex, the metabolic syndrome, and obesity. The exact effect of alcohol consumption in persons with the PI ZZ genotype remains to be determined, but the available evidence suggests that it promotes disease progression. Handling of the Z variant of AAT is delayed in hepatocytes derived from induced pluripotent stem cells from persons who have liver disease associated with AAT deficiency.[17] Whether this finding, along with DNA methylation marks,[18] can be used to identify persons at risk for the development of liver disease is not known.

PATHOPHYSIOLOGY AND CLINICAL FEATURES OF LUNG DISEASE

AAT was first described as a serine protease inhibitor, and it is the loss of this activity in the lung that has informed our understanding of the pathophysiology of the associated lung disease (Fig. 1). AAT inhibits neutrophil elastase, which, when unopposed, can cleave many of the structural proteins of the lung as well as innate immune proteins. Airspace enlargement ensues when elastase is instilled into the lung in animal models, and neutrophil elastase–knockout mice are protected from emphysema that is induced by exposure to cigarette smoke.[19] In the classic loss-of-function concept, the Z form of AAT fails to reach the lung in sufficient quantities and takes longer to inhibit neutrophil elastase.[20] Oxidants in cigarette smoke can further incapacitate Z AAT by oxidizing its active-site methionines and increasing polymerization,[21] both of which render it unable to inhibit neutrophil elastase. This illustrates the pivotal role of smoking in AAT deficiency, which contributes to earlier and more severe lung disease.

Other lung proteases, such as proteinase-3, metalloproteases, cysteine proteases, and bacterial proteases, have proteolytic activity that is similar to the activity of neutrophil elastase and may also play a role.[22] Some, such as cysteinyl proteases and metalloproteases, are induced and activated by neutrophil elastase, and inhibition of neutrophil elastase decreases their activity in vivo. AAT also binds a variety of proteins and fatty acids,[23] and loss or moderation of these

functions as a result of AAT deficiency may increase inflammation. In persons with AAT deficiency, AAT is inactivated in the lung not just by oxidation but also by proteolytic cleavage and polymerization.[24] Moreover, polymerized Z AAT has a distinct proinflammatory effect, acting as a potent neutrophil chemoattractant.[24] Thus, in persons with AAT deficiency, the neutrophil number is increased, protease activity is unopposed, and susceptibility to infection and structural damage develop (Fig. 1).

The clinical manifestations of lung disease associated with AAT deficiency are mainly indistinguishable from those of nonhereditary emphysema. This is partly why severe AAT deficiency remains undiagnosed in approximately 90% of cases, with an interval of 5 to 7 years from the onset of symptoms to diagnosis. The classic clinical description of lung disease associated with AAT deficiency is of early-onset obstructive lung disease in persons with moderate cigarette consumption and panacinar emphysema affecting mainly the lower lobes. Rigid adherence to these indicators to prompt testing has led to underdiagnosis, late diagnosis, and misdiagnosis of AAT deficiency. Up to 37% of people with severe AAT deficiency have predominantly upper-lobe involvement,[25] with bronchiectasis as a common radiologic manifestation. Even when this diagnostic algorithm is used for young people with chronic obstructive pulmonary disease (COPD), AAT deficiency is often diagnosed late, at a point when the lung disease has become irreversible.[26,27] In the National Heart, Lung, and Blood Institute registry for severe AAT deficiency, the average age at diagnosis was 46 years, but the mean forced expiratory volume in 1 second (FEV_1) and diffusing capacity of the lung for carbon monoxide (D_{LCO}) were 47% and 50% of the predicted value, respectively.[28] Early diagnosis is important in helping people make lifestyle changes, reducing occupational risk, and providing access to new therapies, whereas a delayed diagnosis is associated with poor functional status and COPD-related outcomes.[29]

