

## REVIEW

# Genetic risk factors for chronic obstructive pulmonary disease

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**ABSTRACT:** Cigarette smoking is the major risk factor for chronic obstructive pulmonary disease (COPD). However, only a minority of cigarette smokers develop symptomatic disease. Studies of families and twins suggest that genetic factors also contribute to the development of COPD. We present a detailed literature review of the genes which have been investigated as potential risk factors for this disease.

The only established genetic risk factor for COPD is homozygosity for the Z allele of the  $\alpha_1$ -antitrypsin gene. Heterozygotes for the Z allele may also be at increased risk. Other mutations affecting the structure of  $\alpha_1$ -antitrypsin or the regulation of gene expression have been identified as risk factors.

Genes, including those for  $\alpha_1$ -antichymotrypsin,  $\alpha_2$ -macroglobulin, vitamin D-binding protein and blood group antigens, have also been associated with the development of COPD. Variants of the cystic fibrosis transmembrane regulator gene have been identified as risk factors for disseminated bronchiectasis.

The genetic basis to chronic obstructive pulmonary disease has begun to be elucidated and it is likely that several genes will be implicated in the pathogenesis of this disease. The knowledge gained from such studies may also prove relevant to other inflammatory diseases.

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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 co-factors, 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 as compared to the 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

Study type	[Ref.]
Study showing clustering of COPD in families	[4]
Family studies showing increased incidence of COPD or chronic bronchitis in relatives of cases compared to relatives of controls	[5–12]
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	[4, 7, 13, 14]
Studies showing decreased prevalence of disease or less similarity in lung function with increased genetic distance	[5, 15, 16]
Family studies showing a major gene effect or a genetic component to pulmonary function	[17, 18]
Studies of pulmonary function in monozygotic and dizygotic twins	[15, 19–24]

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 (FEV<sub>1</sub>) 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

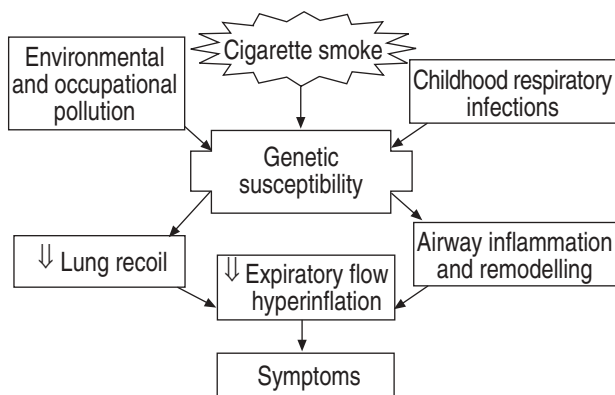


Fig. 1. – Summary of the pathogenic mechanisms in chronic obstructive pulmonary disease (COPD). Exposure to cigarette smoke is the major factor in the pathogenesis of COPD but interacts with other risk factors, including genetic susceptibility, to produce airway obstruction by loss of elastic recoil and/or airway inflammation.

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:  $(a/[a+b])/(c/[c+d])$ ; and the OR is:  $(a/b)/(c/d)$ , 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

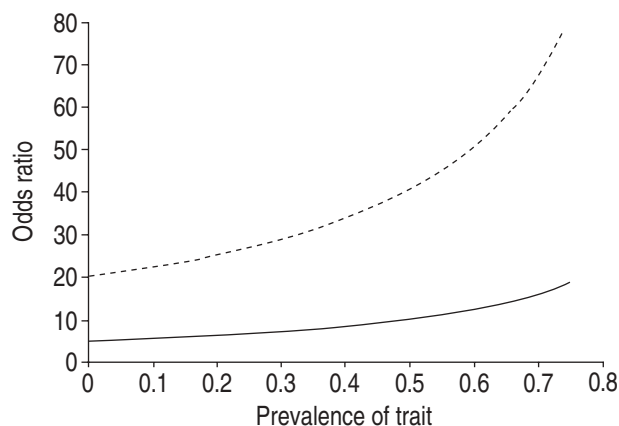


Fig. 2. – Dependence of estimates of relative risk (RR) on the population prevalence of the trait under study. Values for RR and odds ratio (OR) diverge as the prevalence of the trait increases. —: RR=5; - - -: RR=20.

disease-causing mutation. This is because the disease-causing mutation may have first occurred on a chromosome containing the genotype being tested in the study. If the two alleles are very close to each other, then they will remain in association with each other for several generations and are said to be in linkage disequilibrium.

The power of association studies has been clearly demonstrated [28]. Even genetic polymorphisms which impart only a slight increase in RR can be detected if sufficient numbers of patients and controls are obtained. The weakness of the candidate gene approach is that only genes known to be involved in the pathogenic 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 marker 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

#### Genes for which association studies have shown a significant relationship between polymorphisms and COPD

Alpha<sub>1</sub>-antitrypsin  
Alpha<sub>1</sub>-antichymotrypsin  
Cystic fibrosis transmembrane regulator  
Vitamin D-binding protein  
Alpha<sub>2</sub>-macroglobulin  
Cytochrome P450A1  
ABH secretor, Lewis and ABO blood groups  
HLA  
Immunoglobulin deficiency

#### Candidate genes for which there are no significant associations at present

Extracellular superoxide dismutase  
Secretory leucocyte proteinase inhibitor  
Cathepsin G

