Genetic risk factors for chronic obstructive pulmonary disease

A.J. Sandford, T.D. Weir, P.D. Paré

Chronic obstructive pulmonary disease (COPD) is characterized by decreased expiratory flow rates, increased pulmonary resistance and hyperinflation. The most important risk factor for the development of COPD is cigarette smoking [1]. Cigarette smoke, in combination with other factors, leads to two pathophysiological processes in the lung. The first is proteolytic destruction of the lung parenchyma, which increases the size of the airspaces; these eventually coalesce to form emphysematous spaces. The development of emphysema is associated with a loss of lung elastic recoil. The second process is inflammatory narrowing of peripheral airways, which is characterized by oedema, mucus hypersecretion and fibrosis, scarring, distortion and obliteration of peripheral airways. The loss of lung elastic recoil and the narrowing of the peripheral airways combine to decrease maximal expiratory flow from the lung and contribute to hyperinflation. In conjunction with gas exchange abnormalities, hyperinflation produces the symptoms of COPD.

Despite the clear association of smoking and airway obstruction, there remains marked interindividual variation in the response to cigarette smoke. This indicates that there are additional genetic or environmental cofactors, which contribute to the development of COPD. It has been estimated that only 10–20% of chronic heavy smokers will ever develop symptomatic COPD [2, 3]. Co-factors, such as childhood viral respiratory infections and environmental and occupational pollution, undoubtedly play a role in determining this susceptible subset. Furthermore, there is evidence that genetic susceptibility is of major importance. The epidemiological and clinical data that demonstrate a hereditary contribution to the development of COPD are summarized in table 1. Although the results of several of these studies show an aggregation of COPD in families, there is no clear Mendelian pattern of inheritance. Case-control studies have shown an increased prevalence of COPD in the relatives of cases compared to relatives of controls, which cannot be explained by differences in other known risk factors. There is also a higher prevalence

Table 1. – Studies that demonstrate a genetic component to the development of COPD

<table>
<thead>
<tr>
<th>Study type</th>
<th>Ref.</th>
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<tbody>
<tr>
<td>Study showing clustering of COPD in families</td>
<td>[4]</td>
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<tr>
<td>Family studies showing increased incidence of COPD or chronic bronchitis in relatives of cases compared to relatives of controls</td>
<td>[5–12]</td>
</tr>
<tr>
<td>Studies showing significant correlations in lung function between parents and children and between siblings, and higher correlation between parents and children, or between siblings than between spouses</td>
<td>[4, 7, 13, 14]</td>
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<tr>
<td>Studies showing decreased prevalence of disease or less similarity in lung function with increased genetic distance</td>
<td>[5, 15, 16]</td>
</tr>
<tr>
<td>Family studies showing a major gene effect or a genetic component to pulmonary function</td>
<td>[17, 18]</td>
</tr>
<tr>
<td>Studies of pulmonary function in monozygotic and dizygotic twins</td>
<td>[15, 19–24]</td>
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COPD: chronic obstructive pulmonary disease.
of reduced lung function among the children of patients who have COPD than among their spouses. Cross-sectional studies have shown decreasing prevalence of disease and less similarity in lung function with increasing genetic distance. Studies of twins support a large genetic contribution to the variability in lung function. Heritability estimates for forced expiratory volume in one second (FEV1) range 0.5–0.8. Webster et al. [21] studied the effects of smoking on lung function in monozygotic and dizygotic twins. They found that when one monozygotic twin was susceptible to the effects of cigarette smoke, both twins developed reductions in lung function, whereas other monozygotic twin pairs appeared to be nonsusceptible and, despite similar smoking intensity, maintained normal lung function. The same concordance of changes in the lung function with similar smoking intensity was not seen in dizygotic twins. Figure 1 presents the pathogenic mechanisms in COPD schematically.

Our purpose in this article is to review the evidence that specific genes may contribute to genetic susceptibility to COPD.

Identification of susceptibility genes

Complex genetic diseases, such as COPD, are caused by the interaction of environmental factors and genetic susceptibility. Positional cloning has been used to identify the genes for many Mendelian disorders, and has also proved successful in localizing multiple regions of interest in complex diseases, such as hypertension [25] and diabetes mellitus [26]. The positional cloning approach uses multiply-affected families, and compares the inheritance of the disease to the inheritance of genetic markers of known chromosomal location. If a genetic marker is consistently co-inherited with the disease, then it is inferred that the disease gene lies close to that marker on the same chromosome. Additional markers from the region are used to progressively refine the localization, until the gene can be identified.

