

Review

Open Access

## The genetics of chronic obstructive pulmonary disease

Alice M Wood<sup>1</sup> and Robert A Stockley\*<sup>2</sup>

Address: <sup>1</sup>Department of Medical Sciences, University of Birmingham, Birmingham, UK and <sup>2</sup>Lung Investigation Unit, University Hospitals Birmingham, Birmingham, B15 2TH, UK

Email: Alice M Wood - [alice.wood@uhb.nhs.uk](mailto:alice.wood@uhb.nhs.uk); Robert A Stockley\* - [rob.stockley@uhb.nhs.uk](mailto:rob.stockley@uhb.nhs.uk)

\* Corresponding author

Published: 20 October 2006

Received: 22 September 2006

*Respiratory Research* 2006, **7**:130 doi:10.1186/1465-9921-7-130

Accepted: 20 October 2006

This article is available from: <http://respiratory-research.com/content/7/1/130>

© 2006 Wood and Stockley; licensee BioMed Central Ltd.

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/2.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

### Abstract

Chronic obstructive pulmonary disease (COPD) is a heterogeneous disease caused by the interaction of genetic susceptibility and environmental influences. There is increasing evidence that genes link to disease pathogenesis and heterogeneity by causing variation in protease anti-protease systems, defence against oxidative stress and inflammation. The main methods of genomic research for complex disease traits are described, together with the genes implicated in COPD thus far, their roles in disease causation and the future for this area of investigation.

### Background

Chronic obstructive pulmonary disease (COPD) is characterised by airflow limitation that is not fully reversible, which usually progresses, together with an abnormal inflammatory response to noxious particles or gases [1]. Patients may have chronic bronchitis [2], emphysema [3], small airways disease or a combination of these, with or without systemic manifestations of the disease [4]. This results in great variety within the patient population. It is not yet clear what the significance of each disease component is in terms of cause, or effect on management, though research into genetics and pathogenesis is starting to clarify this.

Although cigarette smoking is the main environmental risk factor for developing COPD, only about 15% of smokers develop clinically significant disease [5], suggesting that there are other influences on disease expression. Previous studies estimated that smoking contributes 15% to the variability of lung function [6], whilst genetic factors account for a further 40% [7]. Family based studies support this: they have shown ancestral aggregation of spirometric measures in groups unselected for respiratory

disease [8,9], and higher rates of airflow obstruction in first-degree relatives of patients with COPD [10]. Moreover, the observation of differences in rate of decline of lung function between smokers [11] suggests an interaction between genetic and environmental influences.

A genotype-environment interaction is defined by a non-additive contribution of gene and environment to the clinical phenotype [12]. Thus the two influences together confer a different level of risk than that expected by simply adding them. In a complex disease such as COPD there are likely to be many genes contributing to the overall phenotype, which may have additive or synergistic effects; these are known as epistatic interactions. When interpreting the results of genetic studies in complex diseases it is important to take such effects into account, lest a disease causing locus be missed. There are a variety of statistical methods that can allow for, detect or control for the presence of epistasis [13,14].

There are three main themes within the pathogenesis of COPD. The protease-anti-protease theory suggests that there is an imbalance between proteases that digest elas-

tin, together with other components of the extra-cellular matrix, and anti-proteases that protect against this[15,16]. The origin of this theory was the observation that patients with  $\alpha$ 1-antitrypsin (an anti-protease) deficiency (AATD) develop early onset emphysema [17] implicating a role for its target enzymes (neutrophil elastase and proteinase 3), which can induce many of the features of COPD in animal models[18]. Subsequent work has suggested other important proteases, such as the matrix metalloproteinases (MMP's) [16], cathepsin B and collagenases [19] may also play a role, perhaps as part of a protease/anti-protease cascade.

