Alpha-1 Antitrypsin *(SERPINA1)* Targeted Genotyping

Background

Alpha-1 antitrypsin deficiency (AATD) (OMIM#613490) is one of the most commonly inherited metabolic disorders in people of northern European ancestry, occurring in one in 5000-7000 individuals, and also occurs at lower frequencies in people from other regions. AATD predisposes an individual to chronic obstructive pulmonary disease (COPD), liver disease, panniculitis and C-ANCA-positive vasculitis. AATD is caused by pathogenic mutations in *SERPINA1* (RefSeq NM_000295), the gene that encodes alpha-1 antitrypsin (AAT). Alpha-1 antitrypsin is an inhibitor of neutrophil elastase. Excess neutrophil elastase can destroy the alveolar walls of the lung, causing emphysema. Mutations in *SERPINA1* can also cause accumulation of abnormal proteins in hepatocytes leading to chronic liver disease.

AATD is inherited as an autosomal co-dominant condition and more than 120 variants in *SERPINA1* have been described. Alleles in AATD are named with the prefix PI* for "protease inhibitor." The PI*M allele is present in approximately 95% of unaffected individuals. The majority of patients with AATD have the PI*S and PI*Z alleles. The PI*S allele causes a functionally deficient form of *SERPINA1*, but has a relatively mild phenotype, unless combined with other pathogenic alleles. The PI*Z allele also encodes a functionally deficient form of alpha-1 antitrypsin, but may express as a more severe phenotype, with lung and liver involvement. Approximately 95% of individuals with clinical manifestations of AATD have the PI*ZZ genotype. Serum alpha-1 antitrypsin levels correlate with the AAT phenotype as shown in Table 1.

Other rare variants of *SERPINA1* exist and can also cause lung and liver disease. Null, deficient and dysfunctional forms of alpha-1 antitrypsin occur, but are much less common than the PI*S and PI*Z alleles. Targeted testing for the PI*M, PI*S, and PI*Z alleles will identify the majority of pathogenic mutations. However, if a discordant serum alpha-1 antitrypsin level and phenotype is identified, additional testing by either isoelectric focusing or Sanger sequencing is recommended to better characterize the *SERPINA1* variant.

Clinical Indications

Diagnostic Testing

According to available guidelines, diagnostic testing is recommended for symptomatic adults with emphysema, COPD or asthma that is incompletely responsive to bronchodilators, individuals with unexplained liver disease, asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors, adults with necrotizing panniculitis and siblings of adults with AATD. It is acceptable to test adults with bronchiectasis without clear risk factors for bronchiectasis, adolescents with persistent airflow obstruction, asymptomatic individuals with persistent obstruction on pulmonary function tests with no identifiable risk factors, adults with C-ANCA-positive vascultitis and other conditions enumerated in Table 2.

Table 1. Serum levels of alpha-1 antitrypsin according to genotype

Units	PI*MM	PI*MZ	PI*SS	PI*SZ	PI*ZZ
uM	20–48	17–33	15–33	8–16	2.5–7
mg/dl	150–350	90–210	100–200	75–120	20–45

Table 2. Clinical Indications[5]

Recommended **To be Decided in Discussion with the Patient** **Not Recommended** **Discouraged**

	GENETIC TESTING			
CLINICAL INDICATION				
Pulmonary				
Symptomatic adult with emphysema, COPD, asthma with incompletely reversible airflow obstruction	x			
Asymptomatic with persistent obstruction & risk factors	x			
Symptomatic adult with emphysema, COPD, asthma within completely reversible airflow obstruction (in countries with prevalence < North America or Europe)		x		
Adults with bronchiectasis without evident etiology		x		
Adolescents with persistent airflow obstruction		x		
Asymptomatic with persistent airflow obstruction & no risk factors		x		
Adults with asthma & completely reversible airflow obstruction			x	
Extra-pulmonary				
Unexplained liver disease	x			
Adult with necrotizing panniculitis	x			
Adult with c-ANCA positive vasculitis		x		
Sibling of adult with AATD	x			
Family history of COPD or liver disease not known to be caused by AATD		x		
Distant relative of an individual with the PI*ZZ genotype		x		
Offspring/parent of an individual the PI*ZZ genotype		x		
Sibling, offspring, parent or distant relative of a heterozygous individual (i.e., with the PI*MZ genotype)		x		
Carrier status assessment for reproduction planning				
Individual at high risk for AAT deficiency-related disease		x		
Partner of individual with the PI*ZZ genotype or the PI*MZ genotype		x		
Population screening				
In countries with AAT prevalence >1:1500, prevalent smoking and adequate counseling services		x		
In smokers with normal spirometry			x	
In countries with low AAT prevalence, low prevalence of smoking or inadequate counseling services				x
Other				
Predispositional testing			x	
Predispositional fetal testing				x