The power of positional cloning studies is reduced by polygenic inheritance, genetic heterogeneity and interactions with environmental factors. Cigarette smoking is such an important risk factor for COPD that it is impossible to use family data in which the prevalence of cigarette smoking varies. Ideally, one would need multi-generation families, in which there were similar levels of exposure to cigarette smoke. However, this is extremely unlikely because of age- and gender-related differences in the prevalence of smoking. In addition, most patients with COPD do not come to medical attention until their fifth or sixth decade, by which time it is usually impossible to obtain phenotypic data and deoxyribonucleic acid (DNA) from their parents, and their offspring are generally not old enough to have developed significant symptoms of COPD. An alternative approach would be to use an intermediate phenotype: a trait which is known to predispose to the development of COPD in smokers, such as increased bronchial responsiveness [27].

For these reasons, positional cloning is difficult to apply to genes involved in the pathogenesis of COPD. Therefore, an alternative strategy has been used; association studies of candidate genes. The candidate gene approach involves identifying gene products that are clearly involved in the pathogenesis of a condition, and looking for genetic polymorphisms in the genes that code for these proteins. To determine if these variants contribute to the disease process, case-control studies are performed to test for the association of the polymorphisms with the disease phenotype. The risk imparted by a particular phenotype can be calculated using the relative risk (RR) or odds ratio (OR) equations. RR is given by: \( \frac{a/(a+b)}{c/(c+d)} \); and the OR is: \( \frac{a/b}{c/d} \), where a and b are the number of patients with and without the risk allele, respectively, and c and d are the number of controls with and without the risk allele, respectively.

The calculation of OR and RR yields very similar values when the prevalence of a condition is low; however, the results diverge as the prevalence increases. This is illustrated in figure 2, in which the RR and OR for a genotype are calculated for different prevalences of the trait in the population. An increased OR or RR for a disease in individuals of a specific genotype may indicate that the genotype causes an abnormal gene product or gene regulation, which influences the disease pathogenesis. Alternatively, it is possible that the gene tested in the association study does not contribute to the disease process, but is in association with the true
clination with each other for several
other, then they will remain in asso-
tion may have first occurred on a
type being tested in the study. If the
disease-causing muta-
tion. This is
known to be involved in the patho-
genic process can be examined. The
other major difficulty is ensuring that
the patient and control groups are
adequately matched for every other
variable that could influence the
distribution of the genotype. Chief
among these is ethnic origin. There
is potential for false-positive or
false-negative results if this factor
is not carefully taken into account.
For instance, an association of type 2 diabetes mellitus and an immunoglobulin G (IgG) haplotype was shown to be due to Caucasian admixture in a Native American population [29]. Caucasians have a lower incidence of diabetes and coincidentally a higher prevalence of the IgG haplotype. Therefore, the haplotype appeared to be protective against diabetes, but in fact was only a mar-
er for Caucasian ancestry. The association was shown to be spurious because the protective effect was not seen in individuals with no Caucasian ancestry.

Genetic factors in the pathogenesis of COPD

Table 2 lists genes that have been tested as candidates for involvement in the pathogenesis of COPD. The table

Table 2. – Genes implicated in the pathogenesis of COPD

<table>
<thead>
<tr>
<th>Genes for which association studies have shown a significant relationship between polymorphisms and COPD</th>
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<tbody>
<tr>
<td>Alpha1-antitrypsin</td>
</tr>
<tr>
<td>Candidate genes for which there are no significant associations at present</td>
</tr>
<tr>
<td>Extracellular superoxide dismutase</td>
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</table>

COPD: chronic obstructive pulmonary disease; HLA: human leucocyte antigen.

The recognition by Laurell and Eriksson [30] that patients with extremely low levels of α1-globulin had an increased prevalence of emphysema was the first study to show a genetic risk for COPD. Alpha1-antitrypsin (α1-AT) is a powerful antiprotease and is one of the few enzymes that can inhibit leucocyte elastase. Alpha1-AT is produced in large amounts by the liver, but is also produced by alveolar macrophages and peripheral blood monocytes [31]. It is a highly polymorphic protein and over 70 variants have so far been identified [32] using crossed electrophoresis [33] and isoelectric focusing [34]. The Z variant of α1-AT has deficient antiproteolytic function but, more importantly, it is improperly processed in the rough endoplasmic reticulum and aggregates within the cell. Large amounts of the Z variant of the α1-AT protein accumulate in hepatocytes, where they can cause liver disease [35]. Individuals with homozygous Z mutations have extremely low levels of circulating α1-AT (less than 15% of normal) and have a clearly accelerated rate of decline in lung function even in the absence of smoking [36, 37]. However, it is predomi-
nantly among smokers who are homozygous that symp-
tomatic airflow obstruction develops at a younger age
the prevalence of zygous MS and MZ genotypes. In case-control studies, increased risk for COPD in the relatively common heterozygote Ms compound heterozygotes are rare, but have even lower levels at ~60% of normal.

