

Alpha₁-antitrypsin deficiency: a clinical-genetic overview

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Abstract: Severe α_1 -antitrypsin deficiency (AATD) is an inherited disorder, leading to development of emphysema in smokers at a relatively young age with disability in their forties or fifties. The emphysema results from excessive elastin degradation by neutrophil elastase as a result of the severe deficiency of its major inhibitor α_1 -antitrypsin (AAT). The AAT expression is determined by the *SERPINA1* gene which expresses codominant alleles. The three most common alleles are the normal M, the S with plasma levels of 60% of normal, and the severely deficient Z with levels of about 15% of normal. Homozygosity for the Z mutant allele is associated with retention of abnormal AAT in the liver, which may lead to neonatal hepatitis, liver disease in children, and liver disease in adults. Regular intravenous infusions of purified human AAT (AAT augmentation therapy) have been used to partially correct the biochemical defect and protect the lung against further injury. Two randomized controlled trials showed a trend of slower progression of emphysema by chest computerized tomography. Integrated analysis of these two studies indicated significantly slower progression of emphysema. AAT is quantified by immunologic measurement of AAT in serum, the phenotype characterized by isoelectric focusing, the common genotypes by targeted DNA analysis, and by sequencing the coding region of the gene when the AAT abnormality remains undefined. AATD is often unrecognized, and diagnosis delayed. Testing for AATD is recommended in patients with chronic irreversible airflow obstruction, especially in those with early onset of disease or positive family history. Testing is also recommended for immediate family members of those with AATD, asthmatics with persistent airflow obstruction, and infants and older subjects with unexplained liver disease. There are over 100 different AAT gene variants; most are rare and only some are associated with clinical disease.

Keywords: AAT, AATD, ZZ, early onset emphysema, panacinar emphysema, neonatal jaundice and hepatitis, childhood liver disease, genetics of alpha₁-antitrypsin, alpha₁-antitrypsin laboratory testing and phenotyping

Introduction

The association between α_1 -antitrypsin deficiency (AATD) and emphysema was reported in the early 1960s by Laurell and Eriksson, who discovered five subjects with absent α_1 -globulin peaks when reviewing a large series of serum protein electrophoresis gels.¹ They found out that three of the five had emphysema at an early age, 35–44 years, and that this was an inherited deficiency. The protein was named α_1 -antitrypsin (AAT) because trypsin inhibition was initially used for its measurement; it is also termed α_1 -protease inhibitor, because it is the major protease inhibitor in plasma and its major function is inhibition of neutrophil elastase. Smokers with severe AATD are prone to develop panacinar emphysema with disability in their early forties and fifties. The subject has been reviewed in detail by the joint statement of the American Thoracic Society (ATS)

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and the European Respiratory Society (ERS) in October 2003,² and available free of charge from either Society's website, and in recent concise reviews.^{3,4}

Severe AATD is a relatively common inherited deficiency, based on newborn screening, with the highest frequency of 1/1640 found in Sweden,⁵ and about 1/5000 in the white population in Oregon, USA.⁶ Severe AATD is mostly unrecognized, and there may be several years of delay in the diagnosis after symptoms develop.⁷ Only about 20% of AATD subjects have been recognized in Sweden, which has the highest rate of detection of severe AATD.⁸ The prevalence of severe AATD in clinics of chronic obstructive pulmonary disease (COPD) may depend on the pattern of referral; in one study from California, severe AATD was detected in about 2% of 965 patients with COPD.⁹

This article briefly reviews clinical features of AATD, based partly on a previous article,⁴ provides an update of AAT augmentation therapy, and covers the biochemical and molecular genetic evaluations of AATD.

Clinical review of AATD AATD

The AAT protease inhibitor (Pi) is encoded by the *SERPINA1* gene and secreted into the plasma by the liver cells as a mature glycoprotein of 394 amino acids (molecular weight 52,000).¹⁰ The plasma AAT diffuses passively into the lung interstitium and alveolar lining fluid. Two co-dominant alleles determine the AAT Pi phenotype. The normal AAT genotype is termed MM, while severe deficiency is mostly due to the ZZ genotype having AAT serum levels of about 15% of normal.^{2,3} The Z protein is due to a single amino acid substitution of glutamine to lysine at residue 342 of the processed protein.¹⁰ This substitution results in conformational changes in the molecule leading to its polymerization and retention in the hepatocyte. This retention leads to deficient plasma levels as well as predisposing to liver disease.¹¹ The Z antitrypsin is retained as inclusions in the liver, which stain positively with the periodic acid Schiff stain.

