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Review

Variants of *SERPINA1* and the increasing complexity of testing for alpha-1 antitrypsin deficiency

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Kimberly E. Foil

***Abstract*:** Alpha-1 antitrypsin deficiency (AATD) is caused by mutations in the *SERPINA1* gene, which encodes the alpha-1 antitrypsin (AAT) protein. Currently, over 200 *SERPINA1* variants have been identified, many of which cause the quantitative and/or qualitative changes in AAT responsible for AATD-associated lung and liver disease. The types of these pathogenic mutations are varied, often resulting in misfolding, or truncating of the AAT amino acid sequence, and improvements in sequencing technology are helping to identify known and novel genetic variants. However, due to the diversity and novelty of rare variants, the clinical significance of many is largely unknown. There is, therefore, a lack of guidance on how patients should be monitored and treated when the clinical significance of their variant combination is unclear or variable. Nevertheless, it is important that physicians understand the advantages and disadvantages of the different testing methodologies available to diagnose AATD. Owing to the autosomal inheritance of the genetic mutations responsible for AATD, genetic testing should be offered not only to patients at increased AATD risk (e.g. patients with chronic obstructive pulmonary disease), but also to relatives of those with an abnormal result. Genetic counseling may help patients and family members understand the possible outcomes of testing and the implications for the family. While stress/anxiety can arise from genetic diagnosis or confirmation of carrier status, there can be positive consequences to genetic testing, including improved lifestyle choices, directed medical care, and empowered family planning. As genetic testing technology grows and becomes more popular, testing without physician referral is becoming more prevalent, irrespective of the availability of genetic counseling. Therefore, the Alpha-1 Foundation offers genetic counseling, as well as other support and educational material, for patients with AATD, as well as their families and physicians, to help improve the understanding of potential benefits and consequences of genetic testing.

Keywords: alpha-1 antitrypsin, alpha-1 antitrypsin deficiency, genetic counseling, rare variants, *SERPINA1*, testing

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Introduction

The *SERPINA1* gene that encodes alpha-1 antitrypsin (AAT) is a highly polymorphic 12.2kb gene clustered with other serpin genes on the long arm of chromosome 14 at position 32.13 (14q32.13), and consists of three noncoding regions (1a–c) and four protein-coding regions numbered 2–5 (Figure 1).[1] AAT deficiency (AATD) is caused by variations/mutations in the *SERPINA1* gene sequence, the different types of which lead to different changes in the AAT protein (conformational and concentration), and consequently, have different pathological implications. The normal AAT protease inhibitor (PI) is designated with the letter M and is encoded by the wild-type *SERPINA1* sequences, also termed the M alleles; individuals with biallelic M alleles are therefore designated as PI*MM individuals. Serum AAT

Correspondence to:
Kimberly E. Foil
Division of Pulmonary, Critical Care, Allergy and Sleep Medicine, Medical University of South Carolina, Charleston, SC 29425, USA
foilk@musc.edu

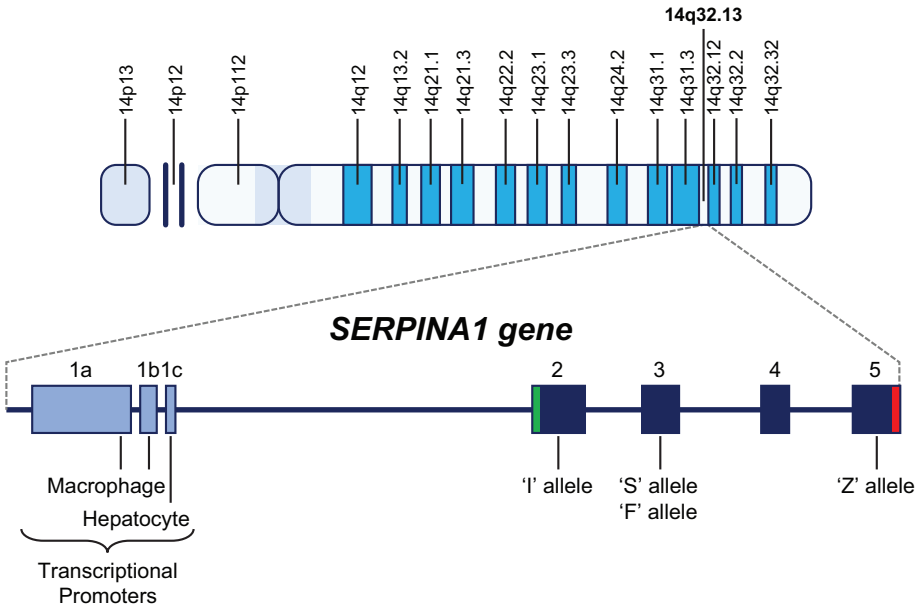

Figure 1. *SERPINA1* gene.
Located on the long arm of chromosome 14 at position 14q32.13, the *SERPINA1* gene consists of three transcriptional promoters (1a–c) upstream of the transcriptional start codon and four protein-coding regions (2–5). The S and F mutations occur in the third protein-coding region, and the I and Z mutations occur in coding regions 2 and 5, respectively. The green bar in coding region 2 represents the AUG transcriptional start codon and the red bar in coding region 5 represents the TAA stop codon.