Two types of studies have attempted to identify an increased risk for COPD in the relatively common heterozygous MS and MZ genotypes. In case-control studies, the prevalence of α1-AT genotypes in individuals with the clinical features of COPD is compared to control subjects without airflow obstruction, who are matched as closely as possible for other potential predictors of COPD. In general, the results of these case-control studies have shown the OR to be significantly increased. As shown in table 3, the OR for COPD ranges 1.5–5.0. The prevalence of the MZ variant in the case populations ranges 3.9–14.2%, whilst in the controls it ranges 1.0–5.3%.

Investigators have also assessed the risk of the MZ genotype by studying lung function in the general population [49–56]. In these studies, a population sample is phenotyped for α1-AT levels and are defined as normal. Patients who are heterozygous MS have mild reductions in α1-AT levels to ~40% of normal, whereas MZ heterozygotes have lower levels at ~60% of normal.

In contrast to these reports, the results of several population studies have demonstrated differences between MZ and MM individuals. KLAYTON et al. [57] found an increased prevalence of COPD in MZ heterozygotes who had smoked, but found no difference in the incidence of COPD between MM and MZ nonsmokers. COOPER et al. [58] found significantly decreased lung function in MZ individuals. However, both of these studies used relatives in the MZ study population and, therefore, the results may not be due to mutations in the α1-AT gene. TATTERSALL et al. [59] found evidence for greater loss of elastic recoil in MZ versus MM smokers, but estimates of airway function were similar in both groups. HALL et al. [60] found that MZ heterozygotes had significantly lower expiratory flow rates, even in the absence of smoking. MADSON et al. [10] found more rapid decline in lung function in MZ individuals in a longitudinal study. Similarly, the results of a 10 year longitudinal study of 28 MZ subjects demonstrated that deterioration in lung function was significantly greater than in a matched MM control group [61].

<table>
<thead>
<tr>
<th>Author</th>
<th>Ref.</th>
<th>Subjects</th>
<th>Genotypes %</th>
<th>OR for MZ</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHIGEOKA [42]</td>
<td>306 COPD patients</td>
<td>3.9</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>196 controls</td>
<td>1.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BARTMANN [43]</td>
<td>526 COPD patients</td>
<td>5.9</td>
<td>3.4</td>
<td></td>
</tr>
<tr>
<td>642 controls</td>
<td>6.5</td>
<td>0.9</td>
<td>5.0</td>
<td></td>
</tr>
<tr>
<td>KOX [44]</td>
<td>114 emphysema or bronchitis patients</td>
<td>4.9</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>721 controls</td>
<td>5.7</td>
<td>6.6</td>
<td>2.6</td>
<td></td>
</tr>
<tr>
<td>JANUS [45]</td>
<td>190 emphysema patients</td>
<td>1.9</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>1,303 controls</td>
<td>7.9</td>
<td>0</td>
<td>3.9</td>
<td></td>
</tr>
<tr>
<td>LIEBERMAN [46]</td>
<td>965 COPD patients</td>
<td>3.9</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>1,380 controls</td>
<td>7.7</td>
<td>0.1</td>
<td>3.3</td>
<td></td>
</tr>
<tr>
<td>MITTMAN [47]</td>
<td>350 COPD patients</td>
<td>3.9</td>
<td>0.4</td>
<td></td>
</tr>
<tr>
<td>2,830 controls</td>
<td>10.0</td>
<td>0.3</td>
<td>3.8</td>
<td></td>
</tr>
<tr>
<td>KUEPPERS [9]</td>
<td>114 COPD patients</td>
<td>3.9</td>
<td>0.1</td>
<td></td>
</tr>
<tr>
<td>114 controls</td>
<td>7.9</td>
<td>0.1</td>
<td>1.5</td>
<td></td>
</tr>
<tr>
<td>BARNETT [48]</td>
<td>107 COPD patients</td>
<td>3.9</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>91 controls</td>
<td>5.6</td>
<td>0.1</td>
<td>4.6</td>
<td></td>
</tr>
</tbody>
</table>

Table 3. – Case-control studies of α1-antitrypsin deficiency genotypes and chronic obstructive pulmonary disease (COPD)

OR: odds ratio.
larger group of 140 patients with pulmonary emphysema and bronchiectasis and found that 20% were heterozygous for the mutation (p=0.0015) [65]. The association has been independently confirmed by POLLER et al. [64] in a group of 137 COPD patients. The mutation was found in 15% of the patients and in only 5% of the healthy controls. In addition, a family was identified in which the mutation segregated with COPD, and, when homozygous, the mutation was associated with the onset of symptoms at a younger age.

