Why is Disease Penetration So Variable? Role of Genetic Modifiers of Lung Function in Alpha-1 Antitrypsin Deficiency

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Abstract

Individuals with alpha-1 antitrypsin deficiency (AATD) have marked heterogeneity in lung function, suspected to be related to a combination of both environmental (e.g., cigarette smoking) and genetic factors. Lung function is heritable in the general population and in persons with severe AATD. Several genetic modifiers of lung function in persons with AATD have been described; however, replication is lacking. A genome-wide association study (GWAS) of lung function in persons with AATD has yet to be performed and may inform whether genetic determinants of lung function are overlapping in persons with AATD and in the general population. As GWASs require large sample sizes for adequate power, genetic risk scores offer an alternate approach to assess the overlap of genetic determinants of lung function in the general population in persons with AATD. Where GWASs are limited to common genetic variant discovery, whole genome sequencing (for rare variant discovery) and integrative genomic studies (examining the influence of genetic variants on gene, protein, and metabolite levels) offer potential for an expanded discovery of genetic modifiers of lung function in AATD. In the following review we examine past descriptions of genetic modifiers of lung function in AATD and describe a path forward to further investigate and define the likely genetic modifiers of lung function in AATD.

Introduction

Severe alpha-1 antitrypsin deficiency (AATD) results in dramatically lower levels of protein, but the variability of clinical presentation, particularly with regard to lung function, is a well-known phenomenon. In 1989, Silverman et al studied lung function levels in 52 type Pi Z (Pi*ZZ or PiZ*Null) individuals with severe AATD, and showed that the majority of individuals not specifically ascertained based on low lung function levels had a forced expiratory volume in 1 second (FEV1) greater than 65% of predicted. 1 A 2017 mortality study by Tanash et al reported that never-smoking PiZZ individuals identified by population or family screening (i.e., not ascertained on respiratory or hepatic symptoms or disease) had a similar survival compared to never-smokers in the general Swedish
population. These studies highlighted not only the variability in lung function levels of persons with severe AATD, but also highlighted the impact of ascertainment bias in the interpretation of studies involving persons with severe AATD.

Cigarette smoking history significantly impacts lung function variability as well as mortality in AATD. Castaldi et al reported a non-linear relation between pack years of smoking and FEV\textsubscript{1} with a steep decline in FEV\textsubscript{1} for 0-20 pack years of smoking and a more gradual decline in FEV\textsubscript{1} for 20+ pack years of smoking (Figure 1). The most important predictors of FEV\textsubscript{1} % of predicted were age, age-by-pack-years interaction, sex, pack years of smoking (0-20 only), index case status (ascertainment adjustment), and childhood pneumonia. Abnormal lung function alone is likely insensitive to the effect of smoking; ever- and never-smokers with Pi*ZZ AATD have variability in symptoms. This was demonstrated in a study of 107 PiZ 30-year-old individuals in Sweden where PiZ ever-smokers reported significantly more phlegm, wheezing, and shortness of breath compared to PiZ never-smokers. Thus, cigarette smoking history and ascertainment bias are important contributors to the reported variability of lung function (and other clinical features) in severe AATD. However, smoking history and ascertainment, even when modeled with other important contributors to reduced lung function, do not account for the totality of lung function variability in AATD.

Heritability of Lung Function in Alpha-1 Antitrypsin Deficiency

In persons without AATD, genetic factors account for a proportion of the observed variability in the development of chronic obstructive pulmonary disease (COPD) and in the population variation in spirometry values including FEV\textsubscript{1}, forced vital capacity (FVC), and ratio of FEV\textsubscript{1}/FVC. The contribution of genetic variation to the variability in clinical features (such as spirometry levels) or the development of disease (such as COPD) is heritability. Heritability estimates for COPD range from 38%-63% with the heritability of FEV\textsubscript{1} and FEV\textsubscript{1}/FVC ratio ranging from 31%-50% and 45%-66%, respectively. In cystic fibrosis, another monogenic respiratory disease with a substantial impact on lung function, studies have found substantial heritability of lung function. In persons with severe AATD, genetic variation is also a likely explanation for the observed variability of the development of impaired lung function. In a familial study of PiZZ AATD individuals in 1990, Silverman et al reported a trend toward reduced FEV\textsubscript{1} in the PiMZ parents of AATD individuals with impaired FEV\textsubscript{1} (< 65% of predicted). The familial influence on reduced lung function was later investigated in the AAT Genetic Modifiers Study, a multicenter family-based cohort study of 378 individuals with severe AATD confirmed to be homozygous for the SERPINA1 Z allele (rs28929474). In this study, heritability...
estimates for post-bronchodilator FEV₁ and FEV₁/FVC ratio were -19% and -26%, respectively, when adjusting for index case ascertainment bias and smoking history.¹⁵ Thus, convincing evidence exists for the heritability of lung function levels in the general population and in persons with severe AATD.