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

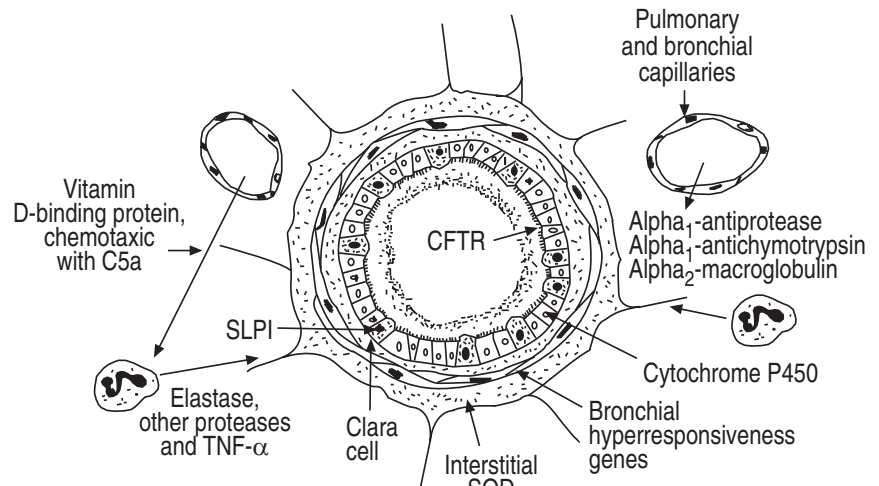


Fig. 3. – Schematic representation of an airway to illustrate how mutations in various genes may contribute to the development of chronic obstructive pulmonary disease (COPD). Alpha<sub>1</sub>-antiprotease ( $\alpha_1$ -antitrypsin),  $\alpha_1$ -antichymotrypsin, and  $\alpha_2$ -macroglobulin are serum proteins that can inhibit inflammatory cell proteases. Deficiencies in their function or level could enhance the proteolytic digestion of the lung parenchyma that characterizes emphysema. Cytochrome P450 is an enzyme present in airway epithelial cells (primarily Clara cells) that converts inhaled toxic chemicals to their metabolites. A gene variant, which enhances the enzyme's activity, could increase the prevalence of lung cancer as well as accelerate the airway inflammation that characterizes COPD. There is an association between mutations in the CFTR gene and bronchiectasis. Variants of the vitamin D-binding protein may influence the susceptibility to COPD. This protein can be converted to a macrophage-activating factor and interact with complement factor 5a (C5a) and C5a des-Arg to enhance chemotaxis of inflammatory cells. CFTR: cystic fibrosis transmembrane regulator; SLPI: secretory leucocyte proteinase inhibitor; TNF- $\alpha$ : tumour necrosis factor- $\alpha$ ; SOD: superoxide dismutase.

indicates those genes for which association studies have shown a significant relationship between specific polymorphisms and COPD, and candidate genes that have the potential to be involved in the pathogenesis of COPD but for which there are no significant associations at present. Figure 3 is a schematic illustration of an airway to depict how enhanced or deficient gene products could contribute to COPD.

### Alpha<sub>1</sub>-antitrypsin

The recognition by LAURELL and ERIKSSON [30] that patients with extremely low levels of  $\alpha_1$ -globulin had an increased prevalence of emphysema was the first study to show a genetic risk for COPD. Alpha<sub>1</sub>-antitrypsin ( $\alpha_1$ -AT) is a powerful antiprotease and is one of the few enzymes that can inhibit leucocyte elastase. Alpha<sub>1</sub>-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  $\alpha_1$ -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  $\alpha_1$ -AT protein accumulate in hepatocytes, where they can cause liver disease [35]. Individuals with homozygous Z mutations have extremely low levels of circulating  $\alpha_1$ -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 predominantly among smokers who are homozygous that symptomatic airflow obstruction develops at a younger age

[38, 39]. Although there is a clear association of homozygosity for this gene variant and the development of COPD, the homozygous state is rare in the population (1 in 1,670 [40] to 1 in 5,097 [41] live births in Caucasian populations) and, thus, can explain only a small percentage of the genetic susceptibility to cigarette smoke.

The discovery that homozygosity for the Z variant leads to increased risk for COPD led to numerous studies in which an association of COPD and heterozygous genotypes was sought. The approximate allele frequencies of the most common gene variants M, S and Z are 0.93, 0.05 and 0.02, respectively. Patients with the MM genotype have the highest  $\alpha_1$ -AT levels and are defined as normal. Patients who are heterozygous MS have mild reductions in  $\alpha_1$ -AT levels to ~80% of normal, whereas MZ heterozygotes have lower levels at ~60% of normal. SZ compound heterozygotes are rare, but have even lower levels at ~40% 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  $\alpha_1$ -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  $\alpha_1$ -AT variants and the prevalence of COPD in those with the MZ phenotype is compared with the prevalence in those with the MM phenotype. Many of these studies were based on small numbers of individuals and had insufficient power to detect an effect of the MZ or MS phenotype. However, even most of

the larger studies showed no significant difference in respiratory symptoms or pulmonary function in the MZ individuals compared to MM subjects. In theory, population-based studies designed to examine the predictive value of a genotype are superior to case-control methods because there is less chance of a systematic bias. However, in COPD, where an environmental factor (*i.e.* cigarette smoking) plays an important role, population studies may have insufficient sensitivity to detect a factor which only increases risk slightly. For example, in a collaborative study to assess risk of lung disease in MZ phenotype subjects, 143 MZ individuals did not have significantly lower lung function than 143 MM individuals drawn from a population study of over 10,000 people [56]. However, only 37% of the subjects were current smokers, 35% had never smoked and 60% were less than 54 yrs of age.

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  $\alpha_1$ -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. MADISON *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].

In addition to mutations that affect the basal serum levels of  $\alpha_1$ -AT, several mutations have been described that affect function [62], but these are relatively rare and can only explain a small percentage of the susceptible subgroup that develops COPD. Two separate groups have reported an association between a mutation in the 3' region of the  $\alpha_1$ -AT gene and COPD [63, 64]. KALSHEKER *et al.* [63] found that this mutation was associated with chronic lung disease. Heterozygosity for the mutation in a group of patients with pulmonary emphysema (18%), and in a group of patients with bronchiectasis (19%), was significantly higher than in normal controls (5%). However, the reason for the association of the mutation with COPD was unclear, since it was not associated with  $\alpha_1$ -AT deficiency or any particular  $\alpha_1$ -AT protein type. Subsequently, these authors studied a

Table 3. – Case-control studies of  $\alpha_1$ -antitrypsin deficiency genotypes and chronic obstructive pulmonary disease (COPD)

First author	[Ref.]	Subjects	Genotypes %					OR for MZ
			MZ	MS	ZZ	SS	SZ	
SHIGEOKA	[42]	306 COPD patients	3.9					4.0
		196 controls	1.0					
BARTMANN	[43]	526 COPD patients	5.9	6.5	0.9		3.4	5.0
		642 controls	1.2	6.5	0.3		0.2	
COX	[44]	114 emphysema or bronchitis patients	4.9	5.7	6.6			2.6
		721 controls	1.9	7.9	0			
JANUS	[45]	190 emphysema patients	14.2	5.3	2.6		1.1	3.9
		1,303 controls	3.9	7	0.1		0.3	
LIEBERMAN	[46]	965 COPD patients	7.7	10.1	1.9	0.3	0.2	3.3
		1,380 controls	2.5	8.0	0	0.1	0.4	
MITTMAN	[47]	350 COPD patients	10.0	6.3	3.4	0.9	0.9	3.8
		2,830 controls	2.9	4.1	0.1	0.1	0.1	
KUEPPERS	[9]	114 COPD patients	7.9	4.4	2.6	0		1.5
		114 controls	5.3	7.0	0	0.9		
BARNETT	[48]	107 COPD patients	9.3	5.6	1.9			4.6
		91 controls	2.2	5.5	0			