The heterozygote state (MZ) occurs in about 2%–3% of the Caucasian population, is more prevalent in northern than southern Europeans,¹² and results in AAT levels about 50%–70% of normal. This genotype is associated with only slightly increased risk of development of COPD in smokers, as reviewed recently.² The S protein variant has an amino acid substitution of glutamine to valine at residue 264 of the processed protein.¹⁰ The MS genotype has a prevalence of about 4%–11% in Europe, being highest in Spain and lowest in Scandinavia.¹² The MS genotype is associated with AAT

levels that are about 80% of normal, and is not a risk factor for disease. In contrast SZ smokers have an increased risk of developing emphysema, greater than MZ subjects but not as great as ZZ subjects, since their AAT serum levels are not as severely reduced. This statement is supported by a recent study comparing computerized tomography (CT) scan data and health status in 63 SZ subjects matched by age, gender, and smoking with ZZ subjects.¹³ The authors reported that less than half of the SZ individuals had CT scan evidence of emphysema, even among index cases, and had more frequent apical predominance of emphysema. In comparison, 86% of the ZZ subjects had emphysema. The SZ subjects had less impairment on pulmonary function testing, but had similar impairment with regard to health status as compared with the ZZ subjects.

Smokers with severe AATD are prone to develop disabling COPD with emphysema in their forties or fifties;^{2,3} however, a significant proportion present in their sixties. Severe deficiency is due to the ZZ genotype in about 95% of cases. Rarely, severe AATD is due to homozygous (“null”) alleles resulting in no detectable plasma AAT. Severe AATD predisposes to hepatocyte injury and panacinar emphysema (involving the whole acinus), which tends to have a basal predominance, in contrast to the upper lobe predominance of centriacinar emphysema (involving the center of the acinus around the respiratory bronchiole), that is characteristic of smokers with MM genotype. However, in the ZZ subjects studied by Holme and Stockley,¹³ 58% had only panacinar emphysema, 16% had both panacinar and centriacinar emphysema, and 26% had only centriacinar emphysema. Only a minority of lifetime ZZ nonsmokers develops severe emphysema, and many do not develop pulmonary symptoms.¹⁴ The yearly decline in forced expiratory volume in 1 second (FEV₁) in ZZ subjects is greater in smokers than in ex-smokers or never-smokers, but differs depending on whether the ZZ individuals were patients presenting with symptoms, or detected by family studies of AATD patients with COPD, or were detected by screening of the general population. A Danish study of ZZ patients reported that the 43 current smokers had a yearly FEV₁ decline of 132 mL/year, significantly greater than the decline of 52 mL/year in the 100 ex-smokers.¹⁵ In a study of 608 adult ZZ individuals from the Swedish national antitrypsin register, only 45% of whom were identified through respiratory symptoms, the mean FEV₁ decline was 70 mL/year in the 46 current smokers, 41 mL/year in the 351 ex-smokers, and 47 mL/year in the 211 never-smokers,⁸ compared with decline of about 30 mL/year in healthy nonsmokers. A recent study from the United

Kingdom related the yearly decline in FEV₁ to the severity of airflow obstruction in ZZ subjects, and found a mean annual decline over 3 years of 49.9 ± -7.4 mL/year.¹⁶ The fastest decline was in the group with moderate obstruction with FEV₁ 50%–80% predicted, and lower in the severe group with FEV₁ < 30% predicted.¹⁶ Genetic variations outside of the *SERPINA1* gene, eg, polymorphisms within the *IL-10*¹⁷ or *SFTPB* (surfactant protein B)¹⁸ genes, are associated with reduced FEV₁, and a specific tumor necrosis factor (TNF)- α genotype is associated with the clinical phenotype of chronic bronchitis.¹⁹ These reports may partly explain the finding that ZZ individuals detected by screening family members of AATD emphysema patients have worse lung function than ZZ subjects detected by screening the general population,²⁰ suggesting other inherited or familial factors. In addition, occupational and environmental air pollution can have an adverse effect on lung function, with a rapid decline in FEV₁ with higher particle pollution ($P = 0.024$), and a rapid decline in lung diffusing capacity with higher ozone levels.²¹

Clinical manifestations of severe AATD

Emphysema with severe airflow obstruction at an early age (starting about 40–50 years) is the most common clinical manifestation of severe AATD in smokers; some patients present in their sixties. Chronic bronchitis may also be present in a significant proportion of patients. There is also an association with bronchiectasis²² and asthma.²³ In a study of 424 ZZ patients who had clinical evaluation, CT scanning, and detailed lung function tests to determine clinical phenotype, 279 had emphysema, 159 had chronic bronchitis, and 83 had bronchiectasis. In this study, 124 subjects had two overlapping conditions, and 33 had all three conditions overlapping.¹⁹

Treatment is similar to the treatment of usual COPD and emphysema, including complete abstinence from smoking, bronchodilator therapy including long acting bronchodilators, antibiotic therapy of respiratory tract infections and pneumonia, and pneumococcal and influenza vaccinations. By analogy to COPD without AATD, combination inhaled corticosteroids and long acting beta-agonists may be helpful in subjects with reversible airflow obstruction or co-existing asthma or history of frequent exacerbations.