AAT DEFICIENCY AS A SYSTEMIC DISORDER

Although lung disease and liver disease are the most prominent disorders associated with AAT deficiency, several other conditions have been reported in persons with the PI ZZ genotype. Among them, neutrophilic panniculitis is characterized by painful subcutaneous nodules and the presence of neutrophil infiltrates in the subcutis; it occurs in less than 1% of persons with the PI ZZ genotype. Disorders that are overrepresented in persons with AAT deficiency include antineutrophil cytoplasmic antibody (ANCA)–associated vasculitis, chronic kidney disease, diabetes,[30] and metabolic alterations, with decreased levels of serum triglycerides and very-low-density lipoproteins.[16]

RISK OF DISEASE AMONG HETEROZYGOTES

The PI MZ genotype (i.e., heterozygous Z carriage) is a common form of AAT deficiency, occurring in up to 4% of the population with European ancestry.[31] These persons are susceptible to multiple disorders (Table 2), partly due to lower levels of circulating AAT but also because the secreted Z AAT is less effective at inhibiting neutrophil elastase.

Although population-based studies have failed to show an association between PI MZ status and COPD or have shown only a weak association with emphysema,[34] studies enriched for persons at risk have shown that PI MZ carriers have a clear predisposition to COPD, at least among smokers.[35,36] These data suggest that PI MZ carriers have a low absolute risk for the development of a clinically relevant lung disease but that the risk increases significantly with additional genetic and environmental factors. This risk pattern is similar to that described for pediatric liver disease, which is rarely seen in PI MZ carriers without coexisting conditions.[40,41] The absolute risk of liver disease among adult PI MZ carriers is unknown, but the risk is increased by the presence of additional coexisting conditions, such as nonalcoholic fatty liver disease, alcohol misuse, and cystic fibrosis.[37-39] Indeed, in a recent genomewide association study, the Z allele was associated with the highest odds ratio for the development of cirrhosis associated with alcoholic or nonalcoholic liver disease.[38]

PI MZ carriers have an increased incidence of gallstone disease[34] and an increased susceptibility to immune disorders such as ANCA-associated vasculitis.[32] Particularly high odds were reported for disorders associated with myeloperoxidase-

Table 2. Overview of Clinical Conditions Associated with AAT Deficiency.*

Disease	Odds Ratio (95% CI)		Study
	PI MZ	PI ZZ	
ANCA-associated vasculitis	2.9 (2.2–3.9)†	ND	Merkel et al.,[32] Rahmattulla et al.[33]
Gallstone disease	1.3 (1.3–1.4)	1.3 (0.7–2.5)	Ferkingstad et al.[34]
Emphysema (population-based studies)	1.4 (1.2–1.7)	28 (18–44)	Ferkingstad et al.[34]
COPD (population-based studies)‡	1–3	4.8 (3.0–7.9)	Ferkingstad et al.,[34] Foreman et al.[35]
COPD (case–control studies)‡	3–10	ND	Molloy et al.[36]
CFLD	5.0 (2.9–8.8)	ND	Bartlett et al.[37]
NAFLD cirrhosis	3–7	ND	Abul-Husn et al.[38]
Alcoholic liver cirrhosis	3.4–6	ND	Strnad et al.[39]
Advanced liver fibrosis (general population)	ND	9–20	Hamesch et al.[16]

* ANCA denotes antineutrophil cytoplasmic antibody, CFLD cystic fibrosis–associated liver disease with portal hypertension, CI confidence interval, COPD chronic obstructive pulmonary disease, NAFLD nonalcoholic fatty liver disease, ND not determined, PI MZ proteinase inhibitor genotype MZ, and PI ZZ proteinase inhibitor genotype ZZ.
† Higher odds ratios have been reported for vasculitis associated with proteinase 3–reactive ANCA with cytoplasmic staining (c-ANCA) and vasculitis associated with myeloperoxidase-reactive ANCA with perinuclear staining (p-ANCA).
‡ Higher odds ratios were reported for current and former smokers.