The 3' mutation could be associated with COPD as a result of linkage disequilibrium with the disease-causing allele. The α1-antichymotrypsin gene has been mapped to within 220 kb of the α1-AT locus [66], and the mutant 3' allele could be in disequilibrium with an α1-antichymotrypsin deficiency allele. Alternatively, KALSHEEKER and co-workers [65] have suggested that the 3' mutation may affect the regulation of α1-AT gene expression. Alpha1-AT is an acute phase protein and its serum concentration increases two- to threefold during inflammation [67]. Presumably, the acute phase response has evolved to attenuate the proteolytic destruction that occurs at sites of acute tissue injury and, thus, prevents excessive tissue destruction. A deficient acute phase increase in α1-AT levels following viral or bacterial respiratory infections could exaggerate the proteolytic tissue destruction that accompanies the release of neutrophil elastase and other enzymes. It is possible that the 3' mutation could affect the acute phase response leading to reduced upregulation of α1-AT synthesis when inflammation is present. Alveolar and lung tissue macrophages are both capable of producing α1-AT [31]. If the α1-AT gene expression in tissue and alveolar macrophages is also affected by the mutation, then a disturbance of the proteolytic-antiproteolytic balance could develop within the microenvironment of the inflamed lung.

MORGAN et al. [68] sequenced the 3' region of the α1-AT gene, and showed that the mutation occurs in a region containing four consensus sequences for DNA-binding proteins, suggesting that it may affect a regulatory element. Gel shift analysis and deoxyribonuclease (DNase) I footprinting experiments confirmed that all four potential regulatory regions bound nuclear factors [69]. However, the mutant sequence demonstrated poor binding, especially in the region of the mutation.

To test for the functional significance of the mutation, both the wild type and mutant 3' regions were cloned into vectors, downstream of a reporter gene. These constructs were used to transfect three different cell lines. In all of the cell types, the wild type sequence showed a 50–100% increase in gene expression compared to a control plasmid. Furthermore, the mutant sequence showed two- to fourfold less activity than the wild type.

The acute phase response is primarily mediated by interleukin 6 [70]. Recently, it has been proposed that transcription factors of the CCAAT box enhancer binding protein (C/EBP) family play an important role in increasing acute-phase gene transcription [71]. The 3' region of the α1-AT gene contains a C/EBP binding site. Interestingly, the mutation in the 3' region appears to influence the binding to neighbouring regions, including the C/EBP site and, therefore, may influence acute phase gene expression.

An additional polymorphism in the 3' region of the α1-AT gene has been shown to be associated with COPD [72]. The polymorphism was found in 3 out of 70 COPD patients but in none of 52 controls. The mutant allele showed loss of more than one restriction site, suggesting the presence of a deletion. Homozygosity for this mutation was associated with early onset COPD. This polymorphism was also associated with normal α1-AT levels.

**Alpha1-antichymotrypsin**

Alpha1-antichymotrypsin, like α1-antitrypsin, is a serine protease inhibitor and acute phase reactant. Alpha1-antichymotrypsin (α1-ACT) is known to inhibit pancreatic chymotrypsin, neutrophil cathepsin G, mast cell chymase and the production of neutrophil superoxide [73]. It is synthesized by hepatocytes and alveolar macrophages [74].

Alpha1-ACT deficiency has a prevalence of approximately 1% in the Swedish population. In cases where hereditary deficiency has been shown, transmission follows an autosomal dominant inheritance pattern [75, 76]. No consistent clinical phenotype is associated with α1-ACT deficiency, although an increased prevalence has been reported in patients with childhood asthma [77] and COPD [78, 79]. In two other studies, deficient patients had increased values of residual volume (RV) and of the RV/total lung capacity (TLC) ratio [75, 76].

Two point mutations in the α1-ACT gene have been associated with decreased α1-ACT serum concentrations and COPD. POLLER and co-workers [78] described an amino acid substitution, Pro227→Ala, which they found in four of 100 unrelated COPD patients and none of 100 controls in a German population (p=0.04). All four patients with the mutant gene had serum α1-ACT concentrations approximately 60% of normal and α1-ACT levels within the normal range. However the prevalence of the Pro227→Ala mutation may vary in different populations, since it was not detected in 102 Russian COPD patients [80]. A second amino acid substitution, Leu55→Pro, was reported by POLLER and co-workers [79] in three out of 200 unrelated COPD patients and none of 100 controls. Mean α1-ACT serum levels in the heterozygotes was 80% of normal, and the mutant protein had an altered pattern on isoelectric focusing and defective function. One of the heterozygotes belonged to a family in which three members were affected with severe early onset COPD. The mutant allele segregated with COPD in this three generation pedigree.