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  $\alpha_1$ -antichymotrypsin gene has been mapped to within 220 kb of the  $\alpha_1$ -AT locus [66], and the mutant 3' allele could be in disequilibrium with an  $\alpha_1$ -antichymotrypsin deficiency allele. Alternatively, KALSHEKER and co-workers [65] have suggested that the 3' mutation may affect the regulation of  $\alpha_1$ -AT gene expression. Alpha<sub>1</sub>-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  $\alpha_1$ -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  $\alpha_1$ -AT synthesis when inflammation is present. Alveolar and lung tissue macrophages are both capable of producing  $\alpha_1$ -AT [31]. If the  $\alpha_1$ -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  $\alpha_1$ -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  $\alpha_1$ -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

$\alpha_1$ -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  $\alpha_1$ -AT levels.

#### *Alpha<sub>1</sub>-antichymotrypsin*

Alpha<sub>1</sub>-antichymotrypsin, like  $\alpha_1$ -antitrypsin, is a serine protease inhibitor and acute phase reactant. Alpha<sub>1</sub>-antichymotrypsin ( $\alpha_1$ -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].

Alpha<sub>1</sub>-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  $\alpha_1$ -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  $\alpha_1$ -ACT gene have been associated with decreased  $\alpha_1$ -ACT serum concentrations and COPD. POLLER and co-workers [78] described an amino acid substitution, Pro<sup>227</sup>→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  $\alpha_1$ -ACT concentrations approximately 60% of normal and  $\alpha_1$ -AT levels within the normal range. However the prevalence of the Pro<sup>227</sup>→Ala mutation may vary in different populations, since it was not detected in 102 Russian COPD patients [80]. A second amino acid substitution, Leu<sup>55</sup>→Pro, was reported by POLLER and co-workers [79] in three out of 200 unrelated COPD patients and none of 100 controls. Mean  $\alpha_1$ -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

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 FEV<sub>1</sub> and forced mid-expiratory flow (FEF<sub>25–75</sub>) [91].

More than 580 variants of the CFTR gene have been described; the most common mutation,  $\Delta F508$ , is found on approximately 70% of all CF chromosomes [92]. Heterozygosity for the  $\Delta F508$  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  $\Delta F508$  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  $\Delta F508$  to be increased (four out of 47) in patients with bronchiectasis and not increased (seven out of 161) in patients with chronic bronchitis. The  $\Delta F508$  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  $\Delta F508$ . ARTLICH *et al.* [97] examined 100 patients with chronic bronchitis for the more common CFTR mutations ( $\Delta F508$ , R553X, G551D, G542D, G542X, N1303K and 621+1G $\rightarrow$ T). The only mutation,  $\Delta F508$ , was found in one patient who also had bronchiectasis, suggesting that none of these CFTR mutations predisposes 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  $\Delta F508$ , R75Q, M1137V, 3667ins4, R1066C). 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 expres-

sion. 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  $\Delta F508$  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, HORNE *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].

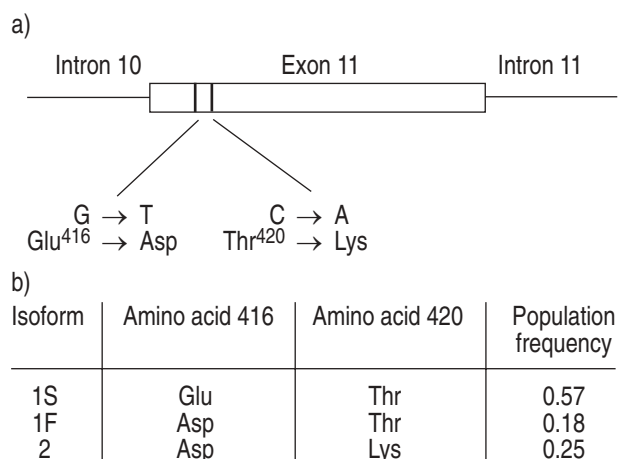


Fig. 4. – Polymorphisms in the vitamin D-binding protein gene. a) Two point-mutations in exon 11 of the gene result in amino acid substitutions at positions 416 and 420 of the protein. b) Amino acids present at position 416 and 420 in the three isoforms of the vitamin D-binding protein, and the frequencies of the isoforms in Caucasian populations. G: guanine; T: thymine; C: cytosine; A: adenine; Glu: 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 isoform is glycosylated and able to form macrophage-activating factor [103], which is consistent with a protective effect for the 2 allele.

#### *Alpha<sub>2</sub>-macroglobulin*

Alpha<sub>2</sub>-macroglobulin is a broad spectrum serum protease inhibitor. Normal serum levels of alpha<sub>2</sub>-macroglobulin are higher in females and decrease with age. Synthesized by hepatocytes, alveolar macrophages [106] and human lung fibroblasts [107], alpha<sub>2</sub>-macroglobulin is thought to have a protective role in the lung. The large size of alpha<sub>2</sub>-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 alpha<sub>2</sub>-macroglobulin to enter the interstitial space [108]. An increase in alpha<sub>2</sub>-macroglobulin levels can be detected in the sputum of patients with acute chest infections [109]. Elevated alpha<sub>2</sub>-macroglobulin levels, up to two times control, have been reported in the serum of patients with alpha<sub>1</sub>-AT deficiency, irrespective of the presence or absence of COPD [110, 111]. Such an elevation is not seen in patients with emphysema unrelated to alpha<sub>1</sub>-AT deficiency.

Alpha<sub>2</sub>-macroglobulin serum deficiency is rare and the cause is unknown. Two case studies described hereditary alpha<sub>2</sub>-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 alpha<sub>2</sub>-macroglobulin has not been described and may be incompatible with life.