reactive ANCA with perinuclear staining (p-ANCA) and those associated with proteinase 3–reactive ANCA with cytoplasmic staining (c-ANCA). ANCA-associated vasculitis can occur with other AAT variants such as the S allele[33] and may represent a loss-of-function phenotype, since proteinase 3 is a target protease for AAT. Although the mechanisms leading to lung or liver disease in PI MZ carriers are probably analogous to those identified in PI ZZ carriers, the factors leading to the increased incidence of gallstones in PI MZ carriers remain to be determined. The compound heterozygous PI SZ genotype is more common than PI ZZ and is characterized by AAT serum levels that are intermediate between those associated with the PI MZ and PI ZZ genotypes. In keeping with this observation, lung disease is less likely to develop in PI SZ carriers than in PI ZZ homozygotes,[42] but as with MZ carriers, smoking significantly increases the risk of COPD. Children who are PI SZ carriers rarely have clinically relevant liver disease.[40,41] Liver disease in adult PI SZ heterozygotes has been reported in small studies and remains to be systematically assessed.

DIAGNOSIS

LUNG DISEASE

AAT deficiency is underdiagnosed, and it is important not merely to consider the condition but also to test specific patient groups for AAT deficiency. All persons with COPD, liver disease, poorly responsive asthma, c-ANCA vasculitis (in >90% of cases, the antibody is specific for proteinase 3), panniculitis, or bronchiectasis, in addition to first-degree relatives of people with AAT deficiency, should be tested.[26,27]

The first step in testing is measurement of the AAT level in serum. This measurement should be accompanied by an assessment of C-reactive protein, since AAT is an acute-phase reactant that increases during infection or inflammation. A serum level higher than or equal to 1.1 g per liter in the presence of a normal C-reactive protein level can be taken as evidence of normal AAT status.[43] If the serum AAT level is less than 1.1 g per liter, or if there is a strong clinical concern, then the clinician should request either phenotyping or genotyping in a specialist laboratory. In inconclusive cases, gene sequencing should be performed. Patients should be referred to a center specializing in AAT deficiency for follow-up.[27] Some guidelines suggest simultaneous testing of AAT levels and genotyping.[44]

Smoking cessation is central for all forms of AAT deficiency. People with severe AAT deficiency (the PI ZZ, ZNull, or NullNull genotype) should be monitored with spirometry, DLco, the 6-minute walk test, and health-related quality-of-life questionnaires.[27] The frequency of moni-

toring depends on the degree of impairment, but monitoring every 6 months for the first few years is helpful for establishing a baseline and identifying signs of rapid decline, with a change to once-a-year evaluations thereafter. The role of computed tomographic (CT) assessment of lung density in monitoring remains uncertain because of a lack of correlation with other surrogate markers that are used more routinely in clinical trials, such as lung function or quality of life.[45]

Follow-up for patients with the PI MZ genotype is more contentious. MZ and SZ heterozygotes who do not smoke have no increased risk of lung disease, but those who do smoke have a significantly increased risk, as compared with relatives who are PI MM carriers.[34,36] Patients with established COPD should be followed according to standard protocols, but it is unclear whether they require closer follow-up, since it is uncertain whether the decline in lung function is accelerated after smoking cessation, as compared with lung function in PI MM carriers with COPD. More rare AAT variants, such as the F (Arg223Cys), I (Arg39Cys), and M_{malton} (Δ52Phe) alleles, are reported to be associated with increased susceptibility to COPD only when inherited with a Z or null allele. Null homozygotes lack circulating AAT and have more severe lung disease than PI ZZ or PI SZ carriers but do not have an increased risk of liver disease.[27] Even with definitive identification of an AAT deficiency genotype, substantial variation is noted in the phenotypic presentation of the disease. The most important differentiator is cigarette smoking, but occupational exposures, such as exposure to kerosene or dust, can also play a role in the phenotypic presentation.[46] A number of polymorphisms have been identified in lung and liver disease associated with AAT deficiency,[47,48] but their importance has yet to be fully defined.