Severe AATD can lead to neonatal jaundice and hepatitis in infancy and childhood, or liver disease and cirrhosis in childhood, but most children recover without overt clinical liver disease.⁵ Although adults with severe deficiency are at increased risk for liver cirrhosis, clinical liver disease is not common. The liver disease is due to the abnormal retained

Z AAT polymerizing and causing injury to liver cells.²⁴ Research on prevention of polymerization and autophagy of the retained AAT, may lead to future therapy by increasing the AAT plasma level and preventing progression of liver and lung disease.²⁵ At autopsy of cases with liver cirrhosis, hepatoma is frequently present.²⁶

There is also an association of severe AATD with the rare condition, necrotizing panniculitis (skin and subcutaneous inflammation),²⁷ and with anti-neutrophil-cytoplasmic-antibody-positive vasculitis.²⁸ Additional information is provided in a recent review.³

AATD protease–antiprotease imbalance as a pathogenic mechanism amenable to AAT augmentation therapy

In severe AATD, the predominant pathogenic mechanism in emphysema is elastolytic lung injury due to an imbalance between proteolytic and elastolytic enzymes released in the lung, and the reduced antiprotease protection in the lung.²⁹ An imbalance, favoring elastin degradation and emphysema, occurs because of the severe deficiency of AAT, the lung's major antiprotease, combined with an increased release of neutrophil elastase in the lung. Neutrophil elastase release is induced by smoking and by the polymerized abnormal Z AAT acting as a neutrophil chemo-attractant.³⁰ This pathogenic mechanism of protease–antiprotease imbalance may be partially corrected by regular intravenous infusions of purified human AAT (AAT augmentation therapy) to restore blood and lung antiprotease activity, and by smoking cessation to decrease the lung burden of neutrophil elastase release.

AAT intravenous augmentation therapy became available in America in 1989³¹ after it was shown that regular intravenous therapy with purified human AAT at a weekly dose of 60 mg/kg in AATD subjects, partially corrected the AATD and the elastase inhibiting capacity in blood as well as bronchoalveolar lavage fluid.³² The half-life of the infused AAT was about 5 days.³² At that time, a randomized controlled clinical trial was considered not feasible because of the logistics and cost.³¹ Until about 10 years ago, there was only one commercial preparation of human AAT available (Prolastin[®], formerly by Bayer Inc and now by Talecris Biotherapeutics). It is prepared from pooled plasma that had individual units screened for viruses, and the purified preparation treated rigorously to protect against virus transmission. The aim of weekly intravenous therapy is to increase the AAT serum levels above a theoretically protective threshold level,³¹ of 0.8 g/L using the old commercial standard, and 11 μ mol/L (0.6 g/L) using a purified National Institutes of Health

standard. This protective level is putative and contentious, and was based on studies in SZ subjects with moderate AATD, who exceeded this level and had only a slightly increased risk of developing emphysema.³¹ The aim of therapy is to reduce the excessive decline in lung function or slow down the progression of emphysema, but it is not expected to improve lung function or repair emphysema. Although monthly infusions of 250 mg/kg³³ and biweekly infusions of 120 mg/kg³⁴ have also been used, they are not officially approved since they do not achieve consistent serum protective levels.^{34,35} Recently, three other commercial preparations of AAT purified from pooled human plasma have been approved in the United States.

In 1989, the American Thoracic Society (ATS) recommended³¹ AAT augmentation therapy for severely deficient patients with significant airflow obstruction, if they had quit smoking and were receiving optimal medical therapy. In contrast, the Canadian Thoracic Society (CTS) in 1992 considered that augmentation therapy was still not established as definitive therapy, and required confirmation by a randomized clinical trial.³⁶

Review of studies evaluating AAT augmentation

In 2001, the CTS published its position paper on AAT augmentation,³⁷ reviewing three studies evaluating the clinical efficacy of AAT intravenous augmentation therapy. The same authors published an updated review in 2005,³⁸ and used evidence-based criteria,³⁹ to assess the studies with the highest level of evidence assigned Category A, and the lowest, Category D. Level A is from well designed randomized controlled trials with adequate numbers and consistent findings, level B is from randomized controlled trials with limited numbers or inconsistent results, and level C is from nonrandomized trials or observational studies.