levels in PI*MM individuals are approximately 100–220 mg/dL, or 20–53 μM.[2] The M allele encompasses several common benign subvariants, such as M1 (Ala213), M1 (Val213), M2, M3 and M4, which can be differentiated by some but not all testing methodologies.[2] Other benign allelic variants also exist, such as E, G, and Zpratt, in which the respective sequence variation encodes a variant AAT protein that does not diminish AAT levels or function.[3–5]

The allele most commonly associated with severe AATD is the Z allele. Individuals that are homozygous for the Z allele (PI*ZZ) are at high risk of lung and liver disease, and have serum AAT levels that are approximately 10–20% of normal (20–45 mg/dL, or 2.5–7 μM).[2,6] Other alleles associated with severe AATD include M_{Malton}, $M_{Heerlen}$, select other rare variants, and the family of null alleles, which produce no AAT protein product. Some other alleles, such as the S variant, are associated with less severe deficiency and intermediate forms of AATD, resulting in serum AAT levels that are reduced compared to PI*MM levels but higher than PI*ZZ levels. Such alleles may contribute to AATD when combined with a pathogenic variant on the other allele. A serum AAT level of <57 mg/dL, or <11 μM, conventionally defines AATD,[2] but attention to moderate AAT levels and corresponding genotypes will continue to elucidate the full spectrum of AATD-associated risks and phenotypes. Yet other *SERPINA1* variants have unique molecular mechanisms, or significance, that is not yet greatly understood.

Nomenclature of *SERPINA1* alleles is unconventional. AAT protein variants were originally identified and named based on their electrophoretic properties long before the encoding *SERPINA1* gene was identified. The normal AAT protein (M) received its name based on its migration to the middle of the isoelectric focusing (IEF) gel. Other alleles were designated with a letter A–L or N–Z depending on their proximal or distal location, respectively, to the M protein band. When challenged with no unused letter for a new allele, numerical figures (for polymorphisms with >0.01 allele frequency) or place of origin names (for rare alleles) were used with the letter of the closest anodal allele, giving rise to allele names such as M1 and $M_{Procida}$.[7] Upon identification of the

SERPINA1 gene, the sequence variant corresponding to each PI allele received the same name for fidelity of allele and protein product (e.g. Z allele encodes PI*Z AAT) in AATD testing, literature and clinical practice. Null alleles, which produce no AAT protein, were originally collectively named 'null' (or −), and later, when corresponding sequence variations were elucidated, were individually named with the prefix 'Q0' and place of origin suffix (i.e. the birthplace of the proband). Genomic nomenclature has evolved with expanding use of genetic testing technologies, and the Human Genome Variation Society (HGVS) has published guidelines for the consistent and unambiguous description of DNA variants.[8] Many more recently identified *SERPINA1* variants are named in accordance with newer guidelines, but conventional AAT nomenclature still widely persists in the literature and in AAT specialty laboratories, and previously named alleles now have multiple aliases (e.g. PI*Z, Z, and c.1096G>A refer to the same variant).

In the United States, the most common abnormal alleles linked to AATD in order of prevalence are S, Z, F and I;[9] beyond these, numerous other *SERPINA1* variants exist. Over 200 variants of *SERPINA1* are now described in the ClinVar database of the National Center for Biotechnology Information,[10] and with next-generation sequencing (NGS) technology, more novel variants are likely to be found.

As previously discussed in the first part of this review series by Tejwani and Stoller,[11] AATD cannot be diagnosed in the clinic based on symptomology alone; a range of quantitative and qualitative methods are required to identify the presence of AATD. However, each of these techniques have advantages and disadvantages, which will be discussed within this part. In addition, an overview of the diversity of the genetic changes that can lead to AATD will also be discussed, along with the implications for clinical practice, as well as the role that genetic counseling can play in the care of patients with AATD and their families.

The spectrum of variants in AATD

AATD is most commonly associated with single-nucleotide polymorphisms (SNPs); for example, the Z and S allelic variants result from the base pair substitutions c.1096G>A and c.863A>T leading to the amino acid changes p.Glu366Lys and p.Glu288Val, respectively, in the AAT protein (ClinVar accession VCV000017967.13 and VCV000017969.9; Table 1).[10] Small changes to the AAT amino acid sequence can affect the structure/folding of the protein and reduce its secretion from hepatocytes, and/or reduce the binding affinity of AAT for neutrophil elastase (NE), with or without protein polymerization. However, AATD can also be caused by null variants (or nonexpressing *SERPINA1* alleles), which can arise from missense mutations, nonsense mutations, frameshifts, large deletions, and complete absence of the *SERPINA1* gene.[12]

Many rare AAT variants, such as the more common/well-characterized $M_{Heerlen}$, M_{Malton}, $M_{Procida}$, $M_{Würzburg}$, $P_{Brescia}$, and P_{Lowell} variants (not a comprehensive list) have been linked to the development of lung disease and/or liver disease as a result of changes in AAT quantity, polymerization, and/or a reduction in ability to inhibit NE (Table 1).[13–19] In particular, the M_{Malton} single amino acid deletion at position 76, is strongly linked to emphysema and liver disease, and is the highest frequency rare variant reported in a recent Portuguese study.[18] Null variants are designated with the prefix 'Q0' and the examples of these given in Table 1, $Q0_{Amersfoort}$, $Q0_{Mattawa}$ and $Q0_{Bellingham}$ (not a comprehensive list), all result in premature termination of the AAT amino acid sequence, and consequently, undetectable levels of serum AAT. In addition, null variants are strongly linked to the development of emphysema,[12,14,20,21] but in line with all null variants, do not confer a risk of liver disease as they do not induce the polymerization of AAT responsible for liver damage.[12] There is, therefore, a wide spectrum of *SERPINA1* genetic variants that cause AATD (Table 1), but currently there is only clear clinical guidance for the management and treatment of the most common (e.g. PI*ZZ) or severe (PI*Znull) forms of the disease.[22] Detailed information on the management and treatment of AATD can be found in the next part of this review series authored by Barjaktarevic and Campos.[23]