LIVER DISEASE

All PI ZZ carriers should be monitored for liver disease at a center that specializes in AAT deficiency. Given its invasive nature, liver biopsy is not acceptable for follow-up of asymptomatic patients. Transient elastography is useful to rule out advanced fibrosis (stage F3 or F4) but is less effective at lower levels of fibrosis, for which it performs similarly to the aspartate aminotransferase (AST)–to-platelet ratio index (APRI), calculated as (AST ÷ the upper limit of the normal range × 100) ÷ the platelet count, and the Fibrosis-4 (FIB-4) score, calculated as the patient's years of age × AST × $ALT^{-0.5}$ ÷ the platelet count (in which ALT denotes alanine aminotransferase).[15] For both equations, platelet count is measured at 10^9 per liter, and aminotransferase in units per liter. The γ-glutamyltransferase level performs better as a noninvasive marker than the APRI, the FIB-4 score, or aminotransferase measurements. The use of these biomarkers in liver disease has been reviewed recently,[49] but cutoff values remain to be defined for liver disease associated with AAT deficiency. Yearly liver ultrasound screening has been proposed, with scans every 6 months to screen for hepatocellular carcinoma in patients with cirrhosis, portal hypertension, or persistently abnormal liver-function tests.

TREATMENT

Treatment for the lung disease associated with AAT deficiency is the same as treatment for COPD. The only licensed disease-specific therapy for AAT deficiency is intravenous augmentation therapy with plasma-purified AAT. This therapy was approved by the Food and Drug Administration in 1987 for lung disease associated with AAT deficiency on the basis of biochemical efficacy and pharmacokinetics, without proof of clinical efficacy. Randomized, controlled trials have focused on decreased loss of lung density as the primary efficacy outcome. The largest of these studies strongly suggested a decreased loss of lung density with augmentation therapy in people with AAT deficiency but no effects on other measures, such as FEV_1, quality of life, or exacerbation of COPD.[45,50]

The liver disease associated with the accumulation of mutant AAT within hepatocytes is exacerbated by secondary factors that also affect hepatic function, such as fat and alcohol. It is recommended that persons with AAT deficiency and normal liver function maintain a normal body-mass index and consume alcohol within recommended limits. Persons with advanced liver disease associated with AAT deficiency should abstain from alcohol. No therapy is currently approved for liver disease associated with AAT deficiency other than transplantation for persons with advanced disease.

An understanding of the molecular and structural basis for the disease has underpinned new

approaches that may come to fruition in the next 5 to 10 years (Fig. 2). Some of these approaches are at the preclinical stage of investigation, and others are in early-phase clinical trials. The underlying genetic defect may be corrected by means of CRISPR (clustered regularly interspaced short palindromic repeats), stem-cell technology,[52] or replacement hepatocytes,[53] and the production of mutant protein may be "switched off" by means of gene silencing.[54] The polymerization intermediates may be stabilized with small molecules[55] or intrabodies (antibodies expressed inside a cell to alter its function),[56] intracellular polymers may be cleared by stimulating autophagy with the use of sirolimus and carbamazepine,[57,58] and secretion may be enhanced by manipulating proteostasis networks.[59] Attempts to read through premature stop codons of null variants have so far been unsuccessful.[60] All these approaches require detailed evaluation before they can be introduced into clinical practice.

FUTURE DIRECTIONS FOR RESEARCH

The antiinflammatory and tissue-protective properties of AAT are underscored by several studies suggesting its usefulness in transplantation medicine (Fig. 3). AAT can ameliorate experimentally induced kidney and lung ischemia–reperfusion injury[61,62] and augment the function of murine and porcine lung transplants.[63] Treatment with AAT has been shown to have an antiinflammatory effect on insulin-sensitive tissues and was beneficial in experimental islet-transplantation models, reducing islet-cell loss and inducing immune tolerance.[64] These data prompted phase 1