**Candidate Gene Studies**

The candidate gene approach for finding genetic modifiers of disease starts with biologically plausible (i.e., with known function and potentially disease-causing) genes and associates genetic variation (usually single nucleotide polymorphisms [SNPs]) in those genes with the presence or absence of a disease (such as COPD) or with levels of a clinical feature (such as lung function).¹⁶,¹⁷ A positive genetic association analysis is typically taken as evidence for an association of the candidate gene with the disease or trait of interest. Several candidate gene studies, detailed below, have been performed to attempt to explain lung function variability in severe AATD (Table 1).

**Endothelial Nitric Oxide Synthase**

The endothelial nitric oxide synthase (NOS3) gene produces one of the enzymes responsible for the nitric oxide synthesis from L-arginine,¹⁸ and was chosen as a candidate gene in AATD because of nitric oxide’s role in the regulation of pulmonary vascular and airway tone.¹⁹ In a study of 345 persons with AATD compared to 93 controls, the authors focused their association analyses on 2 common genetic variants in NOS3, 774C/T and 894G/T, which are in strong linkage disequilibrium (i.e., the 2 genetic variants are highly correlated due to co-inheritance in a block, or haplotype, of genetic material). The authors reported an association between 2 NOS3 variants (774C/T and 894G/T) and severe lung disease (FEV₁ < 35% of predicted) in persons with AATD, noting that the allele frequencies of these 2 variants in persons with AATD and less severe lung disease (FEV₁ > 35% of predicted) were similar to the observed allele frequencies in a control population.²⁰ Thus, the authors postulated a modulatory effect of NOS3 on severe destructive lung disease.

**Glutathione S-Transferase Pi 1**

The glutathione S-transferase pi 1 (GSTP1) gene produces an enzyme important to cellular protection from cytotoxic agents through detoxification of electrophilic substances (such as exist in cigarette smoke) and is expressed in the lungs.²¹ A specific genetic variant (Ile105Val a.k.a. rs1695) in GSTP1 had been previously associated with COPD.²² With a possible functional role in cigarette smoke susceptibility and with prior association with COPD, Rodriguez et al sought to investigate an association between the frequency of the Ile105Val (rs1695) GSTP1 polymorphism and AATD.²³ The authors reported that the allele frequency of the GSTP1 variant rs1695 was similar in persons with COPD and in the general population (controls) though was significantly increased in persons with AATD; further, rs1695 was associated with reduced FEV₁ in current and former smokers with AATD, but not in never-smokers with AATD, and not in persons with non-AATD COPD.²³ Therefore, the authors concluded that GSTP1 modulated lung disease specifically in AATD current and former smokers.

**Interleukin-10 Gene**

In 2008, DeMeo et al evaluated 75 SNPs in 10 candidate genes including IL10, TNF, GSTP1, NOS1, NOS3, SERPINA3, SERPINE2, SFTPB, TGFB1, and EPHX1 (chosen for prior reported associations with either asthma and/or COPD) for association with either FEV₁ and FEV₁/FVC ratio in the AAT Genetic Modifiers family-based study.²⁴ Correlated SNPs (rs1800871 and rs1518110) in IL10 were significantly associated with FEV₁, FEV₁/FVC ratio, and moderate-to-severe COPD. The IL10 SNPs were the only SNPs showing significant associations with both quantitative spirometry and binary COPD status; further, the rs1800871 FEV₁ and FVC/FVC ratio association p values remained significant after adjustment for multiple testing (either 10 candidate genes or 75 SNPs). The study by DeMeo and colleagues was additionally notable in the lack of replication of the previously reported case-control study associations of SNPs in NOS3 and GSTP1 with lung function levels and COPD in persons with AATD.