The alpha<sub>2</sub>-macroglobulin gene, located on chromosome 12, has been cloned and sequenced [114]. Whilst restriction fragment length polymorphism (RFLP) variants of the alpha<sub>2</sub>-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 alpha<sub>2</sub>-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 alpha<sub>2</sub>-macroglobulin complementary deoxyribonucleic acid (cDNA) probe. All 10 restriction enzymes showed an alteration in the RFLP pattern suggesting a major alteration of the alpha<sub>2</sub>-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 alpha<sub>2</sub>-macroglobulin gene in 30 COPD patients and 30 control subjects [114]. A common amino acid substitution, Val<sup>1000</sup>→Ile, was detected equally in both groups.

One COPD patient had an amino acid substitution, Cys<sup>972</sup>→Tyr, which was predicted to interfere with alpha<sub>2</sub>-macroglobulin function. The serum level of alpha<sub>2</sub>-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, KRZANOWSKI *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 FEV<sub>1</sub> 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 FEV<sub>1</sub> values and lifelong nonsmokers with low FEV<sub>1</sub> values [105]. The authors observed a significant decrease of the HLA-Bw16 allele in those with low FEV<sub>1</sub> 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 FEV<sub>1</sub> values was found by O'KEEFE *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 FEV<sub>1</sub> values [105]. Overall, no significant difference in the frequency of haptoglobin variants was observed between individuals with low FEV<sub>1</sub> values compared to those with high values. Among those with non-O blood groups, a possible protective effect 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

1. Snider GL. Chronic obstructive pulmonary disease: risk factors, pathophysiology and pathogenesis. *Ann Rev Med* 1989; 40: 411–429.
2. Fletcher C, Peto R, Tinker C, Speizer FE. The natural history of chronic bronchitis: an eight year follow-up study of working men in London. Oxford, Oxford University Press, 1976.
3. Bascom R. Differential susceptibility to tobacco smoke: possible mechanisms. *Pharmacogenetics* 1991; 1: 102–106.
4. Tager IB, Rosner B, Tishler PV, Speizer FE, Kass EH. Household aggregation of pulmonary function and chronic bronchitis. *Am Rev Respir Dis* 1976; 114: 485–492.
5. Tager I, Tishler PV, Rosner B, Speizer FE, Litt M. Studies of the familial aggregation of chronic bronchitis and obstructive airways disease. *Int J Epidemiol* 1978; 7: 55–62.
6. Khoury MJ, Beaty TH, Newil CA, Bryant S, Cohen BH. Genetic-environmental interactions in chronic airways obstruction. *Int J Epidemiol* 1986; 15: 65–72.
7. Higgins M, Keller J. Familial occurrence of chronic respiratory disease and familial resemblance in ventilatory capacity. *J Chron Dis* 1975; 28: 239–251.
8. Speizer FE, Rosner B, Tager I. Familial aggregation of chronic respiratory disease: use of national health interview survey data for specific hypothesis testing. *Int J Epidemiol* 1976; 5: 167–172.
9. Kueppers F, Miller RD, Gordon H, Hepper NG, Offord K. Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. *Am J Med* 1977; 63: 336–342.
10. Madison R, Zelman R, Mittman C. Inherited risk factors for chronic lung disease. *Chest* 1980; 77 (2 Suppl.): 255–257.
11. Larson RK, Barman ML, Kueppers F, Fudenberg HH. Genetic and environmental determinants of chronic obstructive pulmonary disease. *Ann Intern Med* 1970; 72: 627–632.
12. Cohen BH, Diamond EL, Graves CG, *et al.* A common familial component in lung cancer and chronic obstructive pulmonary disease. *Lancet* 1977; ii: 523–526.
13. Kauffmann F, Tager IB, Muñoz A, Speizer FE. Familial factors related to lung function in children aged 6–10 years. *Am J Epidemiol* 1989; 129: 1289–1299.
14. Devor EJ, Crawford MH. Family resemblance for normal pulmonary function. *Ann Hum Biol* 1984; 11: 439–448.
15. Redline S, Tishler PV, Rosner B, *et al.* Genotypic and phenotypic similarities in pulmonary function among family members of adult monozygotic and dizygotic twins. *Am J Epidemiol* 1989; 129: 827–836.
16. Silverman E, Chapman H, Drazen J, *et al.* Early-onset chronic obstructive pulmonary disease (COPD): preliminary evidence for genetic factors other than Pi type. *Am J Respir Crit Care Med* 1996; 153: A48.
17. Rybicki BA, Beaty TH, Cohen BH. Major genetic mechanisms in pulmonary function. *J Clin Epidemiol* 1990; 43: 667–675.
18. Astemborski JA, Beaty TH, Cohen BH. Variance components analysis of forced expiration in families. *Am J Med Genet* 1985; 21: 741–753.
19. Zamel N, Webster P, Lorimer E, Man S, Woolf C. Environment *versus* genetics in determining bronchial susceptibility to cigarette smoking. *Chest* 1981; 80: 57S.
20. Redline S, Tishler PV, Lewitter FI, Tager IB, Munoz A, Speizer FE. Assessment of genetic and nongenetic influences on pulmonary function: a twin study. *Am Rev Respir Dis* 1987; 135: 217–222.
21. Webster PM, Lorimer EG, Man SFP, Woolf CR, Zamel N. Pulmonary function in identical twins: comparison of nonsmokers and smokers. *Am Rev Respir Dis* 1979; 119: 223–228.
22. Hankins D, Drage C, Zamel N, Kronenberg R. Pulmonary function in identical twins raised apart. *Am Rev Respir Dis* 1982; 125: 119–121.
23. Hubert HB, Fabsitz RR, Feinleib M, Gwinn C. Genetic and environmental influences on pulmonary function in adult twins. *Am Rev Respir Dis* 1982; 125: 409–415.
24. Man SFP, Zamel N. Genetic influences on normal variability of maximum expiratory flow-volume curves. *J Appl Physiol* 1976; 41: 874–877.
25. Soubrier F, Lathrop GM. The genetic basis of hypertension. *Curr Opin Nephrol Hypertens* 1995; 4: 177–181.