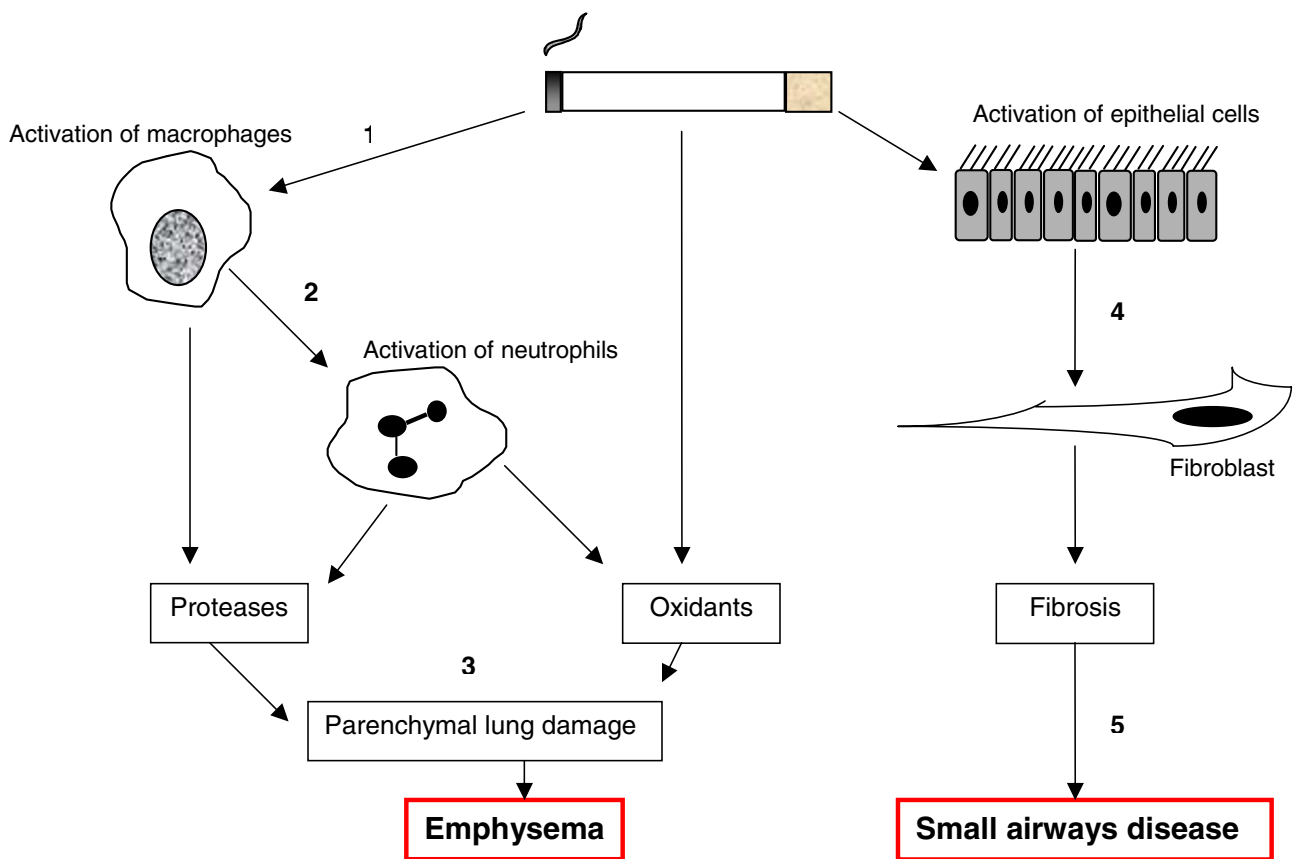
The oxidant-antioxidant theory states that disparity between levels of harmful oxidants and protective antioxidants leads to oxidative stress, which in turn influences the actions of anti-proteases, and expression of proinflammatory mediators[20]. Both of these theories link to the

third observation: the importance of inflammation in the pathogenesis of COPD[21]. These concepts are illustrated in Figure 1.

Polymorphisms in genes relating to proteases, antioxidants and inflammation have been found that relate to the presence of features of COPD, suggesting reasons for the heterogeneity of the observed clinical phenotype. This review will describe some of the methods that have identified candidate genes and summarise the evidence for a genetic basis to COPD (see Table 2).

#### How to identify candidate genes

Candidate genes may be suggested by pathogenesis, or vice versa. Variation, or polymorphism, within the gene can be classified in different ways[22], such as the structural nature of the change in the DNA, or its effect on the protein it codes for. Two common structural changes are



**Figure 1**

**The pathogenesis of COPD.** Cigarette smoke activates macrophages (1), leading to the direct release of proteases or neutrophil chemotactants (2), together with the release of oxidants resulting in subsequent breakdown of connective tissue in the lung (3), causing emphysema. Epithelial cell stimulation promotes fibroblast activity (4), eventually leading to small airways disease (5).

microsatellites – multiple repeats of a short segment of DNA, and single nucleotide polymorphisms (SNP's) – a change of a single base. The latter are the most common type of polymorphism in the human genome[23]. Such changes may occur in coding regions of DNA (those that contribute to the making of a protein) or non-coding regions. If a change occurs in a coding region it can be described as non-synonymous or synonymous, depending on whether it affects the amino acid sequence of the gene product or not. Generally speaking non-synonymous changes in coding regions are more likely to alter the function of a protein[24], and hence to be related to disease. International projects, such as the SNP consortium[25], which catalogues common SNP's in the human genome, and HapMap[26,27], which has genotyped SNP's in 4 major ethnic groups, have contributed to the many databases available on genetic variation. Researchers can use such resources to identify potential disease causing polymorphisms, and their likely population frequencies, allowing the design of case-control association studies, looking for the polymorphism in those with and without the disease. This is a widely used approach, though often producing inconsistent results [28]; this may be because of variation in the definition of cases and controls, underpowered studies, racial differences and population heterogeneity. The issue of power is particularly important when examining a complex disease such as COPD, as each gene may contribute only a small amount to the clinical phenotype: if this results in a genotype relative risk of developing the trait of less than 2, then adequate power may not be achievable[29].

Linkage studies look for haplotypes, or short segments of the genome, conserved between generations by virtue of their size [30] – anything larger has the potential to be changed by recombination during meiosis. If a haplotype can be found that is passed down through a family, alongside a disease, then it suggests that there is a gene within or close to it that may have a functional effect on the disease. Haplotype analyses can also be useful in association studies, though difficult to perform[31]. This is because they allow for the possibility that a combination of SNP's within a gene may be causing the trait in question, rather than one of the SNP's alone[32].