**Tumor Necrosis Factor**

Tumor necrosis factor alpha (TNF, formerly known as TNFa) is an inflammatory cytokine shown to be elevated in sputum, bronchial biopsies and
circulation of COPD patients and was thus an attractive gene for COPD susceptibility. That said, many studies failed to replicate the initial report of TNF genetic variant associations with COPD and COPD-related phenotypes.\textsuperscript{25} Possibly owing to the increased role of inflammation in AATD compared to usual COPD, both DeMeo et al\textsuperscript{24} and Wood et al\textsuperscript{26} investigated TNF variants as potential genetic modifiers of AATD phenotypes. DeMeo and colleagues evaluated 5 TNF SNPs (rs1800629, rs361525, rs1800610, rs3091257, and rs769178) in the AAT Genetic Modifiers study and reported SNP associations with pre-bronchodilator FEV\textsubscript{1} (rs1800610 and rs769178), FEV\textsubscript{1}/FVC ratio (rs3091257 and rs769178), and moderate-to-severe COPD (rs769178). Investigating 4 TNF SNPs (rs1800629, rs361525, rs1799964, and rs3093662), Wood and colleagues reported an association of TNF promoter SNP rs361525 with chronic bronchitis, but found no significant associations of TNF variants with emphysema, bronchiectasis, lung function, or FEV\textsubscript{1} decline.\textsuperscript{26} Notably, the TNF variant rs361525 is in linkage equilibrium (not correlated) with any of the significant TNF variant (rs1800610, rs769178, and rs3091257) associations with lung function levels reported by DeMeo et al.\textsuperscript{24} In summary, in persons with PiZZ AATD, DeMeo et al reported a TNF variant association with chronic bronchitis (but not lung function). The 2 sets of TNF variants were not in linkage disequilibrium (i.e., they were not correlated) with each other (Figure 2) and thus these results represent potentially separate genetic signals.

### The Genome-wide Association Study Era

Though the candidate gene association studies above are based on biologically plausible processes, the results of the above candidate gene studies should be scrutinized on several points. First, none of the above results have been replicated, though replication

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**Table 1. Summary of Previously Reported Genetic Modifiers of Lung Disease in Alpha-1 Antitrypsin Deficiency**

<table>
<thead>
<tr>
<th>Gene(s)</th>
<th>Significant SNPs (as reported - rsid)</th>
<th>Association Phenotypes</th>
<th>Sample Size</th>
<th>Replication?</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NOS3</td>
<td>774C/T - rs1549758 894G/T - rs1799983</td>
<td>FEV\textsubscript{1} &lt; 35% Predicted</td>
<td>345 AATD 93 Controls</td>
<td>No</td>
<td>Novoradovsky et al\textsuperscript{20}</td>
</tr>
<tr>
<td>GSTP1</td>
<td>p.Ile105Val - rs1695</td>
<td>AATD vs. COPD vs. Control FEV\textsubscript{1} % Predicted</td>
<td>69 AATD 98 COPD 198 Controls</td>
<td>No</td>
<td>Rodriguez et al\textsuperscript{23}</td>
</tr>
<tr>
<td>IL10</td>
<td>rs1800871, rs1518110</td>
<td>FEV\textsubscript{1}, FEV\textsubscript{1}/FVC, COPD</td>
<td>378 Pi\textsuperscript{ZZ} Persons (family study)</td>
<td>No</td>
<td>DeMeo et al\textsuperscript{24}</td>
</tr>
<tr>
<td>TNF</td>
<td>rs1800610, rs769178</td>
<td>FEV\textsubscript{1}, FEV\textsubscript{1}/FVC</td>
<td>378 Pi\textsuperscript{ZZ} Persons (family study)</td>
<td>No</td>
<td>DeMeo et al\textsuperscript{24}</td>
</tr>
<tr>
<td></td>
<td>rs361525</td>
<td>Chronic Bronchitis</td>
<td>424 Pi\textsuperscript{ZZ} Persons (unrelated)</td>
<td>No</td>
<td>Wood et al\textsuperscript{26}</td>
</tr>
<tr>
<td>IREB2, HYKK, CHRNA3</td>
<td>rs2568494, rs8034191, rs1051730</td>
<td>pre-BD FEV\textsubscript{1} % Predicted</td>
<td>Discovery: 378 Pi\textsuperscript{ZZ} Persons (family study)</td>
<td>No replication of lung function associations. Gender and smoking interactions with genotype were replicated.</td>
<td>Kim et al\textsuperscript{10}</td>
</tr>
<tr>
<td>IREB2, CHRNA3</td>
<td>rs2568494, rs1051730</td>
<td>post-BD FEV\textsubscript{1} % Predicted</td>
<td>Attempted Replication: 458 Pi\textsuperscript{ZZ} Persons (unrelated)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