26. Davies JL, Kawaguchi Y, Bennett ST, *et al.* A genome-wide search for human type 1 diabetes susceptibility genes. *Nature* 1994; 371: 130–136.
27. Vollmer WM, Johnson LR, Buist AS. Relationship of response to a bronchodilator and decline in forced expiratory volume in one second in population studies. *Am Rev Respir Dis* 1985; 132: 1186–1193.
28. Lander ES, Schork NJ. Genetic dissection of complex traits. *Science* 1994; 265: 2037–2048.
29. Knowler WC, Williams RC, Pettitt DJ, Steinberg AG. *Gm3;5,13,14* and type 2 diabetes mellitus: an association in American Indians with genetic admixture. *Am J Hum Genet* 1988; 43: 520–526.
30. Laurell CB, Eriksson S. The electrophoretic alpha<sub>1</sub>-globulin pattern of serum in alpha<sub>1</sub>-antitrypsin deficiency. *Scand J Clin Invest* 1963; 15: 132–140.
31. Mornex J-F, Chytil-Weir A, Martinet Y, Courtney M, LeCocq J-P, Crystal RG. Expression of the alpha<sub>1</sub>-antitrypsin gene in mononuclear phagocytes of normal and alpha<sub>1</sub>-antitrypsin deficient individuals. *J Clin Invest* 1986; 77: 1952–1961.
32. Cox DW, Johnson AM, Fagerhol MK. Report of nomenclature meeting for alpha<sub>1</sub>-antitrypsin. *Hum Genet* 1980; 53: 429–433.
33. Fagerhol MK, Laurell CB. The Pi system: inherited variants of serum alpha<sub>1</sub>-antitrypsin. *Prog Med Genet* 1970; 7: 96–111.
34. Allen RC, Harley RA, Talamo RC. A new method for determination of alpha<sub>1</sub>-antitrypsin phenotypes using isoelectric focusing on polyacrylamide gel slabs. *Am J Clin Pathol* 1974; 62: 732–739.
35. Birrer P, McElvaney NG, Chang-Stroman LM, Crystal RG. Alpha<sub>1</sub>-antitrypsin deficiency and liver disease. *J Inher Metab Dis* 1991; 14: 512–525.
36. Black LF, Kueppers F. Alpha<sub>1</sub>-antitrypsin deficiency in nonsmokers. *Am Rev Respir Dis* 1978; 117: 421–428.
37. Janus ED, Phillips NT, Carrell RW. Smoking, lung function and alpha<sub>1</sub>-antitrypsin deficiency. *Lancet* 1985; i: 152–154.
38. Brantley ML, Paul LD, Miller BH, Falk RT, Wu M, Crystal RG. Clinical features and history of the destructive lung disease associated with alpha<sub>1</sub>-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis* 1988; 138: 327–336.
39. Tobin MJ, Cook PJJ, Hutchinson DCS. Alpha<sub>1</sub>-antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z: a survey by the British Thoracic Association. *Br J Dis Chest* 1983; 77: 14–27.
40. Sveger T. Liver disease in alpha<sub>1</sub>-antitrypsin deficiency detected by screening of 200,000 infants. *N Engl J Med* 1976; 294: 1316–1321.
41. O'Brien ML, Buist NRM, Murphey WH. Neonatal screening for alpha<sub>1</sub>-antitrypsin deficiency. *J Pediatr* 1978; 92: 1006–1010.
42. Shigeoka JW, Hall WJ, Hyde RW, *et al.* The prevalence of alpha<sub>1</sub>-antitrypsin heterozygotes (PiMZ) in patients with obstructive pulmonary disease. *Am Rev Respir Dis* 1976; 114: 1077–1084.
43. Bartmann K, Fooke-Achterrath M, Koch G, *et al.* Heterozygosity in the Pi system as a pathogenetic cofactor in chronic obstructive pulmonary disease (COPD). *Eur J Respir Dis* 1985; 66: 284–296.
44. Cox DW, Hoepfner VH, Levison H. Protease inhibitors in patients with chronic obstructive pulmonary disease: the alpha<sub>1</sub>-antitrypsin heterozygote controversy. *Am Rev Respir Dis* 1976; 113: 601–606.
45. Janus ED. Alpha<sub>1</sub>-antitrypsin Pi types in COPD patients. *Chest* 1988; 94: 446–447.
46. Lieberman J, Winter B, Sastre A. Alpha<sub>1</sub>-antitrypsin Pi types in 965 COPD patients. *Chest* 1986; 89: 370–373.