Linkage is usually reported as a logarithm of the odds (LOD) score[33], which is a form of likelihood ratio derived from the recombination fraction between the marker and the proposed locus of the disease-causing gene. The threshold level of LOD score needed for genome-wide significance at 5% ( $p = 0.05$ ) varies dependent on the study design, from 3.3 for family studies, using a proposed mode of inheritance (parametric linkage analysis) to 3.6 in sibling pair (non-parametric, no model of inheritance) studies [34]. Linkage analyses need to be fol-

lowed by case-control association analyses for any genes in the area of interest that have a plausible link to disease – established because of potential in pathogenesis, or differential expression in the target tissue [35].

#### **Areas of interest from linkage studies**

The major linkage study in COPD is the Boston early-onset COPD cohort [36-39], which performed genome-wide linkage analysis in 585 members of families with early onset COPD, looking for linkage to pulmonary function test results. Areas of linkage were found for FEV1/FVC on chromosome 2, chromosome 1 and chromosome 17. The area on chromosome 2 was subsequently investigated by the same group, identifying *SERPINE2* as a potential candidate gene. This area also contains the gene for the IL8 receptor, which might contribute to COPD since IL8 is involved in neutrophil chemotaxis to the lung [40](a critical process in delivery of destructive proteases). FEV1 linked to chromosomes 12 and 19 [38] in areas containing the genes for microsomal GST1 and TGF $\beta$  respectively. Mid expiratory flow, which is reduced in small airway disease in COPD, linked to chromosomes 2 and 12, together with a broader area on chromosome 19 [39].

#### **Proteases and anti-proteases**

There are three classes of protease that have been studied in COPD – the serine proteases, which includes neutrophil elastase (NE) and proteinase 3, the cysteine proteases, such as cathepsin-B, and the matrix metalloproteases (MMP's) [41]. In general the serine proteases are capable of degrading elastin and some forms of collagen [41], whilst the MMP's have more of an effect on collagen, gelatin and laminin [16]. Each enzyme is inhibited by one or more anti-proteases, may inactivate other anti-proteases, or activate pro-inflammatory cytokines, such as TNF $\alpha$ , by interacting with proteinase activated receptors (PARs) [42]. The proteases function to clear debris and damaged tissue, but if their action is not effectively controlled they may produce excessive lung damage. The relationships between these enzymes, their inhibitors and some inflammatory mediators are shown in Table 1.

#### **AATD**

AAT is an antiprotease that irreversibly inhibits NE, cathepsin G and proteinase 3. The AAT-NE complex also binds to receptors on neutrophils, thus stimulating further neutrophil migration, and amplifying inflammation. Its main function is to protect the connective tissue from NE released by activated neutrophils. There are four main variants of AAT, traditionally classified by their speed of movement during gel electrophoresis (F = fast, M = medium, S = slow, Z = very slow) [43], which are inherited in a co-dominant fashion. The PiM allele is the wild-type, and is the most prevalent. The PiZ allele is a more com-

**Table 1: Protease-antiprotease interactions**

Proteinase	Class	Activity	Active antiproteases
Neutrophil elastase	Serine	Degrades elastin, collagen type IV & laminin Inactivates TIMP Activates MMP9	AAT SERPINA3 SLPI
Cathepsin G	Serine	Degrades elastin, collagen I, II, IV & laminin Activates MMP9	SERPINA3 SLPI
Proteinase 3	Serine	Degrades elastin & collagen IV Activates TNF $\alpha$	AAT
Cathepsin B	Cysteine	Degrades elastin Inactivates secretory leukocyte proteinase inhibitor (SLPI)	Cystatin C
MMP1	MMP	Degrades collagens I-IV, VII, VIII, X, XI Inactivates AAT Activates TNF $\alpha$	TIMPI-4
MMP9	MMP	Degrades collagen IV, V, X, XIV & elastin Inactivates AAT Activates TNF $\alpha$ & TGF $\beta$	TIMPI-4
MMP12	MMP	Degrades collagen I, IV, elastin & fibrillin Inactivates AAT Activates TNF $\alpha$	TIMPI-4

mon deficiency variant in Northern Europeans, whilst the PiS deficiency variant is more common in South-West Europe [44]. AATD is classified by genotype and by the plasma AAT level. The PiZ variant is associated with significant AAT deficiency, lung and liver disease, though there is considerable disparity in clinical phenotype, which has been reviewed elsewhere [45].

The gene for AAT is on chromosome 14, and is highly pleomorphic. In addition to the common variants described here, there are over 100 SNP's catalogued in public databases [46]. Combinations of such SNP's, which give rise to six new haplotypes, have been associated with a higher risk of developing COPD in subjects without AATD [47]. However, even in patients with the same AAT genotype the phenotypes differ, suggesting that there may be other genetic modifiers present. One way to prove that modifier genes affect a complex disease is to show that traits related to the disease aggregate in families. In AATD the evidence so far is limited. Silverman et al showed some clustering of spirometric parameters in 82 families with PiZ or MZ genotypes, though this did not reach significance when adjusted for smoking status [48].

Case-control genetic studies have not been carried out as frequently in AATD as in usual COPD. One research paper examined polymorphisms in the gene coding for endothelial nitric oxide synthase (NOS) 3, and found a significant correlation between a SNP and severity of lung disease, defined by FEV1 [49]. NOS3 generates nitric oxide and citrulline from the amino acid arginine, as do the other isoforms of NOS [50]. The roles of nitric oxide (NO) in the lung include regulation of vascular tone and inhibition of inflammatory events, such as leukocyte

adhesion; this has been reviewed extensively elsewhere [50]. It might therefore be expected that variation in the pathways that generate NO would have an impact on lung disease. In COPD, whether related to AATD or not, this could conceivably be due to alterations in the inhibition of inflammation. However the authors were unable to show any functional variation in NOS3 with this SNP, and concluded that it must lie in linkage disequilibrium with the gene that caused the association. Other family and case-control studies are underway and may begin to clarify reasons for phenotypic heterogeneity in AATD.

