Genetics of Chronic Obstructive Pulmonary Disease

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ABSTRACT

Variability in the susceptibility to develop chronic obstructive pulmonary disease (COPD) is related to both genetic and environmental factors. COPD is likely a genetically complex disease, but severe alpha 1-antitrypsin (AAT) deficiency [e.g., protease inhibitor (PI) Z] remains the only proven genetic risk factor for COPD. Even among PI Z individuals, substantial variability in lung function is observed, suggesting that genetic modifiers may influence the expression of lung disease in severe AAT deficiency. The variable development of COPD in smokers without alpha 1-antitrypsin deficiency and the familial aggregation of lung function measurements also suggest the presence of genetic influences on lung function growth and decline leading to COPD. Many candidate gene loci have been investigated as potential COPD genetic determinants by case-control genetic association studies. However, inconsistent results of these association studies have been frequent. Genetic heterogeneity and population stratification are two potential reasons for the conflicting findings between association studies. Linkage analysis studies have recently been published that may identify regions of the genome that contain COPD susceptibility genes. Future investigations of genetic influences in COPD should consider the use of family-based designs for association studies and the study of positional candidate genes within regions of linkage.

KEYWORDS: COPD, genetics, linkage analysis, association studies, alpha 1-antitrypsin deficiency

Objectives: Upon completion of this article, the reader should be able to: (1) describe the genetic basis of alpha 1-antitrypsin deficiency and understand its variable expression; (2) appreciate the familial aggregation of COPD; and (3) understand the current state of knowledge in new COPD gene identification.

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Chronic obstructive pulmonary disease (COPD) is a disease of increasing public health importance around the world. Global burden of disease estimates suggest that COPD will rise from the sixth to the third most common cause of death by 2020.1 The most important risk factor for the development of COPD is cigarette smoking, but only a minority of smokers will develop COPD. Although cigarette smoking is the most impor-
tant environmental risk factor for COPD, only 15% of the variation in forced expiratory volume at 1 second (FEV₁) is accounted for by smoking. As reviewed in this chapter, familial aggregation of lung function has been demonstrated, and COPD also demonstrates clustering within families. These findings are consistent with genetic influences for both level of lung function and susceptibility to develop COPD. This article provides an overview of evidence that supports a genetic contribution to pulmonary function, as well as a review of association and linkage studies that have been done to investigate the genetic determinants of COPD.

ALPHA 1-ANTITRYPSIN DEFICIENCY
COPD is a complex human disease associated with mostly irreversible airflow obstruction that is slowly progressive (especially in the setting of ongoing environmental insults like smoking). Severe alpha 1-antitrypsin (AAT) deficiency is the only defined genetic risk factor for COPD, and individuals with susceptible genotypes are at risk for severe, early-onset COPD. AAT is the major defense in the lung against neutrophil elastase. Shortly after the discovery of AAT deficiency, it was demonstrated that AAT deficiency followed a Mendelian pattern of inheritance (generally associated with the Z isoform of the protein) consistent with a single major gene effect. Cigarette smoking is an important impetus for lung function decline in protease inhibitor (PI) Z individuals (genotype PI ZZ or PI Z null) and accounts for some of the variability of disease expression in AAT deficient individuals.

However, individuals severely deficient in AAT develop COPD at variable rates, suggesting that, even among those with AAT deficiency, other genetic factors influence the development of disease. The decline in lung function among PI Z nonsmokers is also highly variable, suggesting the presence of other genetic modifying loci for this disease. As shown in Figure 1, Piitulainen and colleagues have demonstrated marked variability in pulmonary function among PI Z nonsmokers; in their population of PI Z subjects, they found that reduced pulmonary function was related to male gender, wheezing symptoms, and occupational exposure to respiratory irritants. In an investigation of potential genetic modifiers of severe AAT deficiency, one group has suggested that endothelial nitric oxide synthase (NOS3) may be an important modifying locus for the development of COPD in PI Z individuals. Further studies will be required to identify the genetic modifiers of AAT deficiency. Although AAT deficiency only accounts for a small percentage of COPD cases in the general population, it demonstrates that genetic factors can have important influences on the development of COPD. Moreover, because of the importance of AAT as a protector of lung integrity, identification of other genetic influences of disease expression in this condition may provide useful insight regarding the pathogenetic mechanisms of COPD for both AAT deficient and nondeficient individuals.