**Cystic fibrosis transmembrane regulator**

The cystic fibrosis transmembrane regulator (CFTR) gene product forms a chloride channel at the apical surface of airway epithelial cells and is intricately involved in the control of airway secretions. Homozygous deficiency or defective function of this protein results in cystic fibrosis (CF), characterized by elevated sweat chloride levels and early onset obstructive lung disease, secondary to chronic bacterial infection and bronchiectasis. The prevalence of CF is 1 in 2,000 to 1 in 3,000, with the carrier frequency estimated at 1 in 20 to 1 in
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30 in populations of Northern European descent [81]. It has been hypothesized that this relatively high prevalence arose from a selective advantage of carrying a CF allele. Resistance to pulmonary tuberculosis [82], influenza [83], and cholera [84] have each been suggested as a selective advantage. In an animal model, mice that were heterozygous for a mutant CFTR allele secreted 50% less intestinal fluid and chloride ion in response to cholera toxin [85].

CF heterozygotes could have altered airway water and ion regulation, altered mucociliary clearance and an increased susceptibility to challenges that are attenuated by these mechanisms. In the 1960s, several groups investigated the hypothesis that CF heterozygotes may be predisposed to respiratory disease. Comparisons of parents of CF patients versus controls (mean age 34–36 yrs) did not reveal any significant differences in lung function or history of asthma or chronic bronchitis [86–89]. However, obligate heterozygotes have been shown to have increased bronchial reactivity to methacholine [90], and increased incidence of wheeze accompanied by decreased FEV1 and forced mid-expiratory flow (FEF25–75) [91].

More than 580 variants of the CFTR gene have been described; the most common mutation, ΔF508, is found on approximately 70% of all CF chromosomes [92]. Heterozygosity for the ΔF508 mutation was identified in four of eight patients with disseminated bronchiectasis [93], and in five of 65 patients with bronchial hypersecretion [94]. In both studies, it is unclear whether the ΔF508 heterozygotes are predisposed to lung disease or whether they have mild, previously undiagnosed CF with unidentified CFTR mutations on their other chromosomes. In a study of patients with normal sweat chloride levels, Gervais et al. [95] found the prevalence of ΔF508 to be increased (four out of 47) in patients with bronchiectasis and not increased (seven out of 161) in patients with chronic bronchitis. The ΔF508 mutation was not found in any of 21 Japanese patients with diffuse panbronchiolitis, a disease with pathological and clinical characteristics similar to mild CF [96].

Recently, investigators have searched for associations between respiratory disease and other CFTR variants, in addition to ΔF508. Articchio et al. [97] examined 100 patients with chronic bronchitis for the more common CFTR mutations (ΔF508, R553X, G551D, G542D, G542X, N1303K and 621+1G→T). The only mutation, ΔF508, was found in one patient who also had bronchiectasis, suggesting that none of these CFTR mutations predispose to chronic bronchitis [97, 98]. Pignatti and co-workers [99] performed detailed screening for approximately 70 CFTR mutations. Although variants were found in two of 12 patients with COPD without bronchiectasis, and in two of 36 patients with non-obstructive pulmonary disease, the frequency of the mutations was not significantly different from that expected. However, CFTR mutations were found in five of 16 patients with disseminated bronchiectasis and normal sweat chloride levels (one each with mutations ΔF508, R75Q, M1137V, 3667ins4, R1066G). In a subsequent study, five of the same 16 patients were also found to have the IVS8-5T variant (three of whom were previously negative for other CFTR mutations) [100]. The IVS8-5T allele results in reduced CFTR gene expression. This variant was not found to be significantly increased in five of 33 COPD patients. Early work by the same authors did not support the involvement of CFTR in COPD by linkage analysis with a CF locus marker [101].

In summary, heterozygosity for ΔF508 appears to predispose for disseminated bronchiectasis, but the involvement of CFTR in other obstructive pulmonary diseases remains unproven. Studies of CFTR mutations in COPD patients who have documented lifelong airway challenges, such as cigarette smoking, have not been performed.

Vitamin D-binding protein (group-specific component)

Vitamin D-binding protein (VDBP), also known as group-specific component, is a 55 kDa protein secreted by the liver, that is able to bind extracellular actin and endotoxin in addition to vitamin D. VDBP enhances the chemotactic activity of complement factor 5a (C5a) and C5a des-Arg for neutrophils by one to two orders of magnitude [102]. In addition, VDBP can act as a macrophage-activating factor [103]. Thus, besides its vitamin D-binding function, VDBP can have important influences on the intensity of the inflammatory reaction.