#### MMP's

The actions of MMP's include degradation of collagen, inactivation of AAT and activation of TNF $\alpha$ . Their action is reduced by tissue inhibitors of metalloproteinases (TIMP's). Studies using knockout mouse models have supported a role for MMP's in COPD. Mice over-expressing MMP1 develop emphysema [51], whilst those deficient in MMP12 are relatively protected [52]. Further support comes from clinical studies showing increased concentrations of MMP's in the bronchoalveolar lavage fluid of COPD patients [53].

The most widely studied MMP gene polymorphism is in the MMP9 gene, located on chromosome 20. A SNP in the promoter region (C $\rightarrow$ T, position -1562), which increases its activity has been described [54], and linked to COPD in both Chinese [55] and Japanese populations [56]. The Chinese cases were defined by airflow obstruction, according to the GOLD criteria, whilst the Japanese cases were defined by the degree of emphysema on CT scan. This latter group found that airflow obstruction did not correlate with presence of the T allele, though gas transfer

corrected for alveolar volume (KCO) did. A later study has narrowed the emphysema phenotype linked to this polymorphism to upper zone predominant disease [57].

SNP's in *MMP1* and *MMP12* have also been studied in COPD. An insertion in the promoter region of *MMP1* (G→GG, position -1607) that increases its transcription [58] by creating an extra transcription factor binding site has been described. This SNP occurs in 30% of the general population [58] and was negatively associated with rapid decline of lung function (defined by FEV1) in one case-control study[59]. This does not, however, have an explanation from its function. It would be expected that the GG variant would be positively associated if the higher level of *MMP1* transcription lead to more lung damage. A role for polymorphisms of *MMP12* was investigated by the same group, but an association with declining lung function was not seen [59]. A haplotype containing the *MMP1* G→GG SNP, together with an *MMP12* SNP that results in a change in protein composition (Asn357Ser), was found more commonly in the rapid declining group[59]. The authors suggested that this may be because the gene actually responsible for rapid decline lies in linkage disequilibrium with these two SNP's.

#### TIMP2

There are four TIMP's (TIMP1-4) that inhibit active forms of MMP. Although all TIMP's are capable of inhibiting any MMP their affinity for each MMP varies and TIMP2 has been shown to have a greater affinity for MMP2 and MMP9[60]. The contribution of various MMP's, TIMP1 and TIMP2 to emphysema have been investigated, and a key role for the MMP2-TIMP2 system proposed [61]. Two SNP's in *TIMP2* are more common in Japanese subjects with COPD. One in the promoter region (that may cause reduced TIMP2 levels) and a second synonymous change in exon 3 [62]. This result has not yet been reproduced in other ethnic groups, and the functional consequences of each SNP remain theoretical, rather than proven. This should be an area for future research.

#### *α-1 antichymotrypsin*

*α-1 antichymotrypsin* (SERPINA3) inhibits cathepsin G and mast cell chymase in a reversible fashion. Two SNPs in *SERPINA3*, associated with low *SERPINA3* levels, have been associated with COPD in Swedish subjects [63,64], though their cases were defined by a measure of airway resistance, rather than standard spirometric parameters. The positive results for these SNP's were not reproduced in Japan in patients with airflow obstruction and low FEV1 [65,66], though a non-synonymous mutation affecting the signal peptide region was found more commonly in the COPD group. All 3 of these mutations were examined in an Italian study of patients with airflow obstruction and were not found to be associated with disease,

though their cases included subjects with bronchiectasis as well as COPD [66]. The variation in results between the studies could be explained by the different diagnostic criteria used by each group; it may be that the mutations are linked to airway resistance, but not to airflow obstruction, perhaps emphasising the heterogeneity of COPD.

