Improving Diagnosis for AATD through Testing Algorithm

Glenview, IL – Alpha-1 antitrypsin deficiency (AAT/AATD) is an underdiagnosed condition that predisposes individuals to chronic lung, liver, and other rare diseases. AAT is an acute phase protease inhibitor that plays critical anti-inflammatory roles, with its main target being neutrophil elastase (NE). AATD is caused by inherited mutations in *SERPINA1* (encoding AAT) that result in reduced or dysfunctional AAT. The general diagnostic criteria for AATD is a low serum AAT level or the presence of the most common pathogenic alleles by phenotyping, or both. However, when these tests are performed alone, they can miss many cases of AATD, as an array of *SERPINA1* mutations are associated with AATD and serum AAT levels are variable. To improve AATD diagnosis, researchers have developed a comprehensive testing algorithm that screens serum AAT levels, *SERPINA1* mutations, and AAT functional activity.

The new genotyping assay identifies the normal M allele and 19 pathogenic single nucleotide polymorphisms (SNPs). In subjects with suspected AATD where no pathogenic SNP was identified, second-tier testing employs next-generation sequencing of either *SERPINA1* exons or the whole gene (WES and WGS).

Genotypes identified by the SNP genotyping assay were accurately confirmed by the WES and WGS assays. WGS identified a potential splicing mutation in the 3′ UTR of *SERPINA1* in four subjects; three individuals were genotyped as MM and one as $M_{malton}S$, and three had moderately low serum AAT levels. The researchers evaluated the effect of this 3′ UTR mutation on AAT functional activity and found that the MM serum had normal activity while anti-NE activity was reduced for $M_{malton}S$. These data suggest that the newly identified intronic mutation may affect AAT expression, but likely not anti-NE activity. Furthermore, sera from subjects harboring the pathogenic Z, S, or F alleles all showed reduced anti-NE activity compared to the normal genotype (MM), suggesting that serum levels alone do not reflect the concentration of functional AAT for genotypes with these variants.

"We developed a comprehensive AAT testing algorithm that evaluates AAT genotype, expression, and functional activity in subjects with AATD indication," said Lorraine Abushanab, PhD, lead researcher and CHEST 2025 presenter. "Collectively, these assays concordantly established AAT genotype and phenotype, and also uncovered novel associations between *SERPINA1* mutations, AAT expression, and AAT functional activity that may have implications in AATD diagnosis."

AATD is underdiagnosed due in part to the complexity of *SERPINA1* genotypes and clinical manifestations. By combining assays that comprehensively evaluate AAT genotype and phenotype, this multitiered AATD testing algorithm could improve AATD diagnosis.

Further results will be shared at the CHEST Annual Meeting 2025 as part of the Whiffs of Wisdom in Bronchiectasis and Cystic Fibrosis Rapid Fire Original Investigations presentations, titled *A Comprehensive Algorithm Measuring Alpha-1 Antitrypsin (AAT) Serum Level, AAT Activity, and Sequencing* SERPINA1 *Exons or Whole Gene to Improve Diagnosis of AAT Deficiency.* The study abstract can be viewed on the journal *CHEST*® website.