Table 1 summarizes studies using objective data to assess clinical efficacy, published up to November 2010. The first two studies are observational studies evaluating the decline in FEV₁ in subjects receiving augmentation therapy with subjects not receiving therapy,^{40,41} but since this was not a randomized allocation of therapy, the lower decline observed could be due to confounding factors as discussed in detail previously.⁴ In the US AAT Registry study subjects receiving augmentation therapy may have had better economic status and access to medical care which could have resulted in decreased mortality⁴² and the lower decline in FEV₁. The third was a randomized double blind placebo controlled trial of AAT augmentation (250 mg/kg every 4 weeks over 3 years)

in 56 ZZ ex-smokers with moderate emphysema. In this study, CT scanning and computerized quantification was used to assess progression of emphysema.⁴³ CT of the chest showed a trend ($P = 0.07$) favoring a slower progression of emphysema with AAT augmentation.⁴³ (Level of evidence B; not enough power, small number of subjects). A fourth retrospective study compared the rate of decline in FEV₁ in 96 patients with AATD, who were followed for a minimum of 12 months with a minimum of two lung function tests, both before and after weekly AAT augmentation.⁴⁴

Chapman et al⁴⁵ reported a meta-analysis of the effect of augmentation therapy on the decline in FEV₁ from published studies^{40,41,43,44} and data from their AAT Registry, but only one⁴³ was a prospective randomized and placebo controlled trial, while all the others were observational studies with possible confounding factors. AAT augmentation reduced the rate of decline in FEV₁ by 26% (17.9 mL/year) in the whole group of 1509 subjects; this effect was due to the subjects with FEV₁ 30%–65% of predicted (level of evidence C).

Recently, a randomized trial of AAT augmentation therapy over a period of 2.0–2.5 years was performed in 77 subjects with ZZ emphysema, to explore the role of CT densitometry in evaluating progression of emphysema.⁴⁶ Thirty-eight subjects received weekly infusions of purified AAT and 39 received placebo. Four different techniques were used to evaluate the changes in CT densitometry, and there was a trend suggestive of a reduction in the rate of progression of emphysema with AAT augmentation compared with placebo, and P values ranged from 0.049 to 0.084 (Level of evidence B). A Cochrane review of this study and the previous 1999 randomized controlled trial,⁴³ reported that when the two studies were combined in a meta-analysis, lung density measured by CT scan deteriorated a little less in the treated group than in the placebo group (difference 1.14 g/L; 95% confidence interval 0.14–2.14; $P = 0.03$).⁴⁷ However, the Cochrane reviewers concluded that AAT augmentation therapy could not be recommended, because of the lack of evidence of clinical benefit, and the cost of treatment.⁴⁷ An integrated analysis of the two randomized placebo controlled trials^{43,46} using CT scan progression of emphysema as the primary endpoint was recently published,⁴⁸ and showed a lower rate of progression of emphysema as indicated by lung density ($P = 0.006$).

In conclusion, AAT augmentation has been generally safe and well tolerated in a large number of patients, both in the US⁴¹ and in Germany,⁴⁹ with only a low incidence of adverse effects. The CTS guidelines in 2001³⁷ concluded that

Table 1 Summary of studies evaluating AAT augmentation in severe antitrypsin deficiency

Study	Comparisons	Results treated by AAT augmentation versus untreated	Evidence level
Seersholm et al ⁴⁰	FEV ₁ decline in German patients treated with AAT and Danish untreated patients	FEV ₁ decline in treated Germans was 56 mL/year, less than the 75 mL/year in Danes ($P = 0.02$)	C
Alpha-1-antitrypsin deficiency registry study group ⁴¹	Nonrandomized comparison of patients receiving AAT augmentation with untreated patients	Decreased mortality (risk ratio 0.64), ($P = 0.02$) In patients with FEV ₁ 35%–49% predicted decline in FEV ₁ was less with augmentation: 66 versus 93 mL/year, ($P = 0.03$)	C
Dirksen et al ⁴³	Randomized placebo controlled trial in 56 patients over 3 years	CT scan progression of emphysema less in treated patients, ($P = 0.07$); no difference in FEV ₁	B
Wencker et al ⁴⁴	Retrospective comparison of decline in FEV ₁ before and after treatment	Decline in FEV ₁ 34 mL/year less after therapy than the 49 mL/year before therapy ($P = 0.019$)	C
Chapman et al ⁴⁵	Meta-analysis of the above studies and patients on AAT treatment with matched controls from the Canadian AAT registry (total n = 1509)	Positive effect of AAT augmentation with reduction in FEV ₁ decline by 26% (17.9 mL/year). Effect due to subjects with FEV ₁ 30%–65% of predicted	C
Dirksen et al ⁴⁶	Randomized weekly AAT augmentation versus placebo for 2.0–2.5 years (total n = 77)	Trend suggestive of reduction in progression of emphysema by CT densitometry ($P = 0.049$ – 0.084)	B
Gotzsche and Johansen ⁴⁷	Cochrane meta-analysis of Dirksen's two placebo controlled randomized trials	Lung density deteriorated less in treated group ($P = 0.03$)	B
Stockley et al ⁴⁸	Integrated analysis of Dirksen's two placebo controlled randomized trials	Yearly loss in lung density in treated group significantly less than placebo 1.73 g/L versus 2.74 g/L, ($P = 0.006$)	B