Establishing clinical relevance

Guidelines are clear for the most common or severe AATD-causing genotypes with regard to

Table 1. Examples of common and rare *SERPINA1* variants and their clinical significance.

SERPINA1 variants	Type of mutation[10] (nucleotide/amino acid change)	Clinical significance	Approximate serum AAT level	Reference	ClinVar accession number
Common					
I	Point mutation (c.187C>T; p.Arg63Cys)	Uncertain	<120 mg/dL, (22 μM)[24]	Graham *et al.*[25]	VCV000017974.6
F	Point mutation (c.739C>T; p.Arg247Cys)	Uncertain	160 mg/dL, (30 μM)[26]	Okayama *et al.*[26]	VCV000017961.5
S	Point mutation (c.863A>T; p.Glu288Val)	Uncertain	82 mg/dL, (15 μM)[27]	Tan *et al.*[28]	VCV000017969.9
Z	Point mutation (c.1096G>A; p.Glu366Lys)	Pathogenic: linked to development of emphysema and liver disease	20–45 mg/dL, (2.5–7 μM)[6]	Laurell and Eriksson[29]	VCV000017967.13
Rare					
$M_{Heerlen}$	Point mutation (c.1178C>T; p.Pro393Leu)	Pathogenic: linked to development of lung disease	5 mg/dL, (~1 μM)[30]	Hofker *et al.*[30]	VCV000017965.2
M_{Malton}	Point mutation (c.226_228del; p.Phe76_del)	Pathogenic: linked to development of emphysema and liver disease	64–87 mg/dL, (11–16 μM)[31]	Curiel *et al.*[15]	RCV000201853.1
$M_{Procida}$	Point mutation (c.194T>C; p.Leu65Pro)	Pathogenic: intermediate pathogenicity	<10 mg/dL, (<2 μM)[19]	Takahashi *et al.*[19]	RCV000019571.3
$M_{Würzburg}$	Point mutation (c.1177C>T; p.Pro393Ser)	Pathogenic: linked to development of lung disease	94 mg/dL, (17 μM)[17]	Poller *et al.*[17]	RCV000336993.8
N_{Nagato}	Point mutation (c.899T>C; p.Leu300Arg)	Uncertain significance: likely benign	~180 mg/dL, (~33 μM)[32]	Yuasa *et al.*[32]	VCV000594462.3
$P_{Brescia}$	Point mutation (c.745G>C; p.Gly249Arg)	Pathogenic: linked to lung and liver disease	61 mg/dL, (11 μM)[16]	Medicina *et al.*[16]	VCV000189018.1

(Continued)

Table 1. (Continued)

SERPINA1 variants	Type of mutation[10] (nucleotide/ amino acid change)	Clinical significance	Approximate serum AAT level	Reference	ClinVar accession number
P_{Lowell}	Point mutation (c.839A>T; p.Asp280Val)	Pathogenic: linked to lung disease	77 mg/dL, (14 μM)[33]	Cook *et al.*[13]	VCV000017975.4
$Q0_{Amersfoort}$	Null mutation – stop codon insertion (c.552C>G; p.Tyr184Ter) *Occurs in the same codon as $Q0_{Granite\ Falls}$ (c.552delC; p.Tyr184Terfs)	Pathogenic: emphysema	No AAT expression	Prins *et al.*[20]	VCV000017976.1
$Q0_{Mattawa}$	Null mutation – frameshift resulting in a premature stop codon (c.1131A>T; p.Leu377Phe)	Pathogenic: emphysema	No AAT expression	Cox and Levison[34]	VCV000017978.1
$Q0_{Bellingham}$	Null mutation – stop codon insertion (c.721A>T; p.Lys241Ter)	Pathogenic: emphysema	No AAT expression	Satoh *et al.*[21]	VCV000017977.2
S_{Iiyama}	Point mutation (c.230C>T; p.Ser77Phe)	Pathogenic: emphysema	33 mg/dL, (6 μM)[35]	Yuasa *et al.*[36]	VCV000017992.1
V_{Munich}	Point mutation (c.77A>C; p.Asp26Ala)	None	150–250 mg/dL, (29–46 μM)[37]	Holmes *et al.*[37]	VCV000017983.1
$W_{Bethesda}$	Point mutation (c.1078G>A; p.Ala360Thr)	Uncertain	~170 mg/dL, (~32 μM)[38]	Holmes *et al.*[39]	VCV000017985.1
X	Point mutation (c.682G>A; p.Glu228Lys)	None	Serum AAT levels likely within the normal range[40]	Crystal *et al.*[40]	VCV000017963.1
$X_{Christchurch}$	Point mutation (c.1159G>A; p.Glu387Lys)	Uncertain: likely benign	Serum AAT levels likely within the normal range[40]	Brennan and Carrell[41]	VCV000017964.4
$Z_{Augsburg}$	Point mutation (c.1096G>A; p.Glu366Lys)	Pathogenic: linked to development of emphysema and liver disease	83 mg/dL (15 μM)[4]	Faber *et al.*[42]	VCV000017967.13
$Z_{Wrexham}$	Point mutation (c.17C>T; p.Ser6Leu)	Uncertain	Serum AAT levels likely as for PI*Z[43]	Graham *et al.*[43]	VCV000017970.1