47. Mittman C, Lieberman J, Rumsfeld J. Prevalence of abnormal protease inhibitor phenotypes in patients with chronic obstructive lung disease. *Am Rev Respir Dis* 1974; 109: 295–296.
48. Barnett TB, Gottovi D, Johnson AM. Protease inhibitors in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1975; 111: 587–593.
49. Webb DR, Hyde RW, Schwartz RH, Hall WJ, Condemi JJ, Townes TL. Serum alpha<sub>1</sub>-antitrypsin variants. *Am Rev Respir Dis* 1973; 108: 918–925.
50. Cole RB, Nevin NC, Blundell G, Merrett JD, McDonald JR, Johnston WP. Relation of alpha<sub>1</sub>-antitrypsin phenotype to the performance of pulmonary function tests and to the prevalence of respiratory illness in a working population. *Thorax* 1976; 31: 149–157.
51. Morse JO, Lebowitz MD, Knudson RJ, Burrows B. Relation of protease inhibitor phenotypes to obstructive lung diseases in a community. *N Engl J Med* 1977; 296: 1190–1194.
52. Lebowitz MD, Knudson RJ, Morse JO, Armet D. Closing volume and flow volume abnormalities in alpha<sub>1</sub>-antitrypsin phenotype groups in a community population. *Am Rev Respir Dis* 1978; 117: 179–181.
53. Chan-Yeung M, Ashley MJ, Corey P, Maledy H. Pi phenotypes and the prevalence of chest symptoms and lung function abnormalities in workers employed in dusty industries. *Am Rev Respir Dis* 1978; 117: 239–245.
54. McDonagh DJ, Nathan SP, Knudson RJ, Lebowitz MD. Assessment of alpha<sub>1</sub>-antitrypsin deficiency heterozygosity as a risk factor in the etiology of emphysema. *J Clin Invest* 1979; 63: 299–309.
55. Buist AS, Sexton GJ, Azzam A-MH, Adams BE. Pulmonary function in heterozygotes for alpha<sub>1</sub>-antitrypsin deficiency: a case-control study. *Am Rev Respir Dis* 1979; 120: 759–766.
56. Bruce RM, Cohen BH, Diamond EL, *et al.* Collaborative study to assess risk of lung disease in Pi MZ phenotype subjects. *Am Rev Respir Dis* 1984; 130: 386–390.
57. Klayton R, Fallat R, Cohen AB. Determinants of chronic obstructive pulmonary disease in patients with intermediate levels of alpha<sub>1</sub>-antitrypsin. *Am Rev Respir Dis* 1975; 112: 71–75.
58. Cooper DM, Hoepfner V, Cox D, Zamel N, Bryan AC, Levison H. Lung function in alpha<sub>1</sub>-antitrypsin heterozygotes (Pi type MZ). *Am Rev Respir Dis* 1974; 110: 708–715.
59. Tattersall SF, Pereira RP, Hunter D, Blundell G, Pride NB. Lung distensibility and airway function in intermediate alpha<sub>1</sub>-antitrypsin deficiency. *Thorax* 1979; 34: 637–646.
60. Hall WJ, Hyde RW, Schwartz RH, *et al.* Pulmonary abnormalities in intermediate alpha<sub>1</sub>-antitrypsin deficiency. *J Clin Invest* 1976; 58: 1069–1077.
61. Tárjan E, Magyar P, Váci Z, Lantos Á, Vaszar L. Longitudinal lung function study in heterozygous PiMZ phenotype subjects. *Eur Respir J* 1994; 7: 2199–2204.
62. Owen MC, Brennan SO, Lewis JH, Carrell RW. Mutation of antitrypsin to antithrombin: alpha<sub>1</sub>-antitrypsin Pittsburgh (358 met→arg), a fatal bleeding disorder. *N Engl J Med* 1983; 309: 694–698.
63. Kalsheker NA, Hodgson IJ, Watkins GL, White JP, Morrison HM, Stockley RA. Deoxyribonucleic acid (DNA) polymorphism of the alpha<sub>1</sub>-antitrypsin gene in chronic lung disease. *Br Med J* 1987; 294: 1511–1514.
64. Poller W, Meison C, Olek K. DNA polymorphisms of the alpha<sub>1</sub>-antitrypsin gene region in patients with chronic obstructive pulmonary disease. *Eur J Clin Invest* 1990; 20: 1–7.
65. Kalsheker NA, Watkins GL, Hill S, Morgan K, Stockley