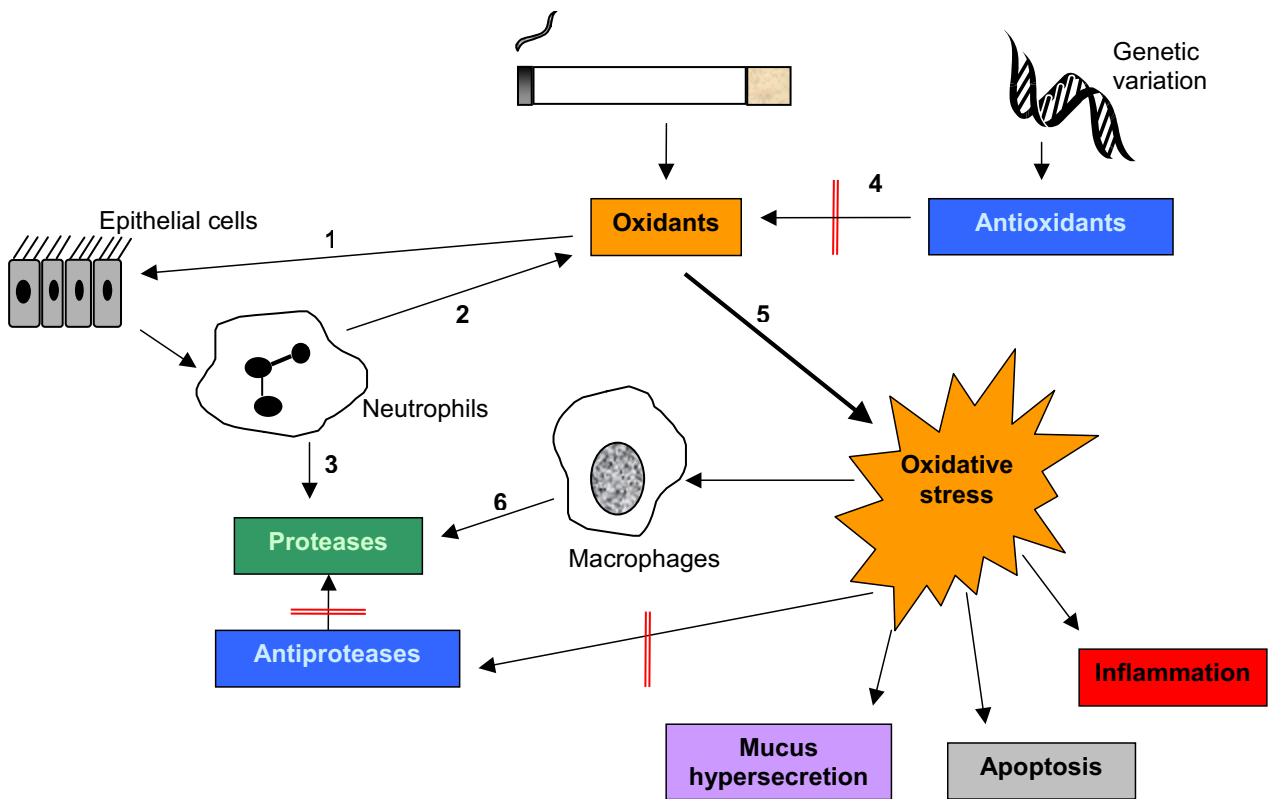
#### Antioxidants

Oxidative stress results from an imbalance between exogenous, harmful, oxidants and endogenous, protective, antioxidants[20]. This process, illustrated in Figure 2, can damage components of the lung matrix (such as elastin), injure the airway epithelium and enhance inflammation in the lung via up-regulation of genes for pro-inflammatory cytokines[20]. Cigarette smoke is a major source of oxidants (mainly free radicals and nitric oxide). Oxygen radicals are also released by inflammatory leukocytes, which are known to accumulate in the lungs of smokers[67], thus exacerbating the process of oxidative damage. Antioxidant enzymes present in the airway include glutathione-S-transferase, superoxide dismutase and catalase[68], amongst others. Gene polymorphisms affecting the function of such proteins might alter the amount of oxidative stress and so have been examined for their link to COPD.

#### *Glutathione-S-transferases*

The glutathione-S-transferase (GST) genes code for a family of enzymes that detoxify some of the harmful contents of tobacco smoke [69]. Polymorphisms in the genes are known to have functional consequences, and have been examined in COPD [70-72]. The two variants with the most evidence supporting a role in the disease are *GSTP1* and *GSTM1*.

*GSTP1* contains two known SNP's, though only one is known to have an effect on the catalytic activity of the enzyme. This is an A→G change at nucleotide +313, resulting in a single amino acid substitution (Ile105Val) [73] shown to increase the metabolism of carcinogenic aromatic epoxides [74]. Studies of the relationship of this variant to lung disease have varied in their results. It would be expected that the 105Ile variant would be associated with higher levels of lung damage, since it is less active against oxidants – this was confirmed by an association with airflow obstruction in a Japanese population [71]and replicated in a Caucasian population in the Lung Health Study (LHS), where this polymorphism together with a family history of COPD was linked to rapid decline of FEV1 (OR = 2.20, p = 0.01)[70]. Conversely the same group showed that the 105Val variant was associated with low baseline lung function (OR = 1.69, p = 0.016) and rapid decline in the higher baseline group (p = 0.017) [72], whilst Gilliland [75]demonstrated reduced annual growth rates for FEV1 and FVC in children homozygous



**Figure 2**

**Oxidative stress and its effects.** Oxidants contained within cigarette smoke irritate epithelial cells (1), releasing activating cytokines that prompt the recruitment of neutrophils and the release of cell derived oxidants (2) and proteases (3). Antioxidants inhibit oxidant mediated damage to the lung (4), but when an imbalance arises (perhaps because of gene polymorphisms) oxidative stress results (5). The consequences of oxidative stress include activation of macrophages (6), leading to the production of more proteases, mucus hypersecretion, epithelial cell apoptosis, inflammation and inhibition of the action of antiproteases.

for the 105Val variant. The latter results are difficult to explain on the basis of this gene's action alone, but might be understandable if there are gene-smoking or gene-gene interactions affecting the expression of the gene product. No gene-smoking affects were seen in the LHS [72], but there may be an additive effect of polymorphisms in *GSTP1* and other GST genes [70], suggesting that a consequence might not be seen unless a change in several gene products were present.