Amino acid numbers described in protein mutations have been updated according to recommendations outlined by the Human Genome Variation Society and therefore may differ slightly in comparison to corresponding mutations reported in the literature.[8]
AAT, alpha-1 antitrypsin.

how patients should be monitored and treated, but they are unclear for variants with variable or uncertain clinical consequence, and even for combinations of moderate frequency alleles associated with moderate deficiency (e.g. PI*SF). The first indicator of whether a rare variant is pathogenic is often the associated serum AAT level. In general, patients who are heterozygous for a pathogenic *SERPINA1* variant and the normal allele (e.g. PI*MZ) are unlikely to have severely low serum AAT levels, but may have mild-to-moderate reduction compared with normal due to the codominant allelic expression of AAT. When pathogenic rare variants are present in combination with other pathogenic variants (e.g. the Z allele), serum AAT levels may be well below normal; such patients may benefit from, or should be considered for, intravenous AAT therapy when clinical criteria for augmentation therapy are met. For example, an individual with the PI*ZM$_{\text{Procida}}$ genotype was recently reported as having a serum AAT level of 24 mg/dL (~4.4 μM).[44] Likewise, when rare pathogenic variants are present in combination with null variants, AAT levels may be very low or undetectable (although this is extremely rare).[12] Case reports have reported an individual with the PI*ZQ0$_{\text{Ourém}}$ genotype as having a serum AAT level of 14.5 mg/dL (~2.7 μM),[45] and individuals homozygous for the Q0$_{\text{Cairo}}$ allele with serum AAT levels below 10 mg/dL (~1.8 μM).[46]

Although AAT level is often useful in interpretation of results and inferring clinical risk, several pathogenic variants result in near-normal levels of serum AAT but are functionally deficient in their ability to inhibit NE. This is thought to be the case with the F variant;[47] individuals with AATD involving the F allele may have serum AAT levels that appear adequate, despite their genetic risk. Testing assays measuring AAT function are available, but not widely used in testing setups. Other factors can also affect serum AAT levels, such as inflammation, cigarette smoke and estrogen.[48,49] As AAT is an acute phase reactive protein, serum AAT levels increase during stages of acute inflammation. Therefore, the presence of systemic inflammation at the time of sample collection for AATD testing may mask usually low baseline AAT levels.[48] Furthermore, different AAT protein phenotypes react differently in response to inflammation, with M-type AAT proteins showing a strong response and Z-type AAT proteins exhibiting a reduced reaction.[48] Serum AAT levels have also been shown to increase with tobacco smoke exposure in a dose-dependent manner.[49] In a population-based cohort of 5187 adults, those who were exposed to environmental tobacco smoke had higher serum AAT levels than nonexposed never-smokers, and serum AAT levels were highest in active smokers who consumed at least 15 cigarettes per day.[49] In women within the same cohort, menopausal status was significantly associated with a decrease in serum AAT levels ($p = 0.003$), and intake of female hormones, such as oral contraceptives and hormone replacement therapy, was significantly associated with an increase in serum AAT ($p < 0.001$ for both).[49] Therefore, serum AAT levels should be interpreted with mindfulness of these factors and should not be used alone for establishing the clinical relevance of rare and novel *SERPINA1* variants.

Determining the clinical significance of novel variants where there are no previous reports of pathogenicity can be challenging; however, computational approaches are being utilized to identify variants with severe pathogenic potential, which can be tested for secretory deficiencies in cell culture models.[50,51] For example, PolyPhen-2 is an easy-to-use and freely accessible software that can predict the possible impact of amino acid substitutions on protein structure and function, and is one of the interpretation tools used in several studies of *SERPINA1* variants.[18,51–53] However, many different programs are available and being used to apply pathogenicity predictions on *SERPINA1* mutations, developing algorithms to identify variants of interest for *in vitro* studies.[50] Although it is increasingly possible to predict the pathogenicity of rare and novel *SERPINA1* variants, much investigation is still required to fully understand their clinical consequences and what the appropriate management of patients with these variants should entail.