SNP=single nucleotide polymorphisms; FEV\textsubscript{1}=forced expiratory volume in 1 second; AATD=alpha-1 antitrypsin deficiency; COPD=chronic obstructive pulmonary disease; FEV\textsubscript{1}/FVC=ratio of FEV\textsubscript{1} to forced vital capacity; BD=bronchodilator
Figure 2. Correlation Structure of Genetic Variants Near *TNF* Previously Reported to Be Associated with Lung Function Levels in Persons with Alpha-1 Antitrypsin Deficiency
was attempted (and failed) for the association of NOS3 and GSTP1 with lung function in AATD. The reported associations with IL10 and TNF have yet to be subjected to replication.

One potential reason for failed replication of genetic modifiers of AATD is that many of the candidate gene studies were not controlled for potential confounding due to smoking history and few were controlled for confounding due to ancestry-related allele frequency differences known as “population stratification.”

Another major limitation is that the genetic variants often do not affect the nearest gene, as has been shown in the complex disease schizophrenia. Thus, several of the variants tested may actually not affect the gene of interest. Other limitations include lack of power and inadequate correction for multiple testing burden. Thus, as candidate gene studies have been described to have replication rates of approximately 1.2% and false positive to false negative ratios potentially exceeding 100:1, it is likely that the candidate gene studies of genetic modifiers of lung function in AATD represent false positive associations that will not be replicated.

One possible solution to address the poor replicability of candidate gene studies is to perform a genome-wide association study (GWAS) of lung function in AATD. GWASs associate genome-wide, common (greater than 5% population frequency) genetic variants with a disease (such as COPD) or a trait (such as lung function levels). In addition to more stringent techniques to adjust for population substructure and multiple testing, one of the major findings of a GWAS is that most of the findings are in novel genes; that is, not previously identified by the literature. These qualities have led many modern GWASs, with strict adjustment of \( p \) values (often to \( p < 5 \times 10^{-8} \)) to avoid false positive associations as well as the practice of replication in an independent sample to avoid unmeasured biases, to result in much more robust replication of putative genotype-phenotype associations.

Specific to obstructive lung disease, recent GWASs have provided important insights into the genetic susceptibility to reduced lung function and COPD.

Given the strength of these replicated GWAS findings, one question has been to determine whether these variants have similar impact in severe AATD. One of the first and still strongest associations with COPD is at the 15q25 locus. In 2012, Kim and colleagues examined genetic variants in the 15q25 region for possible genetic modifiers of lung function in AATD. This was a different type of candidate gene study compared to the aforementioned studies in 2 aspects: (1) the chromosome 15q25 region was chosen based on evidence from a GWAS of COPD, and (2) the study design included test (AAT Genetic Modifiers Study \( n=378 \)) and replication (UK AAT National Registry \( n=458 \)) cohorts. Evaluating 9 total SNPs in the chromosome 15q25 locus, Kim et al reported 2 nominal (i.e., \( p \) values were not significant when adjusting for multiple comparisons) SNP associations (rs2568494 in IREB2 and rs1051730 in CHRNA3) with both post-bronchodilator FEV1 and pre-bronchodilator FEV1/FVC ratio in the AAT Genetic Modifiers Study. These lung function associations were not replicated in the UK National Registry dataset, though the IREB2 SNP (rs2568494) was associated with emphysema (males only) and exhibited an SNP by smoking interaction. Thus, despite the use of GWAS evidence to inform the choice of a candidate genetic region, this study reinforced the difficulty in replication of genetic association analysis results in the search for genetic modifiers of lung function in AATD.

