

## Review

# The genetics of chronic obstructive pulmonary disease

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## Abstract

Chronic obstructive pulmonary disease (COPD) is a significant cause of global morbidity and mortality. Previous studies have shown that COPD aggregates in families, suggesting a genetic predisposition to airflow obstruction. Many candidate genes have been assessed, but the data are often conflicting. We review the genetic factors that predispose smokers to COPD and highlight the future role of genomic scans in identifying novel susceptibility genes.

**Keywords:** association studies, chronic obstructive pulmonary disease, emphysema, genetics, lungs

## Introduction

Chronic obstructive pulmonary disease (COPD) is defined as airflow obstruction that does not change appreciably over a period of several months [1]. It is a syndrome composed of chronic bronchitis, small airways disease (bronchiolitis) and emphysema, which vary in proportion between affected individuals. COPD is a major cause of global morbidity and mortality, and affected 44 million people in 1990. Indeed, 14 million people suffer from COPD in the United States alone, where this condition resulted in nearly 92 thousand deaths in 1995 [2]. It is estimated that 2.88 million people in the world will die from COPD this year and the numbers are growing [3]. COPD is becoming more prevalent amongst Western women and is set to explode in developing countries such as India, Mexico, Cuba, Egypt, South Africa and China [4]. Severe  $\alpha_1$ -antitrypsin deficiency is the only proven genetic risk factor for the development of COPD. Here we review the evidence from human studies that other genetic determinants are also important in the pathogenesis of this condition. Although genetic studies using animal models may be very useful [5–7], they are beyond the scope of this review.

## Environmental factors that predispose to COPD

The major environmental risk factor for the development of COPD is cigarette smoking. In non-smokers, the forced expiratory volume in 1 s ( $FEV_1$ ) declines at a mean rate of approximately 20–30 ml per year during adult life. In most smokers, this mean rate of decline is increased to 30–45 ml per year, but in the subset of cigarette smokers who are susceptible to developing COPD the rate of decline is 80–100 ml per year. There is evidence of a dose–response relationship between the severity of lung disease and the pack-years of cigarettes smoked [8–11], but only 15% of the variability in  $FEV_1$  is accounted for by smoking history. It remains unclear whether susceptible smokers represent a discrete subset of individuals, or if susceptibility to COPD is a continuous trait. Postmortem studies of smokers have demonstrated substantial variability in the severity of emphysema, but most heavy smokers had at least some pathological evidence of disease [12,13].

Other environmental factors have also been implicated in the development of chronic irreversible airflow obstruction.

There has been an association of COPD with environmental pollution since the great London smogs of the 1950s [14]. Domestic and cooking fumes may also be important risk factors, especially in regions where indoor wood stoves are used with poor ventilation [15]. In certain cities in China, non-smoker emphysema death rates are almost 100 times greater than those of the non-smoker in the USA [4]. Exposure to dust in the coal and gold mining industries, and to gas in cadmium mining, has been linked to the development of airflow obstruction [16–18]. Exposure to dust and gases by underground tunnel workers has similarly been associated with respiratory symptoms and COPD, as well as with an accelerated decline in FEV<sub>1</sub>, compared to matched controls who worked above ground [19]. COPD is more common in individuals of lower socio-economic status [16] and has a poorer prognosis when associated with low body-mass index [20] and with bronchial hyper-reactivity [21,22]. There is also evidence that previous viral infections predispose smokers to COPD [23], and an increasing awareness that diet [24] and factors involved during *in utero* [25,26] and adolescent lung development [27] may be important for the subsequent predisposition to obstructive lung disease. These other environmental factors are likely to be much less important than cigarette smoking, but they may interact with smoking to increase the risk of COPD [28,29].

### Familial clustering of COPD

The observation that only a minority of cigarette smokers develop COPD suggests that additional factors contribute to the impact of smoking on the development of chronic airflow obstruction. The most important genetic factor in the development of emphysema is the Z allele of  $\alpha_1$ -antitrypsin, which results in plasma levels of this protein that are 10–15% of that produced by the normal M allele [30]. The levels are low because 85% of the synthesised mutant Z  $\alpha_1$ -antitrypsin is retained as polymers within hepatocytes [31,32]. Homozygotes for the Z allele (denoted PI Z) are greatly predisposed to developing emphysema if they smoke [33,34]. However, severe PI Z  $\alpha_1$ -antitrypsin deficiency makes up only 1–2% of all cases of COPD and there is considerable variability in FEV<sub>1</sub> between current and ex-smokers with the same PI Z genotype [35]. This suggests that other coexisting genetic factors must predispose to lung disease in PI Z individuals.

