Alpha-1 Antitrypsin Mutations: Is One Too Many?

The association between severe deficiency of alpha-1 antitrypsin and early-onset pulmonary emphysema has been known for several decades (1). Alpha-1 antitrypsin is encoded by the *SERPINA1* gene, and the most common genetic variants causing deficiency are termed S and Z. The molecular basis of the deficiency is the abnormal folding, polymerization, and retention of the variant proteins in the endoplasmic reticulum of hepatocytes (2). The Z variant causes a change in the amino acid sequence (Glu366Lys) and is associated with plasma levels that are 10–15% of the normal M allele, whereas the S variant (Glu288Val) causes a milder deficiency with plasma levels that are 50–60% of normal.

Although the risk for lung disease in ZZ homozygotes is clearly established, the role of additional rare variants of *SERPINA1* has not been adequately explored. This is an important issue, as there are more than 100 other rare variants in the *SERPINA1* gene that have known or predicted deleterious effects (3). In addition, whether there is an increased risk for lung disease because of a single copy of the Z allele has been a source of debate in the literature for many years (4).

In this issue of the *Journal*, Ortega and colleagues (pp. 540–554) (5) present a comprehensive survey of all genetic variants in the *SERPINA1* gene in a large study sample. The authors sequenced the entire *SERPINA1* gene in 2,168 heavy smokers (≥20 pack-years) drawn from the SPIROMICS (Subpopulations and Intermediate Outcome Measures In COPD Study) study. The subjects represent three ethnic groups, and measures of circulating alpha-1 antitrypsin levels were available for 64% of the study participants. This landmark study sheds light on the role of non-Z *SERPINA1* variants and heterozygosity for the Z allele in the pathogenesis of chronic obstructive pulmonary disease.

The sequencing identified 26 variants in the *SERPINA1* gene that cause an amino acid substitution in the alpha-1 antitrypsin protein and one frame-shift mutation that results in complete absence of protein. Several of the rare variants (allele frequency, <5%) were unique to a particular ethnic group. In the initial analysis, the aggregate effect of rare variants together with the Z and S alleles on measures of lung function and computed tomography scan evidence of emphysema was tested in each ethnic group. In non-Hispanic whites, highly significant additive effects were observed for several lung function and emphysema outcomes. In addition, the level of alpha-1 antitrypsin decreased with the presence of each additional variant. There were no similar associations in the African American and Hispanic groups, but the sample sizes were much smaller, and there were no individuals with two variants in either group.

When considered individually, only the Z allele was associated with any of the outcome measures. As expected, the presence of two copies of the Z allele was associated with large decrements in lung function and increased emphysema. Interestingly, a single copy of the Z allele was also associated with adverse effects on the outcome variables, with values intermediate between those for the normal genotype (MM) and ZZ homozygotes. These data support the results of previous studies (6, 7) and confirm that MZ heterozygotes are at increased risk for chronic obstructive pulmonary disease, at least in the presence of heavy smoke exposure.

To achieve a more detailed analysis of the variants, the study participants were categorized into six genotypic groups, separating the effect of the Z allele from the other variants. Using this approach, the authors were able to show that heterozygosity for the Z allele alone was associated with lung function and emphysema in non-Hispanic whites. This result suggests that the disease risk associated with a single copy of the Z allele is not solely a result of compound heterozygotes who have one Z allele and a rare variant in the other copy of *SERPINA1*. Furthermore, heterozygosity for any rare variant associated with low alpha-1 antitrypsin levels (values in the lowest decile) was associated with emphysema independent of the Z and S alleles.

In the African American and Hispanic groups, there were fewer significant associations, which was likely a result of the lower sample sizes. However, in African-Americans, a single base insertion in the 5′ untranslated region of *SERPINA1* was associated with low alpha-1 antitrypsin levels and greater functional small airways disease. *In vitro* data using a reporter gene suggested that the variant alters *SERPINA1* gene regulation, and thus may be the causal variant for this novel clinical association.