Figure 1  Forced expiratory volume at 1 second (FEV₁) as a function of age for 225 nonsmoking protease inhibitor (PI) Z subjects from the Swedish Alpha 1-Antitrypsin (AAT) Deficiency Register. Filled circles represent females, and open squares represent males. Reduced pulmonary function was associated with male gender, occupational exposure to respiratory irritants, and wheezing symptoms in subjects ≥ 50 years of age. (Reproduced with permission from E. Piitulainen et al.)
FAMILIAL AGGREGATION OF PULMONARY FUNCTION

Population and twin studies have contributed supporting evidence for the role of genetics in lung function. Spirometric measures of lung function are valuable phenotypes for assessing genetic determinants of baseline lung function and lung function decline. In general population studies higher correlation of lung function was demonstrated between first-degree relatives (parents and children, and siblings) than between spouses. Twin studies have allowed for quantitative estimates of the heritability of traits such as the FEV₁ and have suggested that approximately 50% of the variation in FEV₁ is related to genetic influences.

FAMILIAL AGGREGATION OF COPD

A hereditary basis for COPD was first suggested in 1845, but this observation remained essentially uninvestigated until the mid–twentieth century. Starting in 1959, observational reports were published of aggregation of COPD within families. Subsequent studies suggested an increased prevalence of airflow obstruction among first-degree relatives of individuals with COPD. These early studies set the stage for more in-depth investigations of COPD within families, which have recently gained momentum with the advent of modern genetic epidemiological techniques.

Silverman and colleagues have collected a cohort of individuals with severe, early–onset COPD, and they have also enrolled their family members. Proband with early–onset disease was used to enrich the population for the most severe phenotypes in an effort to increase the probability of finding significant genetic effects. First-degree relatives (parents, siblings, and children); older second-degree relatives (aunts, uncles, and grandparents); and other affected relatives were included. Control subjects were recruited from prior population–based studies and were matched with probands for age, gender, and smoking status. First-degree relatives of probands had an increased risk for having FEV₁ < 80% of predicted. Stratification by smoking status suggested that this elevated risk was found exclusively in first-degree relatives who smoked. Odds ratios in smoking first-degree relatives compared with smoking control subjects of 4.5 for FEV₁ < 80% predicted and 3.6 for chronic bronchitis were significant, with a nearly significant odds ratio of 3.5 for FEV₁ < 60% predicted. These results suggested familial aggregation of smoking–related decrements in pulmonary function for relatives of probands with early–onset COPD.

More recently, McCloskey and colleagues assessed spirometry in 173 siblings of COPD patients and matched control subjects. They found no airflow obstruction in nonsmoking siblings, but among smoking siblings, an odds ratio of 4.7 for airflow obstruction (defined as FEV₁ < 80% predicted with FEV₁/FVC < 0.7, where FVC represents forced vital capacity) was found compared with smoking control subjects. These findings are important because they suggest that COPD is a disease with both genetic and environmental contributions. Many genes have been studied in the pathogenesis of COPD secondary to their hypothesized roles in proteolytic and inflammatory pathways triggered by the effects of cigarette smoke. Candidate genes investigated in case–control genetic association studies have included genes involved in protease–antiprotease pathways, oxidant–antioxidant pathways, and inflammatory responses to cigarette smoke. A subset of these candidate genes is discussed in the following text.

COMPLEX HUMAN GENETIC DISEASES

The quest to identify genes associated with a disease is especially challenging when the evidence suggests that there is no major gene effect, and multiple environmental influences upon phenotypic expression are involved. Although the identification of such complex disease genes is challenging, two general approaches have been used: positional cloning based on genome scan approaches and candidate gene studies based on known pathophysiology. The most common study design in COPD genetics has been the case–control association study, using candidate genes selected from the known pathophysiology of COPD. As suggested by Silverman and Palmer, case–control genetic association studies can be a very powerful study design, but if complicating factors are not addressed, they may provide false positive results. Recently, linkage analysis results on families with severe, early–onset COPD have been published and suggest a new opportunity for understanding the potential genetic influences in COPD.