*GSTM1* has 3 known alleles, one of which is a null allele, such that homozygotes for the null allele have no detectable *GSTM1* activity. This genotype has been associated with emphysema [76] and chronic bronchitis [77], with conflicting results concerning its role in lung cancer [76,78]. In common with most other genetic studies in

COPD the positive results have been difficult to replicate [28], though this may be because studies looked at different subgroups of patients with COPD. The negative studies defined their cases by airflow obstruction [79] and rapid decline in FEV1 [70], hence might not have picked up a change in gene prevalence in chronic bronchitis. This difference in case definition remains a common theme in COPD genetics studies.

*Superoxide dismutase*

There are three superoxide genes, coding for scavengers of reactive oxygen species (ROS) [68]. Extracellular superoxide dismutase (SOD3) is present at high concentrations in areas of the lung containing large amounts of type 1 collagen, especially around large airways and also adjacent to alveoli [80]. It is thought to have a role in protecting the

lung, particularly during inflammation [81,82]. A SNP (C→G substitution at +760) of *SOD3* that increases plasma enzyme levels has been examined in 2 studies relating to COPD [68,83], and found to have a protective effect. A case-control study was carried out in New Zealand, where the mutation was found more frequently in resistant smokers than in those with COPD (OR = 4.3,  $p < 0.05$ ) [68]. The second study was part of the Copenhagen City Heart Study, which examined 9258 individuals in both cross-sectional and prospective study designs [83]. This demonstrated a reduced risk of developing COPD in smokers (OR = 0.4) and a reduced risk of hospital admission or death due to COPD (hazard ratio = 0.3) in those carrying the mutation. Since this effect was not seen in non-smokers, whose odds ratio of developing COPD when they carried the mutation was 1.5, it suggests a gene-smoking interaction, though this could not be statistically proven.

#### *Microsomal epoxide hydrolase*

Microsomal epoxide hydrolase (EPHX1) is expressed in bronchial epithelial cells and metabolises highly reactive epoxide intermediates in cigarette smoke [84,85]. There are 2 known SNP's in this gene that affect enzyme activity by a single amino acid substitution. The first SNP is in exon 3 (Tyr113His), the second in exon 4 results in a further change in protein constitution (His139Arg). In both cases the His variant is associated with lower levels of enzyme activity [86,87]. Both polymorphisms only account for a modest change in activity level [87], so it may be that there is also variation in the gene's regulatory regions [88].

Patients carrying both His variants were at the highest risk of developing COPD (OR = 4.1,  $p < 0.001$ ) and emphysema (OR = 5,  $p < 0.001$ ) in a Scottish population [89]. This result was replicated in those with more advanced COPD in Japan (OR = 2.9,  $p = 0.02$ ) [90] despite the differing frequency of genotypes between the two racial groups. The LHS demonstrated a relationship with rapid decline in lung function (FEV1) for the same haplotype, though this was only statistically significant for those with a family history of COPD [91]. The His139 variant alone was associated with a spirometric diagnosis of COPD in the Boston early-onset COPD cohort [28].

The contribution of this gene to the heterogeneity of COPD has been examined in more detail in the National Emphysema Treatment Trial (NETT) Genetics Ancillary Study [92]. The authors studied a number of polymorphisms and looked for correlation between genotype and functional capacity phenotypes in two separate patient groups, hypothesising that there is a genetic basis to the observed phenotypes. The exon 3 SNP (Tyr113His) was associated with poor exercise capacity, whilst the exon 4

SNP (His139Arg) was connected to relatively greater gas transfer (DLCO). This study was powered to detect a moderate effect of each genotype on overall phenotype, so taken with the previous positive studies it seems likely that these polymorphisms contribute to the COPD phenotype. Their link to specific subgroups of COPD patients will need further study.

#### *Heme oxygenase-1*

Heme oxygenase-1 (HMOX1) is an enzyme important in heme metabolism, which catalyses the oxidative cleavage of heme, resulting in the release of carbon monoxide, biliverdin and iron [93]. Biliverdin is then broken down into bilirubin, which scavenges local ROS; thus HMOX1 contributes to the generation of antioxidants. It is present at higher concentrations in the lungs of smokers than non-smokers, suggesting up-regulation in these circumstances [94], presumably because of a response to increased ROS.