Testing

Based on the latest evidence, clinical practice guidelines recommend testing for AATD in any patient with chronic obstructive pulmonary disease (COPD), emphysema and/or chronic bronchitis, regardless of age or ethnicity, in patients with incompletely reversible asthma, those with unexplained chronic liver disease (see the part of

this review series by Patel and Teckman),[54] necrotizing panniculitis, granulomatosis with polyangiitis, and unexplained bronchiectasis.[22] Furthermore, parents, siblings, children, and extended family of patients identified with an abnormal AAT gene should be provided with genetic counseling and offered AATD testing. For family testing, testing AAT level alone is not recommended as it does not fully characterize AATD disease risk;[22] further testing is required. For diagnostic testing of symptomatic patients, the 2016 US Clinical Practice Guidelines recommend genotyping for at least the S and Z alleles because >95% of all individuals with severe AATD have PI*ZZ or PI*SZ genotypes, with confirmatory testing considered using PI phenotyping, AAT level testing and/or expanded genotyping.[22] The 2017 European Respiratory Society (ERS) statement identifies AAT level testing as a crucial first test to identify AATD, but notes that it must be supported by qualitative tests to identify the causative mutations.[55] In practice, AATD testing consists of at least one of four fundamental steps: determination of AAT serum levels, IEF phenotyping, allele-specific genotyping, and direct sequencing.[56] Measurement of AAT serum levels can detect some, but not all severe AAT deficiencies. When serum levels are severely low, additional testing is required for comprehensive diagnosis and risk assessment. A clinician may order a specific test(s) based on clinical circumstance (e.g. presence of a null allele in the family, patient history of liver transplant, whether a patient is receiving augmentation therapy).[57] Different laboratories employ different testing algorithms, with NGS increasingly incorporated for select samples at AATD specialty labs, and as a standalone AATD test at select genetics labs.

Quantitative testing

As AATD is most commonly characterized by low levels of antigenic AAT, measuring serum levels of AAT provides an initial indication of AAT deficiency irrespective of a patient's genetic variant status, and is a widely available diagnostic test. Nephelometry is the most widely used method in the literature;[6] however, the most modern technique is immunoturbidimetry. Both techniques measure the turbidity of a sample to determine levels of an analyte; however, whereas nephelometry is a measure of light scattered by a sample, immunoturbidimetry is a measure of light absorbed by a sample. Immunoturbidimetry is a higher-throughput method with lower associated costs but provides similar results to nephelometry.[27,58] In the past, quantitative techniques for identifying AATD were performed on whole blood samples. However, more recently, quantitative techniques using dried blood samples are more widely available, facilitating testing.[55,59] Whole or dried blood spots allows application of any of the major testing methods discussed within this part. Use of saliva and buccal samples is gaining popularity for genetic testing; for AATD, DNA-based testing (i.e. genotyping and sequencing) may be done on a buccal or saliva sample, but blood is required for quantitating AAT serum level and PI phenotyping.

Serum AAT reference ranges reported in the 2003 American Thoracic Society (ATS)/ERS statement are still widely used today; by nephelometry, the normal range is given as 83–220 mg/dL, and the range given for patients homozygous for the Z variant (PI*ZZ), is 20–45 mg/dL.[6] However, these reference ranges were largely based on older studies and may contain data obtained from patients with inflammation, which as mentioned earlier, can affect serum AAT levels.[48] Serum AAT levels can increase by 3–4-fold in response to inflammation and infection in AAT replete individuals,[60] and therefore reduce the accuracy of the reference ranges. Although the acute phase response is negligible in terms of AAT elevation in severe AATD, patients with genotypes such as PI*MZ, PI*MS and PI*SS still retain a substantial acute phase response.[48] Therefore, C-reactive protein (CRP) may be tested simultaneously as an indicator of inflammation at the time of AAT testing. Alternative AAT reference ranges from a cohort of individuals with normal (<5 mg/L) CRP levels are shown in Table 2.

Measuring serum AAT levels alone is useful for identifying 'classic' severe AATD but is inadequate for detecting all types of severe AATD and for identifying genotypes. For example, PI*ZZ and PI*$ZM_{Heerlen}$ would have comparable severe AATD, but differentiating the genotypes is important for accurate family testing and estimating the risk for liver disease to the patient. Measuring serum AAT levels alone is also inadequate for detection of alleles that are associated with variable, or near-normal AAT levels with

Table 2. Alternative AAT reference ranges by genotype based on data from a large US screening program,[48] with reference ranges from the 2003 ATS/ERS statement[6] for comparison.

Normal range				
Genotype		**PI*MM**		
AAT levels: mg/dL (μM)	Sanders *et al.*[48†]	126.9–159.2 (23–29)		
	ATS/ERS statement	150–350 (27–64)		
Intermediate deficiency genotypes				
Genotype		**PI*MS**	**PI*SS**	**PI*MZ**
AAT levels: mg/dL (μM)	Sanders *et al.*[48†]	105.0–131.4 (19–24)	88.2–107.5 (16–20)	75.8–96.6 (14–18)
	ATS/ERS statement	Not provided	100–200 (18–37)	90–210 (17–39)
Severe deficiency genotypes				
Genotype		**PI*ZF**	**PI*SZ**	**PI*ZZ**
AAT levels: mg/dL (μM)	Sanders *et al.*[48†]	64.5–79.2 (12–15)	46.4–61.0 (8–11)	0–28.2[‡] (0–5)
	ATS/ERS statement	Not provided	75–120 (13–22)	20–45 (3–8)

[†]Data are interquartile ranges of AAT levels (measured by immunoturbidimetry) from individuals with normal CRP levels (CRP $<$ 5 mg/dL).
[‡]lower quartile was below the assay detection limit of 20 mg/dL.
AAT, alpha-1 antitrypsin; ATS, American Thoracic Society; CRP, C-reactive protein; ERS, European Respiratory Society; PI, protease inhibitor.

reduced AAT function. The F variant is an example of this, as it is associated with near-normal circulating levels of AAT, but abnormal AAT function, which contributes to risk of lung disease.[47] Measuring serum levels alone also limits the identification of individuals harboring an abnormal genotype that have AAT levels overlapping the normal range.[48] Examples of this include individuals with the PI*MZ genotype, where individuals have enhanced susceptibility and familial risk,[61] in addition to the PI*SS genotype, which is of variable consequence.[27] Furthermore, AAT distribution is not uniform and so serum AAT levels do not reflect levels of AAT in the lungs, which is where AAT primarily functions to inhibit NE.[60] Given these limitations of basing diagnosis on serum AAT levels alone, additional qualitative assessment of AATD genotype/phenotype is essential.