Abbreviations: AAT, α_1 -antitrypsin; CT, computerized tomography; FEV₁, forced expiratory volume in one second.

there was a suggestion of possible benefits to augmentation therapy and recommended reserving it to AATD subjects with FEV₁ 35%–50% predicted who quit smoking but continue to show a rapid decline in FEV₁ > 80 mL/year.³⁷ This rate of decline was based on the mean decline in FEV₁ of about 80 mL/year in the Danish untreated patients with FEV₁ 35%–49% predicted in the German–Danish study.⁴⁰ The same authors recommended in 2005 extending augmentation therapy to subjects with FEV₁ > 35% and <65% predicted (instead of <49%) who had a rapid decline in FEV₁.³⁸ The executive summary of the joint ATS/ERS statement² on AATD recommended AAT augmentation for individuals with severe AATD and airflow obstruction, and suggested it would be of more benefit in subjects with moderate airflow obstruction (FEV₁ 35%–60% of predicted). It should be noted that AAT augmentation therapy was available for treatment in only a few countries in Europe in 2005,⁴ but the number increased by the end of 2008 to 15.

Investigations for AATD

Initial investigations

Multiple routine laboratory tests are available to facilitate the diagnosis of AATD. The tests can be grouped into one

of three general types: an initial investigation, a preliminary diagnostic test, and a definitive diagnostic test (Table 2). The initial investigation is a serum or plasma α_1 -antitrypsin level and is required in all cases. Preliminary diagnostic tests can be performed using targeted DNA analysis or Pi isoelectric focusing (PiIEF) tests. In either case, the preliminary diagnostic test distinguishes between the presence or absence of the common Z and S mutations. Of note, the ATS/ERS lists PiIEF in conjunction with serum AAT levels as the gold standard for the diagnosis of AATD.² However, a more sensitive and specific test is gene sequencing of the coding region of the *SERPINA1* gene, the gene which codes for the AAT protein.⁵⁰ For this reason, we consider the *SERPINA1* gene sequencing to be the definitive test for AATD. Although, the definitive test (gene sequencing) is most sensitive, it is expensive and unnecessary for the vast majority of case investigations. These methods will be discussed individually below before describing how they can be used in concert to optimally diagnose patients with AATD. Other test utilization strategies have been described since the ATS/ERS guidelines were published.^{50–58}

The initial investigation for AATD is a serum (or plasma) AAT level. This test is typically performed by

Table 2 Common laboratory tests available for the investigation of AAT deficiency

Test type	Test method	Patient type	
		Symptomatic patient	1 st degree relative of proband OR preconceptional counseling
Initial investigation	Serum AAT level	AAT level determines need to proceed to diagnostic test	Proceed to diagnostic test regardless of the serum AAT level
Preliminary diagnostic	Targeted DNA analysis for the presence of the <i>SERPINA</i> / S and Z mutations Iso-electric focusing	Stop here if the preliminary diagnostic test result agrees with the serum AAT level	Use a method sensitive to the mutation type present in the proband Stop here if the preliminary diagnostic test result agrees with the serum AAT level
Definitive diagnostic	Gene sequencing	Utilize: i. As an alternative to the preliminary diagnostic test, or ii. To further investigate cases in which the preliminary diagnostic test does not explain a relatively low serum AAT level	

Abbreviation: AAT, α_1 -antitrypsin.

immuno-nephelometry or other antibody-based detection methods. The test quantifies the serum level of the protein that is deficient in the disease and, as such, is the first step in the investigation of any patient at risk for the disorder. AAT levels can also be measured on capillary blood collected on filter paper cards.^{54,55} The test result can be compared to published genotype specific reference intervals.⁵¹ Of note, the serum AAT levels discussed in this paper refer to levels measured with methods calibrated to Certified Reference Material 470.⁵⁹ A new protein reference material has been proposed.⁶⁰ AAT methods standardized to this new reference material will yield results that are modestly different.