Numerous isoforms of VDBP have been identified by isoelectric focusing. Two common substitutions in exon 11 of the gene result in three possible isoforms, termed 1F, 1S and 2. Figure 4 shows a partial gene map of VDBP and the substitutions responsible for protein isoforms. Kueppers et al. [9] found a decreased frequency of the 2-2 genotype in COPD patients compared to controls. Subsequently, Horn et al. [104] performed a case-control study, in which they found that the prevalence of the 1F homozygote was significantly greater among patients with COPD than among controls, yielding a RR of 4.8. In addition, the genotypes that contained the 2 allele (2-1F, 2-1S and 2-2), had a protective effect. However, this association remains controversial, since it was not replicated by Kauffmann et al. [105].

![Vitamin D-binding protein (VDBP) isoforms](image)

**Fig. 4.** Polymorphisms in the vitamin D-binding protein gene. a) Two point-mutations in exon 11 of the gene result in amino acid substitutions at positions 416 and 420 of the protein. b) Amino acids present at position 416 and 420 in the three isoforms of the vitamin D-binding protein, and the frequencies of the isoforms in Caucasian populations. G: glutamic acid; Asp: aspartic acid; Thr: threonine; Lys: lysine.
No studies have so far examined the influence of these genetic variants on the ability of the protein to act as a chemotactic enhancer of C5a or as a macrophage-activating factor. However, the macrophage-activating factor is formed from VDBP by modification of an oligosaccharide side chain. Less than 10% of the 2 isofrom is glycosylated and able to form macrophage-activating factor [103], which is consistent with a protective effect for the 2 allele.

**Alpha2-macroglobulin**

Alpha2-macroglobulin is a broad spectrum serum protease inhibitor. Normal serum levels of alpha2-macroglobulin are higher in females and decrease with age. Synthesized by hepatocytes, alveolar macrophages [106] and human lung fibroblasts [107], alpha2-macroglobulin is thought to have a protective role in the lung. The large size of alpha2-macroglobulin (725 kDa) prevents significant transport from blood to the lung interstitium or alveolar space, so that serum levels do not necessarily reflect its concentration in the lung. However, increased permeability of the vessel wall under inflammatory conditions could allow alpha2-macroglobulin to enter the interstitial space [108]. An increase in alpha2-macroglobulin levels can be detected in the sputum of patients with acute chest infections [109]. Elevated alpha2-macroglobulin levels, up to two times control, have been reported in the serum of patients with alpha1-AT deficiency, irrespective of the presence or absence of COPD [110, 111]. Such an elevation is not seen in patients with emphysema unrelated to alpha1-AT deficiency.

Alpha2-macroglobulin serum deficiency is rare and the cause is unknown. Two case studies described hereditary alpha2-macroglobulin deficiency with autosomal dominant transmission [112, 113]. Although symptoms suggestive of respiratory disease were not found in the deficient individuals, it is possible that the subjects were not old enough to develop COPD. Neither study included pulmonary function tests or smoking histories. Complete lack of alpha2-macroglobulin has not been described and may be incompatible with life.

The alpha2-macroglobulin gene, located on chromosome 12, has been cloned and sequenced [114]. Whilst restriction fragment length polymorphism (RFLP) variants of the alpha2-macroglobulin gene have been described, only one variant has been reported to be associated with chronic lung disease in a single patient [115]. The patient had alpha2-macroglobulin serum levels 50% of normal and chronic pulmonary disease since childhood progressing to very severe COPD at the age of 42 yrs (smoking history not reported). DNA from this patient was digested with 10 restriction enzymes and probed with an alpha2-macroglobulin complementary deoxyribonucleic acid (cDNA) probe. All 10 restriction enzymes showed an alteration in the RFLP pattern suggesting a major alteration of the alpha2-macroglobulin gene. RFLP analysis with nine of the 10 restriction enzymes failed to demonstrate polymorphisms in 40 control and 39 COPD patients.

The same author sequenced two functional domains of the alpha2-macroglobulin gene in 30 COPD patients and 30 control subjects [114]. A common amino acid substitution, Val1000→lle, was detected equally in both groups. One COPD patient had an amino acid substitution, Cys972→Tyr, which was predicted to interfere with alpha2-macroglobulin function. The serum level of alpha2-macroglobulin in this patient was within the normal range.

**Cytochrome P4501A1**

Cytochrome P4501A1 is an enzyme that metabolizes exogenous compounds to enable them to be excreted in the urine or bile. It is found throughout the lung, and may play a role in the activation of procarcinogens to their carcinogenic forms. The enzyme is produced by the CYP1A1 gene and mutations at this locus have been associated with lung cancer [116].

In a recent study, the prevalence of a mutation in exon 7 of CYP1A1 was assessed in lung cancer and COPD patients [117]. This mutation causes a substitution of isoleucine to valine at residue 462, and results in a protein with almost twice the enzymatic activity of the isoleucine protein. The high-activity allele was found to be associated with susceptibility to centriacinar emphysema and lung cancer. The polymorphism was not linked to lung cancer in the absence of emphysema.

**Blood group antigens**

The association of COPD with the ABO, secretor and Lewis genes has been the focus of several studies. The ABO locus on chromosome 9 determines the activity of a glycosyltransferase, which converts glycoprotein H into the A or B antigens. An association between the ABO locus and COPD was found by COHEN et al. [118]. The results of this study suggested that impaired lung function was associated with type A blood group. This was confirmed by the same authors in a 5 yr longitudinal study, in which there was a greater decline in lung function in group A individuals compared with non-A subjects [119]. In direct contrast to these studies, KRYZANOWSKI et al. [120] found that subjects with blood group A had a smaller decline in lung function than individuals with other blood groups. The results of several other studies have failed to confirm any association of ABO alleles and pulmonary function [23, 121, 122].

ABO antigens are present on virtually all cells of the body. Approximately 80% of the population secretes ABO antigens into saliva, plasma and respiratory tract secretions. The ability to do this is determined by the secretor locus on chromosome 19q and is a dominant trait. It has been reported that nonsecretors have impaired lung function compared to secretors [123, 124]. This suggests that the presence of ABO antigens in the secretions of the respiratory tract may have a protective effect against lung impairment. This result was independently confirmed by KAUFFMANN et al. [105], who found significantly more nonsecretors of blood group O in subjects with low FEV1 measurements (OR=15.6). Secretor status was shown to have a protective effect against decline in peak expiratory flow rates [123], but, in this study, the effect was only observed in subjects over 40 yrs of age. These associations are controversial because they have not been replicated in other populations [121, 122, 125].
The Lewis blood group has also been investigated as a possible risk factor for airflow obstruction [126]. In Caucasian populations, ~90% of individuals have the dominant Le allele and produce Lewis a substance. In individuals who are secretors, this is converted to Lewis b substance, and therefore they have a and b substances in their serum. Lewis-positive nonsecretors only have a substance in their serum. Horne et al. [126] found a significant increase in airflow obstruction in Lewis-negative subjects, with a RR of 7.2. The authors suggest that it is the presence of b substance rather than secretor status that protects against airflow obstruction.

Since the blood group systems interact at the protein level, a recent study has considered all three gene loci together [127]. Blood group O individuals who were either Lewis-negative or nonsecretors, were found to have impaired lung function and higher prevalence of wheezing and asthma. Individuals who were both Lewis-negative and nonsecretors, had very low lung function. Lewis-positive secretors were found to have lower lung function if they had blood group A, compared with group O.

The reason for the association of ABO, Lewis and secretor genes with COPD remains unclear, but it may be due to the role of these systems in the adhesion of infectious agents [128]. Recurrent respiratory infections, especially in childhood, are known to be a risk factor for COPD, and particular alleles of these blood groups may increase an individual’s susceptibility to infection.

**Human leucocyte antigen locus**

Associations between the human leucocyte antigen (HLA) class I genes and COPD have been investigated in a study of heavy smokers with high FEV1 values and lifelong nonsmokers with low FEV1 values [105]. The authors observed a significant decrease of the HLA-Bw16 allele in those with low FEV1 values (OR=0.2), and a significant increase of the HLA-B7 antigen in the same group (OR=3.8).

HLA typing was also performed in a population of Japanese patients with diffuse panbronchiolitis [129]. The results demonstrated an increased prevalence of HLA-Bw54 in the patients compared to the control subjects (RR=13.3). This HLA type is only found in Japanese, Chinese and Korean individuals, and may explain why diffuse panbronchiolitis has not been reported in Caucasian or African populations.

It is not yet clear whether these associations are due to variations in the HLA genes themselves or to susceptibility genes in linkage disequilibrium with the HLA alleles.

**Immunoglobulin deficiency**

The role of hereditary immunoglobulin A (IgA) and immunoglobulin G (IgG) deficiency in the aetiology of COPD has been examined in several studies [130, 131]. Patients with IgA deficiency, either alone or in combination with IgG deficiency, are known to have recurrent respiratory infections [132]. In a study of IgA deficient individuals, four were found to have decreased levels of IgG2 and two had decreased levels of IgG3 [130]. All six of the IgG and IgA deficient subjects were found to have abnormal lung function. In addition, a significant correlation of IgG2 levels and FEV1 values was found by O’Keeffe et al. [131]. Selective IgA deficiency has been found to segregate with COPD, in a large, three generation pedigree [133].

**Haptoglobin**

The serum protein haptoglobin has several common polymorphisms. The prevalence of these variants was investigated in a population of subjects with low FEV1 values [105]. Overall, no significant difference in the frequency of haptoglobin variants was observed between individuals with low FEV1 values compared to those with high values. Among those with non-O blood groups, a possible protective affect of the Hp1S allele was detected. However, a similar association was not found in an earlier study [9].

**Other candidate genes for COPD**

**Extracellular superoxide dismutase**

Extracellular superoxide dismutase (EC-SOD) is a secretory glycoprotein found mainly in the interstitial spaces, although ~1% is found in the plasma, lymph and synovial fluids. It is the main extracellular antioxidant enzyme in the lung. EC-SOD has a high affinity for glycosaminoglycans, such as heparan sulphate, and therefore >98% of the enzyme is found bound to heparan sulphate in the connective tissue matrix. EC-SOD is ideally localized to play an important role in attenuating tissue damage secondary to oxygen radicals inhaled in cigarette smoke and generated by activated inflammatory cells.

A polymorphism in the EC-SOD gene results in the substitution of arginine to glycine at amino acid position 213 [134, 135]. Approximately 2% of a random population were found to be heterozygous for the substitution [135]. This mutation (R213G) is located in the heparin-binding domain and results in a ~30 fold increase in the serum enzyme concentration, presumably due to a failure of EC-SOD to remain bound to interstitial glycosaminoglycans. A 10 fold increase in serum EC-SOD has been reported in a lung transplant patient with end-stage emphysema [136]. However, the R213G allele was not present in this patient, suggesting that further variants of this gene remain to be identified.

**Secretory leucocyte proteinase inhibitor**

Secretory leucocyte proteinase inhibitor (SLPI) is a 12 kDa serine antiprotease found in a variety of mucous secretions, including those of the respiratory tract. SLPI is produced locally in the lung by airway epithelial cells and is able to inhibit neutrophil elastase [137]. Therefore, SLPI may play an important role in the prevention of tissue damage by neutrophils during inflammation. ABE
et al. [138] screened 114 individuals for polymorphisms in exons 2, 3 and 4 of the SLPI gene. The subjects included individuals with various \( \alpha_1 \)-antitrypsin genotypes and 10 early onset COPD patients who did not have \( \alpha_1 \)-antitrypsin deficiency. However, no mutations were discovered, which suggests that structural alterations in the SLPI protein do not play a major role in the pathogenesis of COPD.

**Cathepsin G**

Cathepsin G is a serine protease, and mutations in the gene for this enzyme may predispose individuals to COPD [139]. Therefore, exons 1–5 of the cathepsin G gene were screened for mutations in 180 individuals. A mutation was found in exon 4, which resulted in an amino acid substitution at position 125, but it was not associated with COPD.

**Summary**

In this manuscript, we have reviewed evidence for a genetic component to COPD and have described the genes that could contribute to the genetic risk. The diagnosis of COPD is based on decreased expiratory airflow, and it is possible that different pathophysiological processes contribute to this phenotype within and between patients. For example, bronchial smooth muscle cell hypertrophy, inflammatory narrowing of peripheral airways and loss of elastic recoil may contribute to a different extent in certain individuals. Susceptibility to these processes may have differing genetic bases. A search for genes that increase susceptibility to airflow obstruction among smokers may have implications beyond the development of COPD. In epidemiological studies, a decrease in FEV1 has been shown to be a marker of premature mortality from other causes [140]. It is possible that an excessive pulmonary response to inhaled toxins and pollutants will serve as a marker of polymorphisms that increase susceptibility to other inflammatory and degenerative diseases. The development of rapid, inexpensive molecular methods to screen for specific polymorphisms will allow an increased capacity to identify risk genotypes. This has profound relevance for the conduct of clinical investigations of environmental risk, therapeutic interventions and clinical screening.

**References**


70. Perlmutter DH, May LT, Sehgal PB. Interferon-\( \beta \)-2-Interleukin-6 modulates synthesis of \( \alpha_1 \)-antitrypsin in human mononuclear phagocytes and in human hepatoma cells. *J Clin Invest* 1989; 84: 139–144.


90. Gabriel SE, Brigman KN, Koller BH, Boucher RC, Farrow GM. The two C/EBP isoforms, IL-6 DBP/NF-IL-6 and NF-IL-6, contribute to the acute phase gene transcription via different mechanisms. *Nucleic Acids Res* 1993; 21: 289–294.