Qualitative testing

Qualitative analysis is performed to identify AAT protein variants and pathogenic alleles. IEF, also called Pi-typing, or phenotyping, is one method used to identify AAT protein variants. This method is a high-resolution electrophoretic technique that separates proteins based upon their charge, which can be used to identify the AAT protein types, both normal and abnormal, present in patient sera. IEF requires expert interpretation of results due to the large number of AAT variants and technical aspects of such assays.[62] IEF is unable to identify heterozygosity for null AAT variants and is not appropriate for patients receiving AAT therapy. PI*ZZ patients receiving AAT therapy will be identified as PI*MZ through IEF due to the introduction of exogenous M-type AAT. For patients on AAT therapy for whom genotype is unknown, genetic testing must be performed.

Targeted polymerase chain reaction (PCR) testing provides rapid and accurate identification of common/well-known genetic variants (e.g. Z, S, and increasingly a subset of other well-characterized pathogenic variants), but given the diversity of *SERPINA1* variations, it would be too costly to assemble a comprehensive panel of probes to detect all known deleterious *SERPINA1* variants. Furthermore, targeted PCR will not detect novel *SERPINA1* variants.[62] Multiplex PCR, the simultaneous detection of multiple genotypes in a single reaction, is possible for a limited number of AATD genotypes, including rare alleles with a known genetic sequence, and allows for more variants to be tested from a single sample.[63] However, multiplex genotyping is also unable to detect novel *SERPINA1* variants. Furthermore, as the prevalence of AATD genotypes varies worldwide,[64] and the prevalence of rare AATD genotypes is largely unknown, it is likely that the panels of probes in multiplex PCR tests will be better suited to some countries than others. Only complete genetic sequencing can identify all *SERPINA1* variants.

Complete genetic sequencing of *SERPINA1* using the Sanger method can provide full details of all mutations present, including rare/novel SNPs and null variants.[47,65] However, NGS has a higher throughput and lower cost than Sanger sequencing and is therefore becoming the gold standard sequencing methodology for AATD. NGS can also identify novel variants and should allow the discovery of further rare *SERPINA1* variants.[49,50] Nevertheless, irrespective of the sequencing method used, it is essential that defined laboratory protocols are followed in order to ensure error rates are minimized.[55]

Screening for AATD

At present, the majority of epidemiological data on AATD comes from the United States and Western Europe. While AATD has been identified at the highest frequencies in these populations, there is a considerable lack of information available for ~50% of the world's United Nations, hindering our global understanding of the disease.[66,67] Many studies of AATD detection have targeted or drawn populations with enhanced suspicion for AATD, such as relatives of a proband or those with clinical features (e.g. early-onset COPD).[55] Population-wide screening studies for AATD can be challenging to perform on a large scale, but several have been successfully conducted and they can provide a less-biased estimate of disease prevalence and natural history.[68] Due to the differing methodologies used, the reported prevalence of AATD and associated variants may not be directly comparable across studies.[24] It has also been suggested that some rare alleles may not be as rare as previously assumed.[24] Determining the frequency and clinical significance of both rare and common variants is key to understanding the true burden of AATD.

Table 3. AATD testing recommendations.[6,22]

Pulmonary	Extra-pulmonary	Familial testing
Testing should occur in those with: • COPD • Incompletely reversible asthma • Unexplained bronchiectasis	Testing should occur for those with: • Unexplained chronic liver disease • Necrotizing panniculitis • Granulomatosis with polyangiitis	Testing should be offered to: • Parents, children, siblings, and extended family of an individual with an abnormal gene

AATD, alpha-1 antitrypsin deficiency; COPD, chronic obstructive pulmonary disease.

Targeted screening of enriched patient populations with respiratory symptoms results in a far higher detection rate than population-wide screening and so has been employed to great effect in several countries worldwide.[27,69,70] A potential disadvantage of targeted screening programs is missing individuals with severe AATD who have not been diagnosed with emphysema or COPD.[69,71,72] This cohort is likely composed of (1) symptomatic individuals who have not sought care/received diagnosis, (2) pre-symptomatic individuals, and (3) those who will remain asymptomatic, with largely unknown distribution among these groups. Some targeted screening programs also test relatives of diagnosed patients (familial screening), which again has advantages and disadvantages, which will be discussed in the 'Genetic counseling' section.[70]