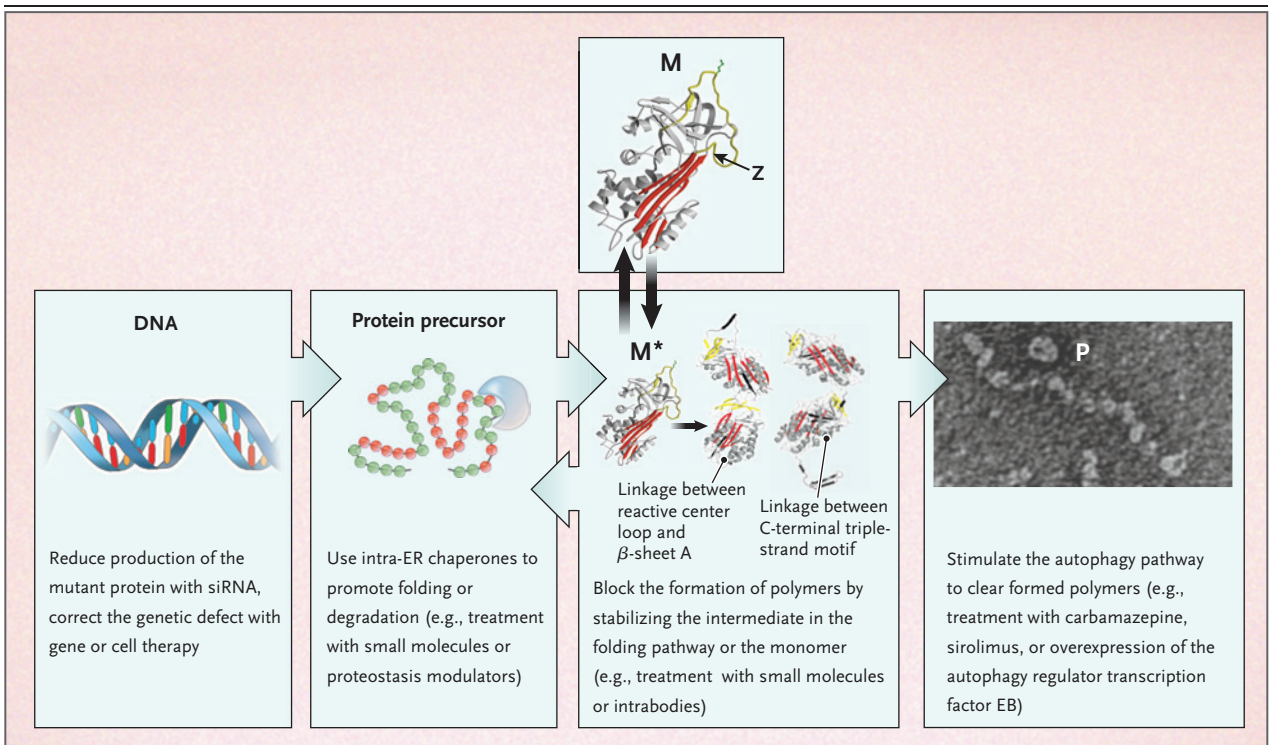


Figure 2. Strategies for Treating AAT Deficiency.

Mature wild-type AAT (M) folds through an intermediate (M*) and is then secreted through the Golgi apparatus. The Z mutation allows the intermediate to form polymers, which accumulate within the cell. Current evidence supports two pathological β-strand linkages: between the reactive center loop and β-sheet A (left)[4] and through a C-terminal triple-strand motif (right).[51] Strategies for treating AAT deficiency address different steps in this pathway: reducing production of the mutant protein with small interfering RNA (siRNA) and correcting the genetic defect with gene or cell therapy, using chaperones within the endoplasmic reticulum (ER) to promote folding or degradation (e.g., treatment with small molecules or proteostasis modulators), blocking the formation of polymers by stabilizing the intermediate in the folding pathway or the monomer (e.g., treatment with small molecules or intrabodies), and stimulating the autophagy pathway to clear formed polymers (P) (e.g., treatment with carbamazepine, sirolimus, or overexpression of the autophagy regulator transcription factor EB).