ASSOCIATION STUDIES IN COPD

A partial list of candidate genes analyzed by association studies will be discussed below. An important point is that almost every gene has at least one study that supports an association and at least one study that refutes the genetic association.

Proteases and Protease Inhibitors

ALPHA 1-ANTITRYPSIN

Although compelling evidence demonstrates an association of severe AAT deficiency with COPD, the evidence for association in heterozygous individuals (e.g., PI MZ) with COPD has been less definitive. Recently, several
Matrix metalloproteinases have provided additional insight into this controversy. Seersholm and colleagues found that PI MZ subjects from the Danish AAT Register were more likely to be admitted to the hospital for obstructive lung disease, but that this increased risk was primarily conferred by relatives of index PI Z subjects (tested for AAT deficiency because they had COPD). Sandford and colleagues found a higher rate of the PI MZ type among Lung Health Study participants with a rapid decline in pulmonary function compared to subjects with a slow decline in pulmonary function. Of note, the association was strengthened in the presence of a family history of COPD. Finally, Dahl and colleagues found a slightly increased rate of FEV₁ decline and airflow obstruction in PI MZ subjects compared with PI M subjects in the Copenhagen Heart Study. Thus it remains unclear if all PI MZ subjects are at slightly increased risk for COPD, or if a subset of PI MZ subjects are at substantially increased risk for COPD due to other genetic or environmental factors.

A mutation in the 3’ region of the AAT gene has also been recognized. This G to A single nucleotide alteration in the PI gene has not been associated with decreased AAT levels. The A allele has been associated with COPD in some populations; in other populations increased AAT levels. The A allele has been associated with COPD due to other genetic or environmental factors. A mutation in the 3’ region of the AAT gene has also been recognized. This G to A single nucleotide alteration in the PI gene has not been associated with decreased AAT levels. The A allele has been associated with COPD in some populations; in other populations increased AAT levels. The A allele has been associated with COPD due to other genetic or environmental factors.

Matrix metalloproteinases Several groups have suggested that matrix metalloproteinases have a key role in the development of emphysema. Metalloproteinases produced by macrophages play an important proteolytic role in the lungs of smokers. Matrix metalloproteinase (MMP)-9 and MMP-12 account for the majority of macrophage elastase in smokers. Investigations into polymorphisms of MMPs have been performed for both MMPs themselves and the tissue inhibitors of metalloproteinases (TIMPs) (of which TIMP-2 is an example). One of the promoter polymorphisms in MMP-9 is considered to be functional (C-1562T). In a cohort of 110 smokers and 94 nonsmokers in Japan, the T allele was associated with a radiographic (CT scan) diagnosis of emphysema (P = 0.02). Logistic regression revealed that the T allele was a significant risk factor for smoking-related emphysema (odds ratio of 2.69, P = 0.02), with a lower diffusing capacity in individuals with one or more T alleles (P = 0.02). In an investigation of MMP-1 (interstitial collagenase), MMP-9 (gelatinase B), and MMP-12 (macrophage elastase), haplotypes consisting of MMP-1 and MMP-12 variants were associated with the rate of lung function decline; an association between single variants in MMP-12 and lung function decline was not observed.

The TIMPs have also been studied as potential genetic determinants of COPD. Hirano and colleagues investigated TIMP-2 in a population of 88 individuals with COPD and 40 control subjects, hypothesizing that mutations in the TIMP-2 gene would result in a down-regulation of TIMP activity, with an increase in lung destruction related to unopposed action of metalloproteinases. They studied two polymorphisms in the coding region of TIMP-2 (G853A and G-418C). For the first polymorphism in exon 3, the G allele occurred at a higher frequency in those with COPD. In the second polymorphism in the promoter region, the C allele predominated in those with COPD compared to control subjects.

Alpha 1-antichymotrypsin Another potentially relevant protease inhibitor in the lung is alpha 1-antichymotrypsin. An alteration in an amino acid at position 227 in the protein (Proline227Alanine) has been associated with deficiency of alpha 1-antichymotrypsin. This polymorphism has been associated with COPD in one population; others have not found a similar association. Ishii and colleagues performed an investigation of 53 individuals with COPD (matched with 65 controls) for polymorphisms in the signaling peptide (Ala15Thr) and two exonic polymorphisms (Pro229Ala and Leu55Pro) of the alpha 1-antichymotrypsin gene. They found that alanine-15 homozygotes had an increased risk of COPD versus controls, with an odds ratio of 2.7 (95% CI 1.2–6.2). However, the variant under investigation may not be causative because it was not associated with a decrease in alpha 1-antichymotrypsin levels; the association could represent linkage disequilibrium with another allele. Replication of this association will be required.