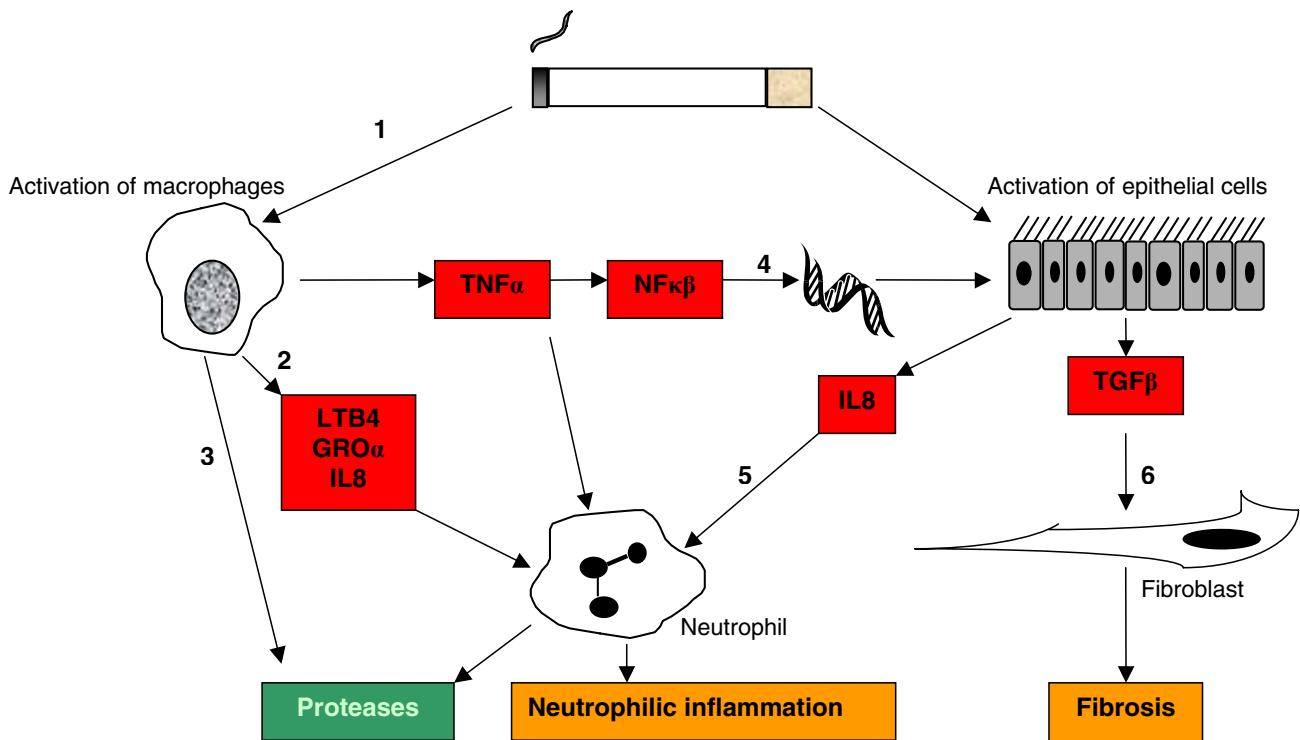
A microsatellite (GT)<sub>n</sub> repeat in the 5' region of *HMOX1* has been described that seems to alter the level of transcription when under thermal stress [95]. When the microsatellite is longer, it is not induced as effectively by ROS [96]. This suggests that in the presence of a long GT repeat (for instance  $n \geq 30$ ) smokers would not be able to protect their lungs from the damage induced by ROS in smoke, and thus would be more susceptible to emphysema. Two clinical studies have shown a link between this *HMOX1* polymorphism and COPD. A Japanese case-control study showed that patients with 30 or more GT repeats in the microsatellite region were more likely to have emphysema, diagnosed by CT scan [96]. A larger study in France showed that 33 or more GT repeats was associated with airflow obstruction and more rapid decline of lung function, particularly in smokers [97]. They were able to show a significant gene-smoking interaction ( $p = 0.0006$  for FEV1/FVC decline). This effect on decline was not, however, reproduced in the LHS [70].

#### **Inflammation and inflammatory mediators**

It is generally accepted that COPD is associated with an abnormal inflammatory response [1]. This extends beyond the lung to systemic manifestations [98]. Many different mediators have been implicated in pathogenesis [99] and their roles are summarised in Figure 3.

#### *TNF $\alpha$*

TNF $\alpha$  mediated inflammation is thought to play a key role in both the respiratory [100] and systemic features of COPD [98]. A SNP in the promoter region of the TNF $\alpha$  gene (G→A at position -308) directly affects gene regulation, and is associated with high TNF $\alpha$  production [101]. This polymorphism has been studied in several COPD related phenotypes, with differing results. An initial case-control study in Taiwan examined subjects with chronic

**Figure 3**

**Inflammatory mediators in COPD.** There are many pro-inflammatory mediators involved in COPD, some of which are illustrated here. Cigarette smoke activates macrophages (1) to release TNF $\alpha$ , LTB4, IL8 and other neutrophil chemotactic factors (2), as well as proteases (3). TNF $\alpha$  promotes further IL8 release from other cells in the respiratory tract by NF $\kappa$ B mediated effects on gene transcription (4). This increases local neutrophilic inflammation (5), and hence the release of proteases. Epithelial cells also stimulate fibroblasts via TGF $\beta$ , leading to fibrosis (6). TNF = tumour necrosis factor alpha, LTB4 = leukotriene B4, IL8 = interleukin 8, GRO = growth related oncogene, TGF = transforming growth factor, NF = nuclear factor

bronchitis, hypothesising that this was linked to increased airway inflammation [102]. They found an increased prevalence of the polymorphism in cases relative to controls ( $p < 0.01$ , OR = 11.1). It has also been linked to airflow obstruction without chronic bronchitis, and severity of emphysema in Japanese subjects [103,104]. Studies in Caucasians have not been able to reproduce these results [28,91] which might be explained by variation in genotype frequencies between races (data available from HapMap[27]), or by linkage disequilibrium with HLA alleles, seen previously in the Caucasian population [105].

### TGF $\beta$

TGF $\beta$ 1 regulates extra-cellular matrix production, cell growth and differentiation, tissue repair and some immune responses [106]. Mice who are unable to activate latent TGF $\beta$  develop emphysema via alterations of MMP12, suggesting that disordered activation relates to the pathogenesis of COPD [107]. A linkage analysis in the Boston early-onset COPD study showed association between an area of chromosome 19 containing the TGF $\beta$ 1

gene and FEV1 [108]. Three SNP's in this gene had a significant association with severe COPD in the NETT cohort [108]. This association was replicated for two of the SNP's by Hersh et al [92], who linked them both to subjective measures of dyspnoea, though not objective measures of exercise capacity. This apparent discordance may be important when defining phenotypes within COPD.