A serum AAT level relatively close to or above 1.5 g/L excludes AATD as the primary cause of symptomatic lung or liver disease and voids the need for further investigation. The threshold value of serum AAT used to identify those at risk for AATD is debatable. Ideally, a testing program will identify all patients who carry the ZZ, Z null, and SZ genotypes. The highest serum AAT levels of these three genotypes would be seen in the SZ patients who have a median serum AAT level of 0.6 g/L with the 97.5th percentile at 0.82 g/L.⁵¹ In the case of an acute phase response, or exposure to high estrogen states, the serum AAT level may double.⁶¹ Therefore, a cutoff of approximately 1.20 g/L would identify the vast majority of the ZZ, Z null, and SZ mutations. Similarly, the MZ patients would also be identified in most cases as the mean serum AAT level (in the absence of an acute phase response) is 0.85 g/L with the 97th percentile being 1.25 g/L.

Therefore, setting the threshold at 1.20 g/L will identify all symptomatic patients with serum AATD and most asymptomatic carrier patients.

Once the serum AAT level has been identified as being relatively low, a preliminary diagnostic test to determine the presence or absence of the S and Z mutations is the most expedient next step. From this preliminary test, a presumptive genotype can be obtained: no Z or S mutation, heterozygous for Z mutation, homozygous Z mutation, heterozygous S mutation, homozygous S mutation, or compound heterozygous for S and Z. The serum AAT level and the patient's presumptive genotype are compared to determine whether the results are discordant or concordant (eg, homozygous Z patients should have serum AAT < 0.7 g/L, whereas no mutation should be >1.0 g/L – see Snyder reference⁵¹). If the presumptive genotyping test is concordant with the observed serum AAT level, then the relatively low serum AAT level does not require further investigation. Patients with discordance between the presumptive genotyping test and the observed serum AAT level may require definitive testing. The roles of the respective tests in the diagnosis of AATD testing are summarized in Table 2. Of note, this algorithm will identify the vast majority of cases of clinical AATD but not all. Patients with rare mutations that do not affect serum AAT concentrations may be missed. To avoid missing these relatively mild and rare cases requires performance of AAT gene sequencing or PiIEF in all cases submitted for investigation. All published algorithms will miss some rare causes of AATD.

Preliminary diagnostic investigations for AATD

Either targeted DNA analysis or PiIEF test can be performed as a preliminary diagnostic test. Both tests are effective in identifying patients with symptomatic AATD due to ZZ or SZ mutations. Both methods are also effective in identifying carriers of either the S or Z mutations. The two methods do have practical and interpretive differences, which are discussed in more detail below. However, for the most part, the two tests can be used interchangeably.

Targeted DNA analysis strategies can take various forms.⁶² These methods require small amounts of DNA and can be done on noninvasive or minimally invasive samples such as a capillary blood collected on a filter paper card. The test results unambiguously indicate the presence or absence of heterozygosity or homozygosity for both the S and the Z mutations.

The analysis of the data from most targeted DNA analysis methods is straightforward in that it is similar to that required for any other gene mutation screening method. This simplicity is in contrast to the more difficult task of interpreting PiIEF tests (see below). The major limitation of the targeted DNA analysis strategy is that this method is unable to detect *SERPINA1* mutations aside from the common S and Z mutations. As such, patients with null mutations and other pathologic mutations (eg, M_{malton}) would go undetected if these methods were utilized in isolation.

The PiIEF test, also known as AAT phenotyping test, is based on the principle that mutations in the AAT gene (*SERPINA1*) will impart changes to the isoelectric point of the AAT protein. Serum samples are electrophoresed usually on a polyacrylamide gel upon which a narrow pH gradient (pH 4.0–5.0) has been established. This pH range has been selected so that the range of isoelectric points of the various AAT variants is encompassed and maximum separation of variants can be achieved. Each of the proteins will migrate through the gel from the point of application toward the pH that is equal to its isoelectric point, whereupon it will stop and focus. Following this focusing step, the gel is stained with Coomassie Blue to visualize the proteins. Visualization can be enhanced by antibody detection. However, immunofixation is not often necessary for interpretation, as the level of AAT protein is adequate (even for the Z variant) to be detectable by stain alone, and because a fairly clean background is obtainable due to the low level of non-AAT proteins in this pH range. The normal M variant yields a characteristic five-band pattern: two major bands and three minor bands. This