A logical follow-on from the association of  $\alpha_1$ -antitrypsin deficiency with emphysema is an assessment of the risk of COPD in heterozygotes who carry an abnormal Z allele and a normal M allele. These individuals have plasma  $\alpha_1$ -antitrypsin levels that are approximately 65% of normal. A population-based study demonstrated that PI MZ heterozygotes do not have a clearly increased risk of lung damage [36]. However, if groups of patients are collected who already have COPD, then the prevalence of PI MZ individuals appears to be increased [37,38]. In addition, a

longitudinal study has demonstrated that among COPD patients (most of whom were smokers), the PI MZ heterozygotes have a more rapid decline in lung function [39]. These data suggest that either all PI MZ individuals are at slightly increased risk for the development of COPD, or that a subset of the PI MZ subjects are at substantially increased risk of pulmonary damage if they smoke. An alternative explanation is that the apparent increased risk among PI MZ subjects reflects ascertainment bias and the elevated rate of PI MZ subjects among COPD cases reflects the influence of other, as yet unidentified, factors.

Several previous studies have suggested that genetic factors other than  $\alpha_1$ -antitrypsin deficiency may be involved in the susceptibility of cigarette smokers to chronic airflow obstruction. These studies have demonstrated a significantly higher prevalence of COPD amongst relatives of index patients than amongst control groups [40–42]. The findings have been confirmed recently in a study of 44 patients with severe COPD (FEV<sub>1</sub> <40% predicted) aged 52 or less [43]. The prevalence of airflow obstruction in smoking siblings was approximately 3-fold greater than in smoking control subjects.

### Association studies

The clustering of COPD in families has resulted in the recognition of a genetic component to this multifactorial disease. There are increasing numbers of association studies assessing candidate genes that may predispose smokers to COPD (Table 1). These studies rely on the comparison between a set of COPD patients and control subjects. Ideally, the case and control populations are matched for age, sex, smoking history and occupational exposures to avoid confounding by non-genetic factors; subjects who have severe  $\alpha_1$ -antitrypsin deficiency are excluded. Matching should also include ethnicity and geographic origin in order to avoid the effects of population stratification, which can provide false-positive evidence for a causative genetic association [44]. However, case-control studies are often complicated by small sample sizes and the difficulty in matching for all known environmental factors that predispose to COPD. Moreover, researchers can only assess genes that have already been described; novel genetic determinants can not be identified directly from the study of known candidate genes. This candidate gene approach is relatively straightforward, but the results are often difficult to interpret.

The first genetic association studies in the 1970s used the small number of genetic polymorphisms that were then known: largely blood group antigens [45]. Subsequently, candidate genes thought to be involved in the pathophysiology of COPD have been examined. For instance, polymorphisms in the proteins that protect the lungs against proteolytic attack have been assessed. A polymorphism that predisposed smokers to develop COPD (Taq-1

**Table 1****Candidate genes that have been associated with COPD in case-control studies**


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<b>PI MZ <math>\alpha_1</math>-antitrypsin deficiency</b>
<b>Tumour necrosis factor <math>\alpha</math></b>
<b>Microsomal epoxide hydrolase</b>
<b>Glutathione S1-transferase</b>
<b>Heme Oxygenase-1</b>
Taq-1 polymorphism of $\alpha_1$ -antitrypsin
Alpha <sub>1</sub> -antichymotrypsin
Vitamin D binding protein
ABO Blood Group
ABH Secretor Status
Cystic fibrosis transmembrane regulator
HLA
Cytochrome P450

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For most of these loci, some studies have supported a significant association while other studies have refuted the association. Candidate genes for which there is the strongest supporting evidence are shown in bold.

G→A) [46] was detected by the restriction enzyme Taq-1 in the 3' non-coding region of the  $\alpha_1$ -antitrypsin gene. The Taq-1 (G→A) allele, conferring the absence of this Taq-1 site, was present in 18% of a population of emphysema patients, but in only 5% of blood donor control subjects. This association was confirmed by a second European group [47]; further studies revealed that the polymorphism was in a regulatory sequence, and that the Taq-1 (G→A) allele reduced the production of  $\alpha_1$ -antitrypsin in response to the inflammatory cytokine interleukin-6 (IL-6) [48]. Subsequent studies by other groups refuted the association with COPD [49,50]. Moreover, although the Taq-1 (G→A) allele reduced the production of  $\alpha_1$ -antitrypsin *in vitro* [48], it had no effect on the plasma level of  $\alpha_1$ -antitrypsin *in vivo* or on the rise in levels of this protein during the inflammatory response [51–53]. Thus the role of this polymorphism in the pathogenesis of COPD remains unproven.