The study showed that the rare variants and the Z and S alleles considered in aggregate were associated not only with cross-sectional outcomes but also with accelerated rate of lung function decline during a 3-year-long period. However, there were no associations of individual variants or subgroups of variants with longitudinal outcomes even in non-Hispanic white patients, indicating that a longer follow-up and/or larger sample size may be needed for these analyses.

One of the main strengths of the study was that a large number of non-Hispanic white patients were sequenced across the entire *SERPINA1* coding region. Larger sample sizes for different ethnic groups need to be studied, but the identification of group-specific variants provides a good rationale for performing such studies. Another strength was that the study participants all had significant smoke exposure, thus reducing the potential for lack of penetrance of the variants. The availability of measurements of circulating alpha-1 antitrypsin was an important asset in this study, as it helped guide the interpretation of the genetic association data.

This study illustrates the difficulty in analyzing rare genetic variants: Although there may be significant associations when considered in aggregate (i.e., gene-based burden testing), there are often too few individuals with each variant to test individually with sufficient power. Nevertheless, rare variants are potentially important for the individual patient, and therefore additional large-scale studies such as this are warranted. ■

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In the Wrong Place at the Wrong Time: Microbial Misplacement and Acute Respiratory Distress Syndrome

Patients with acute respiratory distress syndrome (ARDS) are gravely ill, so any knowledge of actionable factors at play in their lungs will be of great clinical value. It is well recognized that pulmonary bacterial compositions are abnormal in patients with ARDS and that the presence of gut microbiota are a bad prognostic factor. In this issue of the *Journal*, Dickson and his colleagues (pp. 555–563) build on previous work that found gut-specific bacteria were common and abundant in the airways of patients with ARDS and that the presence of enteric organisms correlated with the intensity of inflammation (1–3).

The authors now report a prospective observational study of 91 mechanically ventilated critically ill patients, showing that outcomes were predictable at the time of admission to ICU by measurements in BAL fluid. None of their patients had received antibiotics prior to ICU admission. The authors assessed bacterial burden by quantitative PCR (qPCR), and they estimated the relative abundance of specific gut-associated bacteria by amplification and sequencing of the 16S ribosomal RNA gene. The principal finding was that sequences identifying bowel bacteria predicted the length of time on a ventilator.

The authors are careful and honest about the limitations of the study. The study materials came from the lower respiratory tract, which normally has a relatively low microbial biomass. A significant technical problem arose from the use of dilute miniature BAL specimens that had been previously centrifuged to separate eukaryotic cells. The consequent depletion of bacterial numbers in the studied supernatant magnifies a risk that background contamination may bias the results (4). The risk is addressed carefully in the paper, and a significant message may be that improved protocols are likely to yield even more conclusive results in future investigations.

DNA sequencing of highly variable regions of bacterial genomes (such as the universally present 16S ribosomal RNA gene) allows quantification of almost all the bacteria present in biological samples. Bacteria are identified as operational taxonomic units (OTUs) through database matches. A limitation of 16S sequencing is that OTUs can usually define phyla and genera but rarely identify species. Intraspecific strains of the same species can differ by 20% of their genes, so although 16S shows us the ballpark it tells nothing about individual players.

Bacterial communities can be described with strategies that derive historically from microbial ecology. Measures such as the Shannon diversity index, richness, and evenness capture community structures that are of value in environmental surveys or epidemiological studies. In human microbiology, these parameters may be used to predict microbial community resilience to infection. Their value in the catastrophic events leading to ARDS is uncertain, and it may not be surprising that diversity of lung bacteria did not significantly predict ICU outcomes in this study.

These community parameters are derived from relative abundances (i.e., percent of each sample) after random pruning of sequence reads to match the lowest sample count. This rarefaction can bias the interpretation of disease-associated bacteria, and it is recommended that unrarefied data be used in analysis of differences in taxa between disease states (5).

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