- RA, Fick RB. Independent mutations in the flanking sequence of the  $\alpha_1$ -antitrypsin gene are associated with chronic obstructive airways disease. *Dis Markers* 1990; 8: 151–157.
66. Billingsley GD, Walter MA, Hammond GL, Cox DW. Physical mapping of four serpin genes:  $\alpha_1$ -antitrypsin,  $\alpha_1$ -antichymotrypsin, corticosteroid-binding globulin, and protein C inhibitor, within a 280 kb region on chromosome 14q32.1. *Am J Hum Genet* 1993; 52: 343–353.
  67. Cruickshank AM, Hansell DT, Burns HJG, Shenkin A. Effect of nutritional status on acute phase protein response to elective surgery. *Br J Surg* 1989; 76: 165–168.
  68. Morgan K, Scobie G, Kalsheker N. The characterization of a mutation of the 3' flanking sequence of the  $\alpha_1$ -antitrypsin gene commonly associated with chronic obstructive airways disease. *Eur J Clin Invest* 1992; 22: 134–137.
  69. Morgan K, Scobie G, Kalsheker NA. Point mutation in a 3' flanking sequence of the  $\alpha_1$ -antitrypsin gene associated with chronic respiratory disease occurs in a regulatory sequence. *Hum Mol Genet* 1993; 2: 253–257.
  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.
  71. Ramji DP, Vitelli A, Tronche F, Cortese R, Ciliberto G. The two C/EBP isoforms, IL-6 DBP/NF-IL-6 and C/EBP  $\delta$  NF-IL-6  $\beta$  are induced by IL-6 to promote acute phase gene transcription via different mechanisms. *Nucleic Acids Res* 1993; 21: 289–294.
  72. Buraczynska M, Schött D, Hanzlik AJ, Höltmann B, Ulmer WT. Alpha $_1$ -antitrypsin gene polymorphism related to respiratory system disease. *Klin Wochr* 1987; 65: 538–541.
  73. Kilpatrick L, Johnson JL, Nickbarg EB, et al. Inhibition of human neutrophil superoxide generation by  $\alpha_1$ -antichymotrypsin. *J Immunol* 1991; 146: 2388–2393.
  74. Burnett D, McGillivray DH, Stockley RA. Evidence that alveolar macrophages can synthesize and secrete alpha $_1$ -antichymotrypsin. *Am Rev Respir Dis* 1984; 129: 473–476.
  75. Eriksson S, Lindmark B, Lilja H. Familial  $\alpha_1$ -antichymotrypsin deficiency. *Acta Med Scand* 1986; 220: 447–453.
  76. Lindmark BE, Arborelius M, Eriksson SG. Pulmonary function in middle-aged women with heterozygous deficiency of the serine protease inhibitor alpha $_1$ -antichymotrypsin. *Am Rev Respir Dis* 1990; 141: 884–888.
  77. Lindmark B, Svenonius E, Eriksson S. Heterozygous  $\alpha_1$ -antichymotrypsin and PiZ  $\alpha_1$ -antitrypsin deficiency: prevalence and clinical spectrum in asthmatic children. *Allergy* 1990; 45: 197–203.
  78. Poller W, Faber J-B, Scholz S, et al. Mis-sense mutation of  $\alpha_1$ -antichymotrypsin gene associated with chronic lung disease. *Lancet* 1992; 339: 1538.
  79. Poller W, Faber J-P, Weidinger S, et al. A leucine-to-proline substitution causes a defective  $\alpha_1$ -antichymotrypsin allele associated with familial obstructive lung disease. *Genomics* 1993; 17: 740–743.
  80. Samilchuk EI, Chuchalin AG. Mis-sense mutation of  $\alpha_1$ -antichymotrypsin gene and chronic lung disease. *Lancet* 1993; 342: 624.
  81. Welch MJ, Tsui L-T, Boat TF, Beaudet AL. The Metabolic Basis of Inherited Disease. In: Scriver CL, Beaudet AL, Sly WS, Valle D, eds. New York, McGraw-Hill Inc., 1995; pp. 3799–3876.
  82. Meindl RS. Hypothesis: a selective advantage for cystic fibrosis heterozygotes. *Am J Phys Anthropol* 1987; 74: 39–45.
  83. Shier WT. Increased resistance to influenza as a possible source of heterozygote advantage in cystic fibrosis. *Med Hypotheses* 1979; 5: 661–667.
  84. Quinton PM. Fluid and Electrolyte Abnormalities in Exocrine Glands in Cystic Fibrosis. In: Quinton PM, Martinez RJ, Hopfer U, eds. San Francisco, San Francisco Press, 1982; pp. 53–76.
  85. Gabriel SE, Brigman KN, Koller BH, Boucher RC, Stutts MJ. Cystic fibrosis resistance to cholera toxin in the cystic fibrosis mouse model. *Science* 1994; 266: 107–109.
  86. Anderson CM, Freeman M, Allan J, Hubbard L. Observations on: i) sweat sodium levels in relation to chronic respiratory disease in adults; and ii) the incidence of respiratory and other disease in parents and siblings of patients with fibrocystic disease of the pancreas. *Med J Aust* 1962; 1: 965–969.
  87. Orzaleski MM, Kohner D, Cook CD, Shwachman H. Anamnesis: sweat electrolyte and pulmonary function studies in parents of patients with cystic fibrosis of the pancreas. *Acta Paediatr* 1963; 52: 267–276.
  88. Batten J, Muir D, Simon G, Cedric C. The prevalence of respiratory disease in heterozygotes for the gene for fibrocystic disease of the pancreas. *Lancet* 1963; i: 1348–1350.
  89. Hallett WY, Knudson AG, Massey FJ. Absence of detrimental effect of the carrier state for the cystic fibrosis gene. *Am Rev Respir Dis* 1965; 90: 714–724.
  90. Davies PB. Autonomic and airway reactivity in obligate heterozygotes for cystic fibrosis. *Am Rev Respir Dis* 1984; 129: 911–914.
  91. Davis PB, Vargo K. Pulmonary abnormalities in obligate heterozygotes for cystic fibrosis. *Thorax* 1987; 42: 120–125.
  92. Kerem B-S, Rommens JM, Buchanan JA, et al. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; 245: 1073–1080.
  93. Poller W, Faber J-P, Scholtz S, Olek K, Müller K-M. Sequence analysis of the cystic fibrosis gene in patients with disseminated bronchiectatic lung disease. *Klin Wochr* 1991; 69: 657–663.
  94. Dumur V, Lafitte J-J, Gervais R, et al. Abnormal distribution of cystic fibrosis  $\Delta F508$  allele in adults with chronic bronchial hypersecretion. *Lancet* 1990; 335: 1340.
  95. Gervais R, Lafitte J-J, Dumur V, et al. Sweat chloride and  $\Delta F508$  mutation in chronic bronchitis or bronchiectasis. *Lancet* 1993; 342: 997.
  96. Akai S, Okayama H, Shimura S, Tanno Y, Sasaki H, Takisima T.  $\Delta F508$  mutation of cystic fibrosis gene is not found in chronic bronchitis with severe obstruction in Japan. *Am Rev Respir Dis* 1992; 146: 781–783.
  97. Artlich A, Boysen A, Bunge S, Entzian P, Schlaak M, Schwinger E. Common CFTR mutations are not likely to predispose to chronic bronchitis in Northern Germany. *Hum Genet* 1995; 95: 226–228.
  98. Entzian P, Müller E, Boysen A, Artlich A, Schwinger E, Schlaak M. Frequency of common cystic fibrosis gene mutations in chronic bronchitis patients. *Scand J Lab Invest* 1995; 55: 263–266.
  99. Pignatti PF, Bombieri C, Marigo C, Benetazzo M, Luisetti M. Increased incidence of cystic fibrosis gene mutations in adults with disseminated bronchiectasis. *Hum Mol Genet* 1995; 4: 635–636.
  100. Pignatti PF, Bombieri C, Benetazzo M, et al. CFTR gene variant IVS8-5T in disseminated bronchiectasis. *Am J Hum Genet* 1996; 58: 889–892.
  101. Gasparini P, Savoia A, Luisetti M, Peona V, Pignatti PF. The cystic fibrosis gene is not likely to be involved in chronic obstructive pulmonary disease. *Am J Respir Cell Mol Biol* 1990; 2: 297–299.
  102. Kew RR, Webster RO. Gc-globulin (vitamin D-binding