Oxidant–antioxidant pathways In addition to the importance of the protease-antiprotease pathways in the pathogenesis of COPD, the oxidant–antioxidant pathways may also be critical. Genes in the antioxidant pathway, which are involved in detoxifying highly reactive oxygen species produced by cigarette smoke, seem like logical candidates that may be relevant to the pathogenesis of COPD. One particular candidate gene that has been studied is heme oxygenase-1. This enzyme protects cells from heme- and nonheme-related oxidant stress, as well as being functional for the degradation of heme biliverdin. A short tandem repeat polymorphism (a GT repeat sequence) located in the promoter of the heme oxygenase-1 gene was associated with emphysema in Japanese smokers. This association study included 100 individuals with emphysema and 101 control subjects; the investigators demonstrated that a higher number of GT repeat sequences was found more commonly in individuals with emphysema than in control subjects (odds ratio 2.4). The investigators suggested that the higher number of GT repeats contributed to down-regulation of gene expression; with down-regulation of gene expression, higher levels of oxidative stress would be enabled in the lung, potentially contributing to the development of emphysema.
Several groups have investigated other metabolizing enzyme polymorphisms in genes that are involved in detoxifying toxins associated with tobacco combustion. Microsomal epoxide hydrolase (EPHX1), expressed in bronchial epithelial cells, is important in the metabolism of epoxide intermediates of cigarette smoke. Two particular polymorphisms in the EPHX1 gene (Tyrosine113Histidine and Histidine139Arginine) have been investigated, and they are purportedly associated with rate of metabolism of toxic epoxides. The slower metabolizing form was associated with a higher proportion of individuals with clinical COPD and pathologically confirmed emphysema compared with control subjects (odds ratio of 5). In another study the polymorphism at amino acid 113 was associated with more severe COPD. However, as with other association studies in COPD candidate genes, these results have not been consistent, and the association of enzyme polymorphisms in this gene with disease was not found in other populations. Nonetheless, Sandford and colleagues did demonstrate an association between EPHX1 polymorphisms and lung function decline in cigarette smokers with airflow obstruction in the Lung Health Study.

Glutathione S-transferases also serve an important function in the metabolism of the aromatic hydrocarbons within cigarette smoke. There are several types of glutathione S-transferase, and variants in types M1, T1, and P1 have been investigated in association studies with COPD. In a population of Korean individuals, there was no association of M1 or T1 polymorphisms with COPD. However, in a Caucasian population, a deletion of the M1 gene has been associated with chronic bronchitis (in smokers) and homzygous deletion of the M1 type has been associated with emphysema in individuals diagnosed with lung cancer. In the glutathione S-transferase P1 gene homzygosity at position 105 for isoleucine was associated with COPD in Japanese individuals (Isoleucine 105 Valine).

INFLAMMATORY CYTOKINES

In addition to proteolytic and antioxidant pathways that lead to lung destruction in COPD, airway inflammation has a critical role in disease. Pro-inflammatory cytokines play an important role in perpetuating lung inflammation in COPD, so studying association for disease phenotypes with genes for inflammatory cytokines is a logical pursuit. Several inflammatory cytokines have been investigated; here we review the evidence for tumor necrosis factor (TNF).