microheterogeneity has been attributed (at least in part) to differences in the post-translational sialic acid content of the protein. The same microheterogeneity is also exhibited by the other variants, but the minor bands are more difficult to visualize in the deficient variants because of the amount of sample that is typically applied for analysis. The S and Z variants are readily distinguished because of the characteristic separation of their major bands from the major bands of the normal M variant; the S bands have a higher isoelectric point than the M bands, and the Z bands have an even higher isoelectric point still. Correct identification is further ensured by including patient controls corresponding to these variants to serve as markers with each analytical run. PiIEF techniques thereby result in the straightforward detection of the S and Z mutant proteins as well as normal AAT proteins, including M1, M2, M3, and F variants. Due to the co-dominant expression of AAT alleles, both heterozygotes and homozygotes of these variants may be identified by this methodology. Additionally, atypical banding patterns – ie, those that are not consistent with the aforementioned variants – may be observed, and can suggest the presence of a rare (normal or deficiency-causing) variant, which would be missed by the previously mentioned targeted DNA analysis for S and Z mutations. PiIEF alone, however, cannot positively identify these rare variants without running the corresponding patient control, which is often not available. Therefore, gene sequencing may be needed for definitive determination.

It is important to recognize additional challenges and limitations of the AAT phenotyping test. A high level of technical expertise and experience is required for both performing the test and for the interpretation of results. Despite that, with practice and optimizing of protocols, consistently interpretable gels can be routinely achieved. A recently available commercial method produced by Sebia™ (Norcross, GA, USA), should facilitate consistent high quality PiIEF results. Of note, dried capillary blood collected on filter paper cards may also be used for PiIEF.⁵⁴ This development enables the full range of initial and preliminary diagnostic tests to be available via this minimally invasive and efficient sample collection method.

Still it remains, as for the polymerase chain reaction-based detection method, that routine testing using PiIEF can only definitively detect certain AAT mutations that are associated with clinical AATD. Specifically, it cannot directly detect mutant AAT proteins that result from null mutations, or those mutations that do not result in an altered isoelectric point of the AAT protein (eg, M_{malton}).

Definitive investigations for AATD

Definitive testing is required when neither the preliminary diagnostic tests nor the patient's clinical information explain an observed low serum AAT level. Some patients will have a clinical explanation for a low serum AAT level, including protein energy malnutrition, protein losing states (nephropathy, protein losing enteropathy), and liver disease. In most cases, such clinical explanations will be associated with a general deficiency of hepatic plasma protein levels (eg, albumin). When identified, a clear clinical explanation may obviate the need for gene sequencing. When no clinical explanation is identified, the definitive test of sequencing the coding region of the AAT gene (*SERPINA1*) is required to investigate discordance between the presumptive genotype and the observed serum AAT level.

The *SERPINA1* gene codes for an unprocessed immature protein of 418 amino acid residues. The canonical isoform of the AAT protein results from cleavage of the 24 amino acid signal peptide from the N-terminus, creating the mature protein of 394 amino acids; therefore, current recommended

nomenclature differs from legacy nomenclature by 24 amino acids. The Z and S mutations account for approximately 95% of mutations reported in individuals with AATD; other *SERPINA1* mutations associated with AATD have been reported throughout the gene (Table 3). Of note, the AAT mutations are assigned letter names based on the electrophoretic mobility of the protein product, which is in turn determined by its isoelectric point. As researchers have identified additional mutations, many of them sharing a common letter designation, the naming convention has become less useful. The M designation in particular is a nonspecific term that refers to proteins with severe mutations (eg, M_{malton}) as well as to the common normal variant (M1, M2, M3). In Table 3, the common genetic mutations of the *SERPINA1* gene are listed using standard mutation description nomenclature alongside the traditional or legacy letter names.

Sequencing of the four coding regions and their intron/exon boundaries will detect the majority of all *SERPINA1* mutations reported in association with clinical AATD. The exceptional mutations not detected by sequencing include

Table 3 Sample mutations of the *SERPINA1* gene described with standardized and legacy nomenclature

Nucleotide nomenclature ^a	Protein nomenclature (Unprocessed)	Protein nomenclature (legacy)	Synonyms
17 kb deletion of all coding exons			Nullisola _{di procida} ; Null _{procida}
c.17C>T	p.Ser6Leu	p.Ser-19Leu	Z _{wrexham}
c.194T>C	p.Leu65Pro	p.Leu41Pro	M _{procida}
c.227_229delTCT	p.Phe76del	p.Phe52del	M _{malton} ; M _{palermo}
c.230C>T	p.Ser77Phe	p.Ser53Phe	S _{iiyama}
c.272G>A	p.Gly91Glu	p.Gly67Glu	M _{mineral springs}
c.275C>T	p.Thr92Ile	p.Thr68Ile	Null _{lisbon}
c.347T>A	p.Ile116Asn	p.Ile92Asn	Null _{ludwigshafen}
c.415G>A	p.Gly139Ser	p.Gly115Ser	Null _{devon} ; Null _{newport}
c.552delC	p.Tyr184X	p.Tyr160Ter	Null _{granite falls}
c.646+1G>T			Null _{west}
c.721A>T	p.Lys241X	p.Lys217Ter	Null _{bellingham}
c.739C>T	p.Arg247Cys	p.Arg223Cys	F
c.839A>T	p.Asp280Val	p.Asp256Val	P _{duarte} ; P _{lowell} ; Null _{cardiff}
c.863A>T ^b	p.Glu288Val	p.Glu264Val	S
c.1027_1028delTC	p.Ser343ArgfsX16	p.Ser319ArgfsX16	Null _{hong kong 1}
c.1078G>A	p.Ala360Thr	p.Ala336Thr	W _{bethesda}
c.1096G>A ^b	p.Glu366Lys	p.Glu342Lys	Z
1130dupT	p.Leu377PhefsX24	p.Leu353PhefsX24	Null _{mattawa}
c.1158delC	p.Glu387ArgfsX11	p.Glu363ArgfsX11	Null _{bolton}
c.1158dupC	p.Glu387ArgfsX14	p.Glu363ArgfsX14	Null _{saarbruecken}
c.1178C>T	p.Pro393Leu	p.Pro369Leu	M _{heerlen}

Notes: ^aNucleotide changes are described relative to NM_001127705.1. ^bThe most common *SERPINA1* mutations: i) c.863 A>T (or "S") is most common in individuals of Southern European origin, the allele frequency in white subjects in North America is about 6%; ii) c.1096G>A (or "Z") is most common in individuals of Northern European origin, the allele frequency is about 2% in white subjects in North America.

large scale deletions, insertions, inversions, or complex rearrangements and mutations within the regulatory regions or introns of the gene. To date, only large-scale deletions and mutations within the regulatory regions of the *SERPINA1* gene have been described, representing only four of all described mutations. When sequencing is used routinely in the investigation of AATD, *SERPINA1* mutations as well as silent variant changes are often identified. Resources to discriminate mutations from silent polymorphism are readily available (eg, <https://research.cchmc.org/LOVD/home.php>). The limitations of the definitive method include those of cost, which is related to both the material and technical cost of gene sequencing and to the necessary expertise required to interpret the significance of any identified changes in the gene sequence.

Genetic counseling

The tests discussed above are useful to diagnose patients with symptomatic AATD as well as to identify patients who carry AAT gene mutations but who are currently asymptomatic. Knowledge of AAT mutation status may motivate patients to stop exposure to tobacco smoke and to minimize exposure to other environmental pollutants. The ATS/ERS guidelines² recommend testing for AATD in siblings of affected probands with severe AATD, and consideration and discussion of testing in offspring, parents, and relatives of affected probands. Testing of potentially affected family members would involve utilizing a diagnostic method that is sensitive to the mutation type carried by the proband (eg, if the proband is ZZ, targeted DNA analysis or PiIEF), as well as measurement of the serum AAT level. The preliminary diagnostic test and the serum AAT level can then be compared to determine the need for additional definitive testing.

Prenatal genetic testing may also be required in the case of an expectant parent who carries or is homozygous for the Z or a null mutation. The potential partner would need to be screened for the S and Z mutations by targeted DNA analysis or by PiIEF. These results require correlation with the individual's serum AAT level. Where the serum AAT level is lower than expected relative to the preliminary diagnostic test result, gene sequencing may be necessary to exclude rare mutations.

Conclusion/future directions

AATD is a well studied disorder that remains an area of active research. The clinical awareness of AATD by physicians has been suboptimal for a number of years and has not improved on follow-up.⁷ However, this concern may be alleviated in the future as there are continued efforts to increase the clinical

awareness of AATD, and improve laboratory facilities for AAT evaluation. These efforts are buoyed by the expectation that AAT augmentation therapy will ultimately improve clinical as well as radiologic outcomes. When clinicians do consider AATD, they will have access to screening and confirmatory diagnostic investigations which are both less invasive and more sensitive due to improvements in laboratory technology.

Disclosure

Dr Abboud has participated in two advisory board meetings for Talecris Biotherapeutics. He has also given three presentations on alpha₁-antitrypsin deficiency sponsored by Talecris to Asthma & COPD Educators in Vancouver, to Respiratory Therapists in Edmonton, and to Respiratory Physicians at the University of Alberta in Edmonton in 2008. Lastly, as of 2008, he has been participating as a local principal investigator in Vancouver in a multicenter clinical trial evaluating weekly IV infusions of a purified human alpha₁-antitrypsin preparation in treating emphysema due to severe antitrypsin deficiency, sponsored by CSL Behring Biotherapies for Life.

In 2010, Dr Mattman received an educational grant and has participated in an advisory board meeting on behalf of Talecris Biotherapeutics.

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