The logical follow-on from this work was the assessment of mutations in another plasma proteinase inhibitor,  $\alpha_1$ -antichymotrypsin, to explain the susceptibility of smokers to COPD. No patients who are homozygotes for  $\alpha_1$ -antichymotrypsin deficiency have ever been described, but two point mutations that alter the amino acid sequence (229Pro→Ala [54] and 55Leu→Pro [55]) in the  $\alpha_1$ -antichymotrypsin gene have been associated with COPD. The 55Leu→Pro point mutation causes a conformational change within the protein [56], resulting in low circulating levels of the inhibitor and its retention within hepatocytes. The retention of this protein has been associated with

intracellular hepatic inclusions of  $\alpha_1$ -antichymotrypsin and cirrhosis [54] analogous to that associated with  $\alpha_1$ -antitrypsin deficiency [31]. However, the association of these two polymorphisms with COPD was not replicated in a study of 168 COPD patients and 61 control subjects [57]. Moreover, the mutations are uncommon, making it unlikely that they are a frequent contributor to the pathogenesis of COPD [50,57].

Two other plasma serine proteinase inhibitors, secretory leukoprotease inhibitor and elafin, are also potential candidates, as mutations in these genes may reduce the anti-proteinase screen and predispose smokers to airflow obstruction. No polymorphisms were detected in the secretory leukoprotease inhibitor gene in 10 patients with early onset irreversible airflow obstruction [58]. Moreover, although polymorphisms have been described in the elafin gene [59], they have not been assessed in patients with COPD. Similarly, no polymorphisms have yet been described in tissue inhibitor of metalloproteinase genes in patients with COPD.

The cytokine tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) plays an important role in the inflammatory response. Approximately 10% of the population have a polymorphism (G→A) at position -308 in the 5' promoter region of the gene. This variant is known as tumour necrosis factor 2 and results in a 2-fold increase in the plasma concentration of TNF- $\alpha$  following gene activation [60]. The -308 polymorphism was found to be more prevalent in a group of Taiwanese patients with COPD, when compared to controls matched for age, sex and smoking who did not have airflow obstruction [61]. It is plausible that smokers who have a higher level of TNF- $\alpha$  in the bronchial mucosa have more bronchitis and more airflow obstruction. However, these findings have been refuted by others who have assessed the association between this polymorphism and airflow obstruction in Caucasian populations [62,63].

Each puff of a cigarette contains  $10^{17}$  free radicals, which can cause lung damage. Thus defects in the detoxification of these reactive species may predispose smokers to airflow obstruction and emphysema. Indeed the proportion of patients with slow microsomal epoxide hydrolase activity was significantly higher in patients with COPD and emphysema, when compared to healthy blood donor controls [64]. The smoking history of the blood donor control group was not recorded. These findings have been supported by Paré and colleagues, who have assessed a well-characterised cohort of patients from the Lung Health Study [65]. These patients were all smokers and had spirometric signs of early COPD. They were followed up for five years as part of a longitudinal study and then stratified into two groups: those smokers whose lung function showed a significant decline and those whose did not. Association analysis demonstrated a significantly higher

prevalence of the slow-detoxifying epoxide hydrolase in those patients who showed a progressive decline in lung function compared to those who did not. These findings were not reproduced by another group who assessed the polymorphism in a Korean population [66].

More recently, Yamada and colleagues reported an association between COPD and a short tandem repeat polymorphism in the heme oxygenase-1 gene promoter [67]. The protein that this gene encodes also plays an important antioxidant role in the lung, and there is *in vitro* evidence that the polymorphism in the gene promoter region reduces the upregulation of heme oxygenase-1 in response to reactive oxygen species in cigarette smoke. Although the possibility that microsomal epoxide hydrolase and heme oxygenase-1 might be associated with obstructive lung disease is biologically appealing, further association studies are required in other well-characterised COPD populations with matched control subjects or, ideally, with family-based association study designs.

Finally, mutations in enzymes that generate protective antioxidants have also been associated with the development of COPD. The glutathione S1-transferases (GSTs) are a family of enzymes that catalyse the conjugation of reduced glutathione with various electrophilic compounds. They are divided into the alpha (GSTA), mu (GSTM), pi (GSTP), theta, sigma, and kappa subclasses [68]. A polymorphism in exon 5 (Ile105) of GST P1 is located in the substrate binding pocket and has considerable effects on catalytic activity. It was significantly more common in men with irreversible airflow obstruction than in controls who were current smokers, but who had no evidence of COPD [68].