The largest population-based screening study conducted in AATD was performed in 200,000 Swedish infants in 1976, which identified 127 PI*Z and 48 PI*SZ individuals who have been followed longitudinally.[73] Studies such as this are informative but can be prohibitively expensive and therefore, population-wide screening has seen limited application.[33] Benefits of population-based screening leading to early and widespread identification of affected individuals include lifestyle recommendations (e.g. smoking avoidance) to reduce disease risk, earlier symptom recognition and treatment, and the opportunity for genetic counseling. Limitations include potential psychological effects, potential genetic discrimination, and medical resource utilization in (the often many) pre-symptomatic years, and these have remained barriers to adoption of standardized newborn screening for AATD, so additional data are needed.[74] Current testing recommendations from the Alpha-1 Foundation and the ATS/ERS are shown in Table 3.[6,22]

Several US commercial screening programs currently provide cost-free AATD testing for use in clinical settings (i.e. the test must be ordered by and resulted to a healthcare provider).[65] Clinicians may also order AATD testing through a variety of institutional and for-fee commercial laboratories. AATD testing is also increasingly available direct to consumers without physician referral, and, therefore, without pre-test genetic counseling to fully inform patients of the implications the results may bring. The Alpha-1 Foundation has offered free and confidential testing for AATD for over 15 years through a research program,[75] though with associated online educational materials and access to genetic counseling. More recently, AATD is among the conditions included on genomic health screens offered direct-to-consumer by companies such as 23andMe, and others. Additionally, in recent years, AATD has been added to expanded prenatal/preconception genetic carrier screens. While such screens are aimed at detecting couples with reproductive risk, individuals genetically affected with conditions such as AATD are occasionally identified.[76,77]

The hopeful dissemination of deidentified data from genomic tests that include AATD, but are not performed due to concern for AATD specifically, will contribute to elucidating the true prevalence of AATD variants, and facilitate the continued understanding of AATD's disease burden and natural history through projects such as patient registries. The American College of Medical Genetics and Genomics (ACMG) recommends that all labs performing whole-exome and genome sequencing report secondary findings in 59 genes associated with select highly penetrant genetic disorders, for which established interventions are available to reduce morbidity and mortality.[78] Of note, despite increasing consumer access to AATD results *via* inclusion on

genomic health and expanded carrier screens, the *SERPINA1* gene is not currently on the ACMG list of genes in which to report secondary findings; therefore, individuals receiving clinical exome or genome testing generally do not learn their AATD genotype even though early recognition may improve outcomes. Such tests, however, are usually ordered in an attempt to find the etiology of an unexplained clinical phenotype, and the reporting paradigm will likely change if exomes/genomes are pursued for broader predictive purposes in the future.

Inheritance of AATD variants and genetic counseling

As AATD follows the autosomal pattern of inheritance, one of each parents' two *SERPINA1* alleles is passed to each child. In addition, the *SERPINA1* alleles that encode AAT are co-dominantly expressed,[1] meaning that in AATD heterozygotes, half of AAT produced is normal, while half is abnormal in accordance with the genetic variant. The risks to a patient's offspring therefore depend upon the genetic status of both parents (Figure 2).[79] If only one parent is heterozygous for a pathogenic allele and the other is an unaffected noncarrier, there will be a 50% chance that each offspring will be heterozygous for the pathogenic allele and a 50% chance that they will be an unaffected noncarrier [Figure 2(a)]. If one parent is homozygous (e.g. PI*ZZ) or compound heterozygous (e.g. PI*SZ) for pathogenic alleles and the other parent is an unaffected noncarrier, each offspring will have a 100% chance of carrying one pathogenic allele [Figure 2(b)]. If both parents are heterozygous for a pathogenic allele, there will be a 25% chance of each offspring being an unaffected noncarrier, a 50% chance of being heterozygous for a pathogenic allele, and a 25% chance of being affected with biallelic pathogenic variants [homozygous or compound heterozygous, based on parental genotypes; Figure 2(c)]. More significantly, if one parent themselves is homozygous or compound heterozygous and the other parent is also heterozygous, there is a 50% chance of the offspring being severely deficient in AAT [Figure 2(d)]. In the rare event that both parents have biallelic pathogenic alleles, then each offspring will also be affected. Furthermore, the likelihood of inheriting two different pathogenic alleles is becoming increasingly apparent with the growing number of recognized deficiency genotypes, as well as the commonality of heterozygotes in the general population.