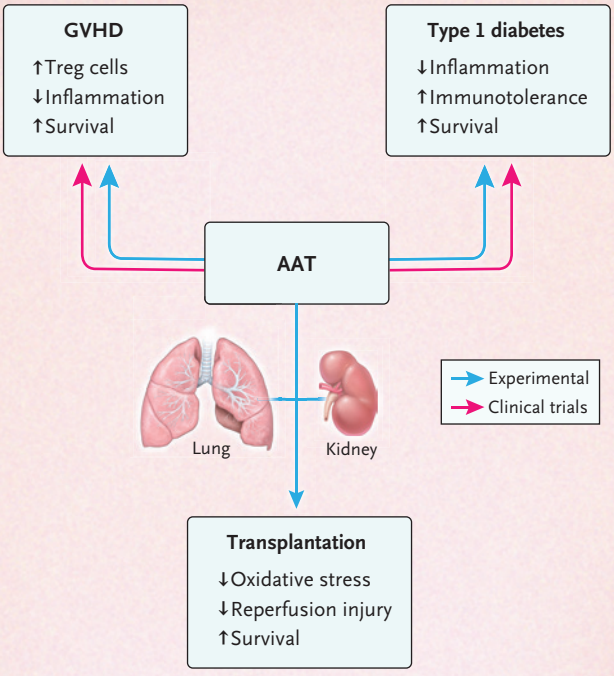


Figure 3. AAT in Immunology and Transplantation.

AAT can ameliorate experimentally induced kidney and lung ischemia–reperfusion injury and augment the function of murine and porcine lung transplants. Treatment with AAT has an antiinflammatory effect on insulin-sensitive tissues and may have a role in the treatment of type 1 diabetes. It also induces immune tolerance and increases regulatory T (Treg) cells and may therefore be effective in the management of graft-versus-host disease (GVHD).

and 2 clinical trials that showed the safety and side-effects profile of AAT administration in children with type 1 diabetes.[65]

Treatment with AAT induces immune tolerance and increases regulatory T cells.[66] This process is of particular relevance in graft-versus-host disease (GVHD), a potentially lethal consequence of allogeneic hematopoietic stem-cell transplantation. In keeping with the data from islet transplantation, AAT administration decreased mortality and reduced proinflammatory cytokine levels in three different mouse models of GVHD.[67] Moreover, it enhanced the recovery of regulatory T cells and decreased the number of alloreactive effector T cells. These findings underpinned a multicenter clinical study showing the safety of intravenous AAT in patients with glucocorticoid-refractory GVHD.[68] The administration of AAT increased the ratio of regulatory T cells to effector T cells, thereby mimicking the findings in experimental models. However, further trials are needed to assess the efficacy of AAT in humans.

END POINTS FOR PHASE 2 TRIALS

The first approval for AAT augmentation therapy was based purely on biochemical efficacy and pharmacokinetics.[69] Early studies of a new drug involve single ascending doses, followed by multiple ascending doses, an approach that addresses safety and tolerability but may also reveal a signal for efficacy and the dose at which this can be achieved. The results of large phase 3 studies suggest that it is unlikely that shorter phase 2 studies will show robust changes in lung density on CT scans, FEV_1, DLCO, pulmonary exacerbations, or quality of life.[50] As a minimum, these phase 2 studies should generate data similar to the original study reported by Wewers et al.,[69] which showed increased levels of AAT in blood and lung epithelial-lining fluid, above a protective threshold, along with an increased capacity for neutrophil elastase inhibition in lung epithelial-lining fluid. The observed downstream antiinflammatory effects of inhibiting neutrophil elastase, such as normalizing neutrophil chemotaxis and degranulation and protecting innate immune proteins from proteolytic inactivation, are also desirable (Fig. 1). Surrogate markers of efficacy might provide early indications of treatment response. These markers include desmosine and isodesmosine, unique cross-linkers of mature elastin fibers that can be measured in plasma, bronchoalveolar-lavage (BAL) fluid, or urine,[70] and Aα-Val^{360}, a specific marker of neutrophil elastase activity in plasma.[70]

These markers may also be effective for evaluating the effects of non–AAT neutrophil elastase inhibitors, which can increase elastase neutralizing capacity in blood and epithelial-lining fluid, while also showing downstream anti–neutrophil elastase effects similar to those of AAT. However, some of the antiinflammatory effects of AAT augmentation therapy may reflect a wider antiprotease and antiinflammatory profile not related solely to inhibition of neutrophil elastase (Fig. 1). Recent studies have shown an inflammatory imprint in plasma and BAL fluid from persons with AAT deficiency, which is

ameliorated by augmentation therapy.[70] The anti-inflammatory effects of therapeutic candidates can be assessed by measuring reductions in inflammatory markers such as proinflammatory cytokines (tumor necrosis factor α, interleukin-17, granulocyte–macrophage colony-stimulating factor, macrophage inflammatory protein 1, and macrophage migration inhibitory factor) and macrophage, lymphocyte, eosinophil, or mast-cell activating cytokines in BAL fluid. A problem with biomarkers in lung disease associated with AAT deficiency is the requirement for BAL, since the procedure for obtaining the samples is difficult to standardize across sites. Attempts to use sputum biomarkers have met with variable success.[71] AAT can be detected in exhaled-breath condensates,[72] and the response of volatile organic compounds to AAT augmentation has been analyzed with the use of composite nanosensor arrays.[73] However, these methods remain to be tested in large-scale clinical trials, and newer plasma-based assays of inflammation are anticipated.

The biomarkers of choice in early-stage studies of liver disease associated with AAT deficiency depend on the intervention being assessed. If the intervention is aimed at promoting secretion of Z AAT from the liver, it would be expected to increase circulating AAT levels and anti–neutrophil elastase capacity in plasma and BAL fluid, decrease desmosine and isodesmosine in these compartments, and have antiinflammatory effects. The liver can be imaged with transient elastography[15,16] or magnetic resonance elastography, and the findings correlate well with the stage of fibrosis and may change even over the short term.[49] The role of blood liver-function tests in phase 2 studies is uncertain, although γ-glutamyl transpeptidase shows some promise as a noninvasive marker of fibrosis.[15] Silencing-RNA approaches would decrease systemic and lung AAT levels and antiprotease protection; unless accompanied by concomitant intravenous augmentation therapy, such approaches might require systemic and BAL evaluations to make sure that there was no increase in lung inflammation.[54] A number of early-phase studies of AAT deficiency–associated liver disease evaluate liver biopsy specimens for polymer burden and fibrosis as biomarkers of response, but these invasive tests are a potential barrier to recruitment. Another obstacle is the relatively small population of persons with well-characterized disease, many of whom are already receiving augmentation therapy and would be loath to stop such therapy if they were assigned to a placebo group.[54]

AAT DEFICIENCY AS A PARADIGM OF CONFORMATIONAL DISEASES

AAT is the archetypal member of the serine proteinase inhibitor, or serpin, superfamily. The process of mutation-driven polymerization in other members of the family has been shown to form a group of diseases, the serpinopathies.[74] Mutants of neuroserpin polymerize in the brain, causing familial encephalopathy with neuroserpin inclusion bodies, an autosomal dominant dementia.[75] Mutants of antithrombin, C1 inhibitor, and alpha$_1$-antichymotrypsin are retained as polymers within hepatocytes, causing a circulating deficiency associated with thrombosis, angioedema, and emphysema, respectively.[74] The aberrant β-strand linkage that underlies AAT deficiency and the serpinopathies has similarities to the linkages formed in amyloidosis and by the proteins underlying prion disease and Parkinson's, Huntington's, and Alzheimer's diseases. Indeed, the approaches being developed to treat these conditions are similar: the use of small molecules to block the aberrant protein linkages, monoclonal antibodies to stabilize intermediates, and more recently, small interfering RNA approaches to silence protein expression. Thus, AAT deficiency and the serpinopathies provide a useful model for the protein linkages, the effect of mutations, the propagation of polymeric structures, and therapeutic strategies for other conformational diseases.

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