- protein) enhances the neutrophil chemotactic activity of C5a and C5a des Arg. *J Clin Invest* 1988; 82: 364–369.
103. Yamamoto N, Homma S. Vitamin D-binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes. *Proc Natl Acad Sci USA* 1991; 88: 8539–8543.
  104. Horne SL, Cockcroft DW, Dosman JA. Possible protective effect against chronic obstructive airways disease by the GC 2 allele. *Hum Hered* 1990; 40: 173–176.
  105. Kauffmann F, Kleisbauer J-P, Cambon-de-Mouzon A, et al. Genetic markers in chronic airflow limitation: a genetic epidemiologic study. *Am Rev Respir Dis* 1983; 127: 263–269.
  106. White R, Janoff A, Godfrey HP. Secretion of alpha<sub>2</sub>-macroglobulin by alveolar macrophages. *Lung* 1980; 158: 9–14.
  107. Mosher DF, Wing WA. Synthesis and secretion of alpha<sub>2</sub>-macroglobulin by cultured human fibroblasts. *J Exp Med* 1976; 143: 462–467.
  108. Böhm N, Shah I, Totović V, Karitzky D. Combined alpha<sub>1</sub>-antitrypsin and alpha<sub>2</sub>-macroglobulin deficiency syndrome: light microscopic evidence of collagenolytic, elastolytic and myolytic tissue lesions. *Path Res Pract* 1980; 168: 17–35.
  109. Burnett D, Stockley RA. Serum and sputum alpha<sub>2</sub>-macroglobulin in patients with chronic obstructive airways disease. *Thorax* 1981; 36: 512–516.
  110. Brissenden JE, Cox DW. Alpha<sub>2</sub>-macroglobulin in patients with obstructive lung disease, with and without alpha<sub>1</sub>-antitrypsin deficiency. *Clin Chim Acta* 1983; 128: 241–248.
  111. Ganrot PO, Laurell CB, Eriksson S. Obstructive lung disease and trypsin inhibitors in alpha<sub>1</sub>-antitrypsin deficiency. *Scand J Clin Lab Invest* 1967; 19: 205–208.
  112. Bergqvist D, Nilsson IM. Hereditary alpha<sub>2</sub>-macroglobulin deficiency. *Scand J Haematol* 1979; 23: 433–436.
  113. Stenbjerg S. Inherited alpha<sub>2</sub>-macroglobulin deficiency. *Thrombosis Res* 1981; 22: 491–495.
  114. Poller W, Faber J-P, Klobeck G, Olek K. Cloning of the human alpha<sub>2</sub>-macroglobulin gene and detection of mutations in two functional domains: the bait region and the thiolester site. *Hum Genet* 1992; 88: 313–319.
  115. Poller W, Barth J, Voss B. Detection of an alteration of the alpha<sub>2</sub>-macroglobulin gene in a patient with chronic lung disease and serum alpha<sub>2</sub>-macroglobulin deficiency. *Hum Genet* 1989; 83: 93–96.
  116. Drakoulis N, Cascorbi I, Brockmöller J, Gross CR, Roots I. Polymorphisms in the human *CYP1A1* gene as susceptibility factors for lung cancer: exon-7 mutation (4889 A to G), and a T to C mutation in the 3'-flanking region. *Clin Invest* 1994; 72: 240–248.
  117. Cantlay AM, Lamb D, Gillooly M, et al. Association between the *CYP1A1* gene polymorphism and susceptibility to emphysema and lung cancer. *J Clin Pathol: Mol Pathol* 1995; 48: M210–214.
  118. Cohen BH, Ball WC, Brashears S, et al. Risk factors in chronic obstructive pulmonary disease (COPD). *Am J Epidemiol* 1977; 105: 223–232.
  119. Beatty TH, Menkes HA, Cohen BH, Newill CA. Risk factors associated with longitudinal change in pulmonary function. *Am Rev Respir Dis* 1984; 129: 660–667.
  120. Krzyzanowski M, Jedrychowski W, Wysocki M. Factors associated with the change in ventilatory function and the development of chronic obstructive pulmonary disease in a 13 year follow-up of the Cracow study. *Am Rev Respir Dis* 1986; 134: 1011–1019.
  121. Higgins MW, Keller JB, Becker M, et al. An index of risk for obstructive airways disease. *Am Rev Respir Dis* 1982; 125: 144–151.
  122. Vestbo J, Hein HO, Suadican P, Sørensen H, Gyntelberg F. Genetic markers for chronic bronchitis and peak expiratory expiratory flow in the Copenhagen Male Study. *Dan Med Bull* 1993; 40: 378–380.
  123. Cohen BH, Bias WB, Chase GA, et al. Is ABH non-secretor status a risk factor for obstructive lung disease? *Am J Epidemiol* 1980; 111: 285–291.
  124. Haines AP, Imeson JD, Meade TW. ABH secretor status and pulmonary function. *Am J Epidemiol* 1982; 115: 367–370.
  125. Abboud RT, Yu P, Chan-Yeung M, Tan F. Lack of relationship between ABH secretor status and lung function in pulp-mill workers. *Am Rev Respir Dis* 1982; 126: 1089–1091.
  126. Horne SL, Cockcroft DW, Lovegrove A, Dosman JA. ABO, Lewis and secretor status and relative incidence of airflow obstruction. *Dis Markers* 1985; 3: 55–62.
  127. Kauffmann F, Frette C, Pham Q-T, Nafissi S, Bertrand J-P, Oriol R. Associations of blood group-related antigens to FEV<sub>1</sub>, wheezing and asthma. *Am J Respir Crit Care Med* 1996; 153: 76–82.
  128. Raza MW, Blackwell CC, Molyneux P, et al. Association between secretor status and respiratory viral illness. *Br Med J* 1991; 303: 815–818.
  129. Sugiyama Y, Kudoh S, Maeda H, Suzaki H, Takaku F. Analysis of HLA antigens in patients with diffuse pan-bronchiolitis. *Am Rev Respir Dis* 1990; 141: 1459–1462.
  130. Björkander J, Bake B, Oxelius VA, Hanson LÅ. Impaired lung function in patients with IgA deficiency and low levels of IgG2 or IgG3. *N Engl J Med* 1985; 313: 720–724.
  131. O'Keefe S, Gzel A, Drury R, Cullina M, Grealley J, Finnegan P. Immunoglobulin G subclasses and spirometry in patients with chronic obstructive pulmonary disease. *Eur Respir J* 1991; 4: 932–938.
  132. Oxelius VA, Laurell AB, Lindquist B, et al. IgG subclasses in selective IgA deficiency. *N Engl J Med* 1981; 304: 1476–1477.
  133. Webb DR, Condemi JJ. Selective immunoglobulin A deficiency and chronic obstructive lung disease. *Ann Intern Med* 1974; 80: 618–621.
  134. Folz RJ, Peno-Green L, Crapo JD. Identification of a homozygous mis-sense mutation (Arg to Gly) in the critical binding region of the human EC-SOD gene (SOD3) and its association with dramatically increased serum enzyme levels. *Hum Mol Genet* 1994; 3: 2251–2254.
  135. Sandström J, Nilsson P, Karlsson K, Marklund SL. Tenfold increase in human plasma extracellular superoxide dismutase content caused by a mutation in heparin-binding domain. *J Biol Chem* 1994; 268: 19163–19166.
  136. Peno-Green L, Crapo JD, Folz RJ. Characterization of human serum extracellular superoxide dismutase variants in normal and lung disease individuals. *Am J Respir Crit Care Med* 1995; 151: A167.
  137. Sallenave J-M, Shulmann J, Crossley J, Jordana M, Gaudie J. Regulation of secretory leukocyte proteinase inhibitor (SLPI) and elastase-specific inhibitor (ESI/Elafin) in human airway epithelial airway cells by cytokines and neutrophilic enzymes. *Am J Respir Cell Mol Biol* 1994; 11: 733–741.
  138. Abe T, Kobayashi N, Yoshimura K, et al. Expression of the secretory leukoprotease inhibitor gene in epithelial cells. *J Clin Invest* 1991; 87: 2207–2215.
  139. Lüdecke B, Poller W, Olek K, Bartholomé K. Sequence variant of the human cathepsin G gene. *Hum Genet* 1993; 91: 83–84.
  140. Weiss ST, Segal MR, Sparrow D, Wager C. Relation of FEV<sub>1</sub> and peripheral blood leukocyte count to total mortality: the normative aging study. *Am J Epidemiol* 1995; 142: 493–498.