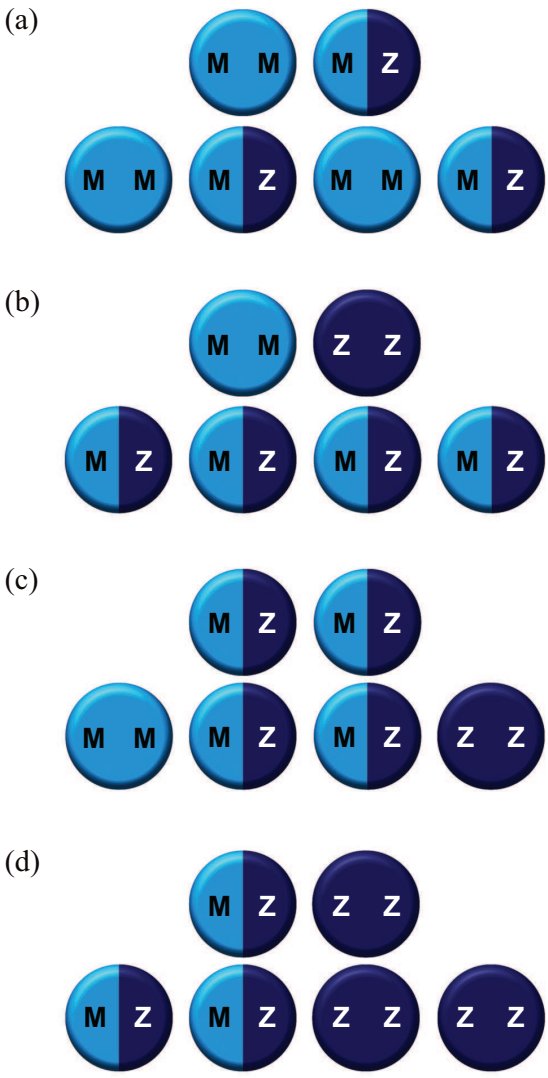

Figure 2. Autosomal inheritance of *SERPINA1* alleles.
Autosomal inheritance of *SERPINA1* alleles if parents are: 'normal' and heterozygous (a), 'normal' and homozygous (b), both heterozygous (c), and heterozygous and homozygous (d). Only eventualities c and d confer risk of the offspring being severely deficient in AAT (25% and 50% chance, respectively).
Estimated worldwide prevalence:[80]
MZ, 42,564,136; ZZ, 181,894.

It is important to note that genetic testing only informs patients whether or not they harbor any pathogenic *SERPINA1* alleles and does not predict the age of AATD disease onset, the type of symptoms patients will present with, the severity of these symptoms, or the rate of AATD disease

progression.[22] However, knowledge of AAT pathogenic variant status allows for risk modification (e.g. smoking avoidance) and allows proper monitoring for disease onset or progression.[22] Owing to the autosomal inheritance described above, testing for AATD should be offered to parents, siblings, children, and extended family members of individuals found to have AATD (whether homozygous or heterozygous), with siblings of severely deficient individuals at highest priority due to their greater genetic risk.[22] However, genetic testing has implications for the whole family and the possible outcomes of the results should be discussed before testing.[6] Patients and family members should be offered genetic counseling for this purpose. For children, ethical considerations are more complicated; predictive testing in adolescents should be undertaken only if both themselves and their parents give informed consent, and if they are mature enough to understand the implications of testing and the possible outcomes. For younger children, parental/guardian consent is required, but it has been shown that parents value genetic testing for their children at risk for AATD when it can be done in a confidential setting.[81] A discussion of potential risks and benefits should occur surrounding predispositional diagnostic testing for a patient of any age, weighing medical utility with potential anxiety, impact on insurability (e.g. life, disability), and, for children, autonomy. Testing children for AATD is only clearly indicated when clinically directed (e.g. in the presence of liver disease), where testing follows the previously discussed guidelines.

Where genetic counseling is unavailable, withholding testing based on the absence of a genetic counselor referral is not recommended and a knowledgeable provider can advise patients on the possible outcomes of testing. However, it is helpful for providers ordering AATD testing to be familiar with key areas of pre- and post-test genetic counseling, including the natural history, pattern of inheritance, reduced penetrance and clinical variability, and genetic risk to family members based on the results. The information patients receive from genetic testing can be personally unsettling and create stresses and anxiety within the family. In accordance with the 2008 Genetic Information Non-discrimination Act (GINA) in the United States, genetic testing results cannot affect a patient's ability to obtain health insurance or employment; however, GINA does not address life, disability or long-term care insurance, or apply to employers with fewer than 15 employees or the military. Potential effect of results on eligibility or premiums for life, disability, or long-term care insurance may further exacerbate stress and anxiety. Despite the potential for negative psychological impacts from AATD testing, there is evidence to suggest that knowledge of AATD status can enable patients to feel peace of mind and in control of their lives, as well as improve medical outcomes.[75] Specific information about genetic counseling for AATD is available through the Alpha-1 Foundation website (www.alpha1.org).

Conclusions

There are many known *SERPINA1* variants associated with AATD and the increasing availability of higher throughput sequencing methodologies, particularly NGS, is contributing to continued detection of rare and novel variants responsible for AATD. Several studies suggest that some variants are not as rare as previously thought, indicating that additional data from standardized studies and/or population-level testing are required to refine prevalence data and determine AATD's true extent and disease burden. Deciding the best course of treatment in individuals with rare/novel variants can be challenging. Computational modeling, quantitative AAT testing and clinical assessment can assist in proper diagnosis and management of patients. To better inform patients and healthcare professionals about the genetic variants related to AATD, a dedicated online database now exists, which is being updated as further data become available (Alpha-1 Alleles; www.alpha1research.org/allele_search). Irrespective of which genetic variant(s) of *SERPINA1* a patient carries, laboratory testing is the cornerstone of AATD diagnosis, and it is important that clinicians have an understanding of the advantages/disadvantages of the different diagnostic tests available. Expert analysis and utilization of multiple methods in inconclusive cases can help provide comprehensive analysis and accurate diagnosis. Where possible, genetic counseling should be offered with genetic testing and to those diagnosed with AATD so that patients can better understand the broader implications of AATD variants to themselves and their families.

Author contributions

KF contributed to the writing of the manuscript, reviewed the manuscript, and approved the manuscript for submission.

Conflict of interest statement

The author declares that there is no conflict of interest.

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