Carrier Testing

Testing is acceptable for individuals who are at high personal risk of having AATD-related disease and for the partners of individuals who are either homozygous or heterozygous for AATD.

Methodology

Targeted variant analysis is performed using real-time polymerase chain reaction (PCR) with fluorescent labeled Taqman^{R} probes for allelic discrimination assay (Applied Biosystems) to identify only the specified alleles of *SERPINA1*, PI*M, PI*S and PI*Z. Genomic DNA is isolated from the peripheral blood and a two-step PCR protocol using specific, fluorescent, dye-labeled probes is performed to differentiate between the three alleles. Fluorescence signals generated by the PCR amplification indicate which alleles are present in the patient's sample.

Interpretation

This assay will not detect other mutations that may cause AATD. Uncommon variants or SNPs may affect binding of Taqman^{R} probes and may result in a false negative or false positive result. Results of this assay do not completely guarantee that the patient has no other *SERPINA1* variants. Correlation of the genotype with the patient's serum alpha-1 antitrypsin level as well as clinical manifestation is strongly recommended. When discrepancies exist between the enzyme level and targeted genotype results, sequencing of the coding regions of the *SERPINA1* gene can be performed to identify rare mutations.

Table 3. Description of Targeted AAT Genotypes

GENOTYPE	INTERPRETATION AND GUIDANCE
PI*MM	• Homozygous for the wild type M allele of SERPINA1 • Typical serum alpha-1 antitrypsin levels between 102-254 mg/dL
PI*MS	• Heterozygous for the S allele (p. Glu264Val (c.862G>A, p.Glu288Val) • Serum alpha-1 antitrypsin levels between 86-218 mg/dL and pulmonary and hepatic disease is not expected • Carrier of the S allele, pulmonary and hepatic disease not expected
PI*MZ	• Heterozygous for the Z allele (p.Glu342Lys (c.1096G>A, p.Glu366Lys) • Serum alpha-1 antitrypsin levels between 62-151 mg/dL • Carrier of the Z allele, pulmonary and hepatic disease not expected
PI*SS	• Homozygous for the S allele (p. Glu264Val (c.862G>A, p.Glu288Val) • Serum alpha-1 antitrypsin levels between 43-154 mg/dL
PI*SZ	• Compound heterozygous for S (p. Glu264Val (c.862G>A, p.Glu288Val) and Z (p.Glu342Lys (c.1096G>A, p.Glu366Lys) • Serum alpha-1 antitrypsin levels between 38-108 mg/dL • May be at increased risk for chronic obstructive pulmonary disease and may be at increased risk for hepatic disease
PI*ZZ	• Homozygous for the Z allele (p.Glu342Lys (c.1096G>A, p.Glu366Lys) • Serum alpha-1 antitrypsin levels between <29-52 mg/dL • Increased risk of chronic obstructive pulmonary disease, hepatic disease, and other conditions caused by alpha-1 antitrypsin deficiency (e.g., panniculitis, c-ANCA positive vasculitits)

References

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4. Stoller JK, Aboussouan LS. A review of α1-antitrypsin deficiency. *Am J Respir Crit Care Med*. 2012;185(3):246-59.
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Test Overview

Test Name	Alpha-1 Antitrypsin (*SERPINA1*) Targeted Genotyping
Methodology	Targeted allelic discrimination assay by real-time PCR with Taqman^{R} probes (Applied Biosystems)
Specimen Requirement	Peripheral Blood: 5 ml in an EDTA tube (purple top)
Billing Code	89910
CPT Code	81332-G0452

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