Whether these findings are because the 15q25 locus has a weaker effect size or truly no effect is still not clear. Sample size is critical for adequate power in genetic association analysis, particularly for GWAS. The discovery of genetic loci related to lung function in AATD may be limited owing to the small sample size of AATD cohorts with genetic data. A sufficiently large GWAS in alpha-1 deficiency has yet to be performed. The utility of GWASs in the discovery of genetic modifiers of monogenic lung disease (such as AATD) has been demonstrated in cystic fibrosis. As the genomics field advances, several additional avenues of exploration are emerging. GWASs typically interrogate only common variants, and the key genetic modifiers of alpha-1 may be rare or may have been poorly captured on existing genotyping platforms. Whole exome and/or genome sequencing offers opportunities to uncover genetic modifiers missed by a GWAS. A recent exome sequencing study in 9 Pi*ZZ individuals offers a first look at the potential of sequencing technology to contribute to our understanding of genetic modifiers of lung function in AATD. However, the small sample size (including only 2 former smokers) and lack of replication make the results difficult to interpret at
In this time. Whole genome sequencing data in large populations are increasingly available through efforts such as the Trans-Omics for Precision Medicine program through the National Heart, Lung, and Blood Institute at the National Institutes of Health and have the potential to enhance rare variant discovery for complex respiratory traits and diseases. Integrative genomic methods leverage genetic data against gene expression, methylation, proteomic, and metabolomics data, and may increase the power (over GWAS alone) to identify relevant genes and/or pathways.\textsuperscript{46-49}

Another path forward to answer the question about the population-derived GWAS loci relevance to lung function in AATD is via aggregate genetic risk scores. Genetic risk scores present a better-powered opportunity to evaluate if known genetic loci contributing to reduced lung function and COPD in the general population are relevant to persons with AATD. Genetic risk scores also may be more powerful ways to assess for the presence of gene environment interaction.\textsuperscript{50} Genetic risk scores are aggregate measures of the genetic risk to a particular trait or disease and are generated for an individual by counting the number of risk alleles (i.e., 0, 1, or 2) a person carries. These genetic risk scores can then be evaluated for association with a trait or disease.

For example, a recent GWAS of spirometry levels in the United Kingdom Biobank identified 279 genetic loci associated with reduced lung function and aggregated these variants into a genetic risk score demonstrating association with reduced FEV\textsubscript{1}/FVC ratio and showed that each standard deviation increase in the genetic risk score was associated with a 1.55 (95% confidence interval 1.48–1.62) increased odds for COPD and a \textit{P}-value of 2.87\textit{e}-75.39 A genetic risk score utilizing a subset of the above-mentioned 279 variants showed an association with lung function, emphysema, and small airways disease on quantitative computed tomography imaging.\textsuperscript{51} An analysis of a population-derived genetic risk score, such as the 279-variant genetic risk score, would allow researchers to establish if genetic modifiers of lung function in AATD are overlapping with the genetic risk for lower lung function in the general population or if the genetic risk for lower lung function in AATD is distinct. Answering this question is critical to guiding future research into the heterogeneity of lung function levels in severe AATD. Evidence of differences in the genetic susceptibility for lower lung function in the general population compared to AATD should prompt ongoing investigations, such as large-scale GWASs, into the distinct genetic modifiers of lung function in AATD.

Conclusion

The heterogeneity of lung function in persons with AATD is partially attributable to genetics. Discovery of genetic modifiers of lung disease may allow for a greater understanding of the underdiagnosis and variable clinical presentation of persons with AATD. Although previous studies have attempted to ascribe genetic variants in a variety of candidate genes to lung function traits in AATD, replication of these findings has been problematic. GWASs to discover novel genetic susceptibility loci as well as genetic risk scores of known COPD and lung function genetic loci may help to establish whether the genetic modifiers of lung function in AATD are overlapping or distinct from the genetic modifiers of lung function in the general population. Sequencing-based studies and integrative genomic studies offer additional exciting opportunities for future descriptions of the biologic underpinnings of lung function heterogeneity in AATD.

Declararion of Interest

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References