The two SNP's identified by Hersh et al both have an effect on TGF $\beta$ 1 levels. The first is a C $\rightarrow$ T change at position -509, in the promoter region, which enhances promoter function, thus increasing levels of TGF $\beta$  [109]. The second is a C $\rightarrow$ T change at position 613, which leads to an amino acid substitution (Leu $\rightarrow$ Pro) and higher production of TGF $\beta$ 1 [110]. If both of these polymorphisms are implicated in COPD, it suggests that TGF $\beta$  may have a protective role. A case-control study examining the latter SNP in COPD subjects, resistant smokers and healthy controls concurred, finding that the Pro allele was less common in COPD subjects relative to resistant smokers (OR = 0.59,  $p = 0.01$ ) and controls (OR = 0.62,  $p = 0.005$ ). Further



research on the role of TGF $\beta$  in COPD may help to clarify if this association has credibility in relevant pathogenic processes.

#### *Vitamin D binding protein*

Vitamin D binding protein, also known as Gc globulin, is a precursor of macrophage activating factor (MAF) [111] and enhances the neutrophil chemotactic properties of C5 derived peptides [112]. The latter function is prevented by neutrophil elastase inhibitors [113], suggesting a relationship between the protease-antiprotease pathway and inflammation. This would fit well with a role for vitamin D binding protein in the pathogenesis of COPD.

A number of studies have looked for links between polymorphisms in this gene (*GC*) and COPD. Two non-synonymous SNP's have been identified, which represent the *GC2* and *GC1S* alleles. The *GC2* allele has been found to be protective in studies of Caucasian subjects [114,115], consistent with the fact that only 10% of this form can be converted to MAF [116]. No role has been proven for this allele in neutrophil chemotaxis [114]. The *GC1S* allele has not been shown to have a significant association with COPD [28]. In Japanese subjects the *GC1F* allele has been linked to an increased risk of developing airflow obstruction, emphysema and a rapid decline of FEV1 [117,118]. Caucasian patients homozygous for this allele were at increased risk of developing COPD in one study [115] but not in another [114]. Neither could the link to rapid decline be reproduced in this racial group [91]. The difference in allele frequency between racial groups may explain why studies in Caucasians (who have a lower frequency of the 1F allele) have been unable to detect an association, as they would have required greater patient numbers to be adequately powered. An alternative explanation is that there is racial variation in gene associations with COPD.

#### *IL13*

Studies in transgenic mice have shown that if IL13 is over expressed, it results in cathepsin and matrix metalloproteinase dependent emphysema with mucus metaplasia [119]. A polymorphism in the promoter region (C $\rightarrow$ T, position -1055) is associated with increased IL13 production [120], with the T genotype being more common in COPD patients [121]. In mice IL13 induced emphysema is characterised by excessive pulmonary mucus production, so further studies looking for the prevalence of this polymorphism in the subgroup of COPD patients with chronic bronchitis might be worthwhile.

#### **Gene products without an identified role in pathogenesis**

##### *Surfactant proteins*

The surfactant proteins are hydrophobic proteins that contribute to regulation of surface tension in the alveoli.

Components of surfactant also have a role in host defence and control of inflammation. Alterations of surfactant might therefore be a factor in COPD, as suggested by mathematical models of emphysema [122] although this has yet to be studied in vivo. A SNP in the gene coding for surfactant protein B (*SFTPB*), which causes a single amino acid substitution (Thr131Ile), has been associated with COPD in the Boston Early-onset COPD cohort [28], and in a case-control study in Mexico [123]. In the NETT cohort this was also seen when gene-environment interaction was taken into account, where it was associated with dyspnoea score and exercise capacity [92]. In the Mexican study a number of SNP's and microsatellites were examined, with mutations in *SFTPB* (or microsatellite markers linked to it) being the most closely associated with COPD.

#### *SERPINE2*

The *SERPINE2* gene was identified as having a potential role in COPD by a novel method. Firstly linkage of airflow obstruction to an area on chromosome 2 in the Boston early-onset COPD cohort [37,38], followed by integration of these results with knowledge of genes expressed during murine lung development, together with human lung microarray datasets from control subjects and those with severe COPD [35]. Multiple SNP's in this gene were examined in patients from the NETT cohort, with several being significantly associated [35]. A subsequent large case-control study did not, however, find any association with COPD in European patients [124] and questioned the validity of some of the results reported in the original study.

*SERPINE2* has not been studied in COPD. It is known to be an inhibitor of trypsin-like serine proteases, but not neutrophil elastase [125], which might have indicated a role in the protease-antiprotease pathways. Its major function is in coagulation and fibrinolysis [126]. Although enhanced prothrombotic markers have been linked to decline of FEV1 in one small study in COPD [127] this has not been widely investigated.

#### **The future**

There are several areas in which methodology of genetic studies is advancing. Animal model genetics may help in clarifying some aspects of pathogenesis. One study has been performed which showed differences in inflammatory cell and cytokine profiles between murine strains after exposure to smoke [128]. If this type of study were followed by quantitative trait locus analysis it may help to identