Cleveland Clinic

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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 3000-5000 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 variants in *SERPINA1* (RefSeq NM_001127701.0; GRCh38/hg38), 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. Pathogenic variants in *SERPINA1* can also cause accumulation of abnormal proteins in hepatocytes leading to chronic liver disease. AATD is inherited as an autosomal recessive condition and more than 150 variants in *SERPINA1* have been described to date.

Alleles in AATD are named with the prefix PI* for "protease inhibitor," another name for the AAT protein. Many pathogenic variants in *SERPINA1* result in structurally abnormal AAT protein, which impairs secretion and results in plasma deficiency. The majority of patients with AATD have the PI*S (c.863A>T, p.Glu288Val, g.94380925) or PI*Z (c.1096G>A, p.Glu366Lys, g.94378610) alleles. The PI*S allele causes a structurally abnormal AAT with mild functional impact and low disease risk, unless combined with other pathogenic alleles. The PI*Z allele also encodes a structurally abnormal form of AAT with more severe dysfunction associated with higher risk of lung and liver disease. Approximately 95% of individuals with clinical manifestations of AATD have the PI*ZZ genotype. Other rare variants of *SERPINA1* exist and can also cause lung and liver disease. The PI*F allele (c.739C>T, p.Arg247Cys, g.94381049) results in a quantitatively normal but functionally abnormal AAT, causing decreased binding to neutrophil elastase. Individuals with this variant may have normal AAT levels but have an increased risk for lung disease, especially when inherited along with PI*Z allele. The PI*I allele (c.187C>T, p.Arg63Cys, g.94383051), similar to the PI*S allele, causes some structural abnormality in AAT with mild functional impact. However, when inherited along with the PI*Z allele, PI*I confers a higher risk of lung and liver disease.

Serum alpha-1 antitrypsin levels typically correlate with the *SERPINA1* genotypes as shown in Table 1. However, AAT levels can be significantly elevated in some clinical circumstances, which could mask AATD. Serum levels in patients with acute inflammation, cancer, non-AATD related liver disease, pregnancy, estrogen therapy, or blood transfusions may be discordant from genotype results.

Clinical Indications

According to available guidelines, diagnostic testing is recommended for:

- 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
- siblings of adults with AATD

Testing may be considered for:

- 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 vasculitis
- parents or children of adults with AATD
- screening for individuals >11 years of age in areas of AATD prevalence or in areas of high smoking rates

Table 1

GENOTYPE	Expected AAT Levels (based on Bornhorst 2003 data)	Disease Risks
PI*MM	102-254 mg/dL *Standard laboratory reference range: 90-200 mg/dL (per Dati 1996)*	No increased disease risks
PI*MS	86-218 mg/dL	No increased disease risks
PI*MZ	62-151 mg/dL	No increased disease risks
PI*MF	102-254 mg/dL	No increased disease risks
PI*MI	86-218 mg/dL	No increased disease risks
PI*SS	43-154 mg/dL	Possible pulmonary disease risk
PI*SZ	38-108 mg/dL	Possible pulmonary and hepatic disease risks
PI*ZZ	<52 mg/dL	Pulmonary and hepatic disease risks, risk of other AATD-related conditions (e.g., panniculitis)
PI*FS	86-218 mg/dL	Pulmonary disease risk
PI*IS	43-154 mg/dL	Possible pulmonary disease risk
PI*FZ	38-151 mg/dL	Pulmonary disease risk, possible hepatic disease risk
PI*IZ	38-108 mg/dL	Possible pulmonary and hepatic disease risks
PI*FF	102-254 mg/dL	Pulmonary disease risk

Methodology

Targeted variant analysis is performed using LightMix® and LightSNiP® melt curve technology (TIB MOLBIOL) on the LightCycler480 II (Roche) to identify four alleles of the *SERPINA1* gene, PI*S, PI*F, PI*I and PI*Z, which combine to create the genotypes listed in Table 1. Genomic DNA is isolated from the peripheral blood and four regions containing the variants of interest are amplified by PCR. Following PCR, the amplified DNA sequences are subjected to a temperature gradient, allowing fluorescently labeled probes targeted to each variant to bind to the double stranded DNA sequence and fluoresce. As the temperature increases, the probes dissociate, or "melt", at a specific temperature based on the nucleotide sequence of the probe and fluorescence decreases. Measurement of fluorescence throughout the gradient allows the specific temperature at which melting occurs to be recorded. Mismatches between the probe and the DNA sequence cause the probe/DNA hybrid to be less stable and the probe to melt at a lower temperature, allowing discrimination between variant and normal.

Interpretation

This laboratory developed test will not detect other mutations that may cause AATD. Uncommon variants or polymorphisms in the regions of interest may affect binding of LightMix® or LightSNiP® probes and may result in a false negative, false positive, or indeterminate result. Absence of the S, Z, F, and I alleles is consistent with (but does not confirm) the normal, aka wild type, PI*MM genotype. Importantly, there are over 100 known rare variants of *SERPINA1* that are not detected

by this PCR test. Therefore, correlation of the genotype with the patient's serum alpha-1 antitrypsin level and clinical manifestations is strongly recommended. When discrepancies exist between the enzyme level and targeted genotype results (e.g., the serum AAT level is low but no abnormal allele is identified by PCR), sequencing of the coding regions of the *SERPINA1* gene to identify rare mutations should be considered.

References

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Test Overview

Test Name	Alpha-1 Antitrypsin (*SERPINA1*) Targeted Genotyping
Ordering Mnemonic	HA1AT
Methodology	Targeted allelic discrimination assay by real-time PCR with high resolution melt analysis and fluorescence monitoring
CPT Code	81332

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