Tumor Necrosis Factor

TNF is a pro-inflammatory cytokine that has been associated with activities that lead to neutrophil release and activation. As already discussed, neutrophils provide one of the most important sources of elastases in the lung; neutrophil elastase is inactivated by AAT. Any genetic effect that may modulate the amount of circulating TNF would potentially influence elastase levels in the lung. TNF alpha has a polymorphism in the promoter region (G to A at position –308), and TNF beta has a polymorphism in its first intron (A252G). In vitro, these particular polymorphisms have been associated with levels of TNF production. An association of the A variant of G-308A in TNF alpha has been reported with COPD in Taiwanese and Japanese individuals. Another group investigating this polymorphism in 106 patients with COPD and 99 controls found no increase in frequency of the A allele in individuals with COPD. However, they did note that individuals homozygous for the A allele had less reversibility of airflow obstruction (P < 0.05) and greater mortality from all-cause and respiratory-related deaths. Investigations in other populations have not revealed a similar association. More specifically, no association was found for this polymorphism with COPD in a Caucasian population, or in a population of Japanese individuals with COPD. In the Lung Health Study this polymorphism was not associated with lung function decline. Although intriguing from a pathobiological point of view, the involvement of TNF polymorphisms (like other inflammatory cytokines) in determining COPD-related phenotypes needs further investigation.

SUMMARY OF ASSOCIATION STUDIES IN COPD

The preceding data represent only a selection of the genes that have been investigated in COPD genetic association studies. A more complete list is available in Table 1. For most candidate genes that have been studied for genetic association in COPD, there are association studies that support an association of a particular genetic variant with COPD, whereas other studies refute that association. There are several methodological reasons why there might be conflicting results among different case-control studies. These include the influence of genetic heterogeneity between different populations, which may prevent replication of studies. Many of the reported case-control association studies have included relatively modest sample sizes, and corrections for the multiple comparisons involved in studying multiple genetic loci and multiple phenotypes have not been consistently applied. Another potentially important aspect of case-control studies is the appearance of positive associations secondary to the effects of population stratification. Population stratification may result from differences in ethnicity or geographic origin between case and control subjects. All of the reported genetic association studies in COPD have used the case-control design using candidate genes from known pathophysiology. Family-based genetic association studies, which are not susceptibility to supporting
Table 1  Candidate Genes Associated with COPD in Case-Control Studies

| Gene                                      | Reference(s) |
|-------------------------------------------|---------------|
| Alpha 1-antitrypsin                        | 11, 63-65     |
| Matrix metalloproteinase                   | -141          |
| Matrix metalloproteinase                   | -940          |
| Matrix metalloproteinase                   | -1241         |
| Tissue inhibitor of metalloproteinase-2 (TIMP-2) | 42            |
| Alpha 1-antichymotrypsin                   | 33, 36, 43, 44|
| Heme-oxygenase                            | 47            |
| Microsomal epoxide hydrolase              | 48-51         |
| Glutathione S-transferase (M1, T1, P1)    | 50, 52-54, 66, 67 |
| Tumor necrosis factor alpha                | 58-60, 68, 69 |
| Cystic fibrosis transmembrane conductance regulator | 70 |
| Human β2 adrenoreceptor                   | 71            |
| Human defensin                            | 172           |
| Cytochrome P4501A1                        | 73            |
| Vitamin D binding protein                 | 31, 74-76     |
| HLA                                        | 76-78         |
| Lewis ABO blood groups                     | 22, 79        |

associations due to population stratification, have not yet been reported in COPD. However, the results from genome-wide linkage studies for quantitative and qualitative COPD-related phenotypes should provide new directions in COPD research that are not limited to our current theories regarding COPD pathogenesis.

LINKAGE ANALYSIS OF COPD-RELATED PHENOTYPES

Recently, genome-wide linkage analysis was completed to search for those genetic loci that may contribute to lung function. Linkage of genetic markers to spirometric measures of lung function was assessed in 1578 members of 330 families unselected for any respiratory illnesses.\textsuperscript{61} FEV\textsubscript{1} demonstrated the strongest linkage to chromosome 6 (LOD of 2.4) and FVC to chromosome 21 (LOD 2.6). As genetic determinants of pulmonary function are identified, it will be intriguing to determine whether they contribute to normal variation of spirometric values, pathological reductions of pulmonary function as in COPD, or both. Regardless of their relationship to disease, studies of genetic determinants of pulmonary function within the normal range may provide important insight into the genetic determinants of lung growth and development.