### What do candidate gene association studies tell us about disease processes in COPD?

This complex picture is starting to show similarities to the quagmire that bedevils the field of asthma genetics. However, unlike asthma genetics, linkage studies in COPD have not been performed to identify regions of the genome likely to contain susceptibility genes, in which association studies with candidate genes may be more productive. The inconsistent results from case-control association studies are likely to relate to differences between study populations and the relatively small sizes of the populations under consideration. In addition, failure to account for population stratification differences between cases and controls within a particular study, and failure to correct adequately for the multiple comparisons involved in studying multiple polymorphisms with multiple phenotypes, is also likely to be problematic. However, several messages can be drawn from the association studies that have been undertaken to date. It is clear that many researchers continue to focus on the well-established hypotheses of lung damage: proteinase-anti-proteinase and oxidant-antioxidant imbalance. At least some smokers

with a MZ  $\alpha_1$ -antitrypsin phenotype may be more likely to develop COPD than smoking matched controls, but the Taq-1 polymorphism in the 3' non-coding region of the PI locus has not been proven to confer an increased risk of lung disease. Heterozygote deficiency of  $\alpha_1$ -antitrypsin is so uncommon that even if it is ultimately shown to have a pathophysiological effect, it will contribute to the development of airflow obstruction in only a few smokers. There is growing evidence for the role of antioxidant imbalance in the pathogenesis of airflow obstruction, which is supported by association studies between COPD and variants in epoxide hydrolase and GSTs that detoxify free radicals and other tobacco products. Before these associations are generally accepted, they must be subjected to scrutiny with further association studies.

### Genomic scans to identify genes that predispose smokers to COPD

The association studies described above have all been conducted with variants in known candidate genes. Clearly our understanding of COPD would be revolutionised if a new gene or genes could be discovered that explained the predisposition of a minority of smokers to develop COPD. An alternative approach to this problem is to detect novel genes using linkage analysis in families of COPD patients, using polymorphic markers throughout the genome. If a marker segregates with COPD in affected relatives, then it indicates that this marker is located near to one or more genes that cause this disease. In order for this approach to be successful, it requires a large number of well-characterised affected relatives; either extended pedigrees or nuclear families can be used.

One of our research groups (EKS) has been focusing on linkage analysis of extended pedigrees of patients with severe, early-onset COPD. A genome screen of 72 extended pedigrees (600 individuals) has been performed by the National Heart, Lung, and Blood Institute (NHLBI) Mammalian Genotyping Service; analysis of this data is currently underway. However, the sample size is modest and it is unclear how far generalisations can be made from this population to older COPD subjects.

To study the genetics of COPD in subjects at ages more typical for the development of this disease, a large number of families will be required. The magnitude and organisation of a network to recruit the thousands of patients that are required for such studies is extremely expensive. A pharmaceutical company (Glaxo-Wellcome) has funded a consortium that spans 10 centres in seven North American and European countries. The consortium, which is led by the authors, involves collaboration between universities and industry designed to recruit nuclear families of COPD patients. This consortium has started to recruit 3000 families in order to identify 1500

affected sib pairs with COPD. The index cases (proband) and their siblings are being screened with respiratory questionnaires, spirometry and high resolution chest CT scans. The collection of this data from 3000 patients with COPD and their siblings will provide unique insights into the pathophysiology of airflow obstruction and, most importantly, the genetics of this condition. The search for new genes that predispose smokers to COPD will be undertaken using linkage analysis of COPD with genomic scan data from DNA-based polymorphisms throughout the genome. Strong linkage between regions of the genome and COPD-related phenotypes will identify locations on chromosomes that need to be assessed in more detail. Clearly, the rapidly advancing project to fully sequence the human genome will provide a 'road map' of the genes in the regions of interest, thereby rapidly accelerating the identification of genes that result in COPD.

### Benefits of cloning genes that predispose smokers to COPD

Why have so many workers put so much effort and resources into searching for genes that predispose to COPD? There are several answers. The identification of new genes would greatly improve our understanding of a condition that has for 37 years rested largely on the observation that deficiency of a protective anti-proteinase ( $\alpha_1$ -antitrypsin) is associated with emphysema. Novel genes would allow the assessment of new mechanisms and pathways in disease and provide new therapeutic opportunities. At-risk individuals could be identified by screening and strongly advised to abstain from smoking and avoid occupations where there are high loads of environmental dusts. Finally, new genes may help to explain other diseases. There is epidemiological evidence that COPD and lung cancer share a common familial component other than smoking [69,70]. The discovery of novel genes that predispose to COPD may therefore have a major impact on our understanding of the pathogenesis of cancer.

### Conclusion

COPD is an enormous cause of global morbidity and mortality that is becoming an even greater health problem with the growing use of cigarettes around the world. Mutations in the anti-proteinase and antioxidant screen are currently the best candidates to explain part of the genetic risk of COPD. However, new candidates need to be assessed in order to improve our understanding of the development of this disease. The recruitment of large numbers of affected siblings with COPD will provide the basis for whole genome scans to discover novel genes that predispose smokers to airflow obstruction. This will be greatly aided by the rapid completion of the human genome project. Taken together, it is a very exciting time for all those interested in the pathogenesis of this all too common disabling condition.

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