Two recent papers from the Boston Early-Onset COPD Study presented genome-wide linkage analysis results within early-onset COPD families. Silverman and colleagues presented results for genome scan linkage analysis of qualitative and quantitative traits.\textsuperscript{28, 29} A genome-wide scan of short tandem repeat polymorphic markers was analyzed in 585 individuals who were members of pedigrees ascertained through severe, early-onset COPD probands (without AAT deficiency). Qualitative phenotypes included mild airflow obstruction (FEV\textsubscript{1} < 80\% predicted, FEV\textsubscript{1}/FVC < 90\% predicted) and moderate airflow obstruction (FEV\textsubscript{1} < 60\% predicted, FEV\textsubscript{1}/FVC < 90\% predicted) and the presence or absence of chronic bronchitis. Table 2 summarizes the results from this assessment of qualitative phenotypes. The strongest evidence for linkage to moderate airflow obstruction in all subjects was on chromosome 12 (LOD = 1.70). When this analysis was limited to smokers, linkage to mild airflow obstruction resulted in a maximum LOD of 1.64 on chromosome 19; the maximum lod score for chronic bronchitis was 2.08 on chromosome 22. Additional markers were analyzed in the 12p region and revealed an LOD

Table 2  Candidate Chromosomal Regions from Linkage Analysis of Severe, Early-Onset COPD Families\textsuperscript{28, 29}

| Phenotype               | Chromosomal Regions of Linkage | LOD Score |
|-------------------------|--------------------------------|-----------|
| Qualitative phenotypes  |                                |           |
| Mild airflow obstruction| 8                              | 1.36      |
| Moderate airflow obstruction| 12                         | 1.70 (2.13*) |
| Chronic Bronchitis      | 19                             | 1.54      |
| Quantitative phenotypes |                                |           |
| FEV\textsubscript{1}    | 12                             | 1.53 (2.43*) |
| FVC                     | 1                              | 2.05      |
| FEV\textsubscript{1}/FVC| 2                              | 4.12      |
|                          | 1                              | 1.92      |
|                          | 17                             | 2.03      |

\*LOD score for linkage to this region increased after the inclusion of 12 additional markers in the analysis. FEV\textsubscript{1}, forced expiratory volume at 1 second; FVC, forced vital capacity.
score in all subjects of 2.13 for moderate airflow obstruction and 1.43 for mild airflow obstruction, using nonparametric analytic techniques. In smokers, the maximum two-point LOD score for mild airflow obstruction was 3.14 for marker D12S1715. Taken together, these results provide suggestive evidence for a susceptibility locus for early-onset COPD on chromosome 12p. However, LOD score thresholds of 3.3 have been recommended for significant linkage and 1.9 for suggestive linkage for pedigree-based linkage studies. Of note, linkage of this region to qualitative airflow obstruction phenotypes was not obtained. Therefore, this linkage on chromosome 2q may represent an airflow obstruction locus that was detected because quantitative phenotypes have increased power to detect linkage. Another possibility is that this region may include a genetic determinant of dysanaptic lung growth. As reduced FEV<sub>1</sub>/FVC ratios have been associated with subsequent decline in pulmonary function, it is likely that an important disease locus for COPD is located in this chromosomal region. One of the advantages of linkage analysis is that it can reveal regions of the genome that should be analyzed more closely in association studies. In this case, the interesting candidate genes in the regions of 2q and 12p include the interleukin-8 receptor on chromosome 2 and microsomal glutathione S-transferase-1 on chromosome 12p.

CONCLUSIONS

Multiple studies have demonstrated heritability of lung function and familial aggregation of COPD. The only proven genetic risk factor for COPD remains severe AAT deficiency; however, even in the setting of this known genetic susceptibility, the development of lung disease is highly variable. This variable susceptibility is likely a function of genotype-by-environment interactions, where critical exposures such as cigarette smoke, air pollution, and toxic inhalations contribute to lung function decline and disease progression. Much like AAT deficiency, individuals who are not deficient in AAT have variable expression of lung disease in the setting of exposures such as cigarette smoke. Case–control association studies have provided a means to investigate genetic variants that may play a role in COPD, but results have not been consistent across studies. Linkage analysis results from genome-wide linkage analysis may provide useful new directions to lead to the identification of genes involved in the development of pulmonary emphysema and chronic bronchitis. New insight from genetic studies, together with the knowledge about the different pathophysiological pathways that contribute to COPD, may lead to improved understanding of disease susceptibility, interactions with relevant environmental factors, and treatment options for this debilitating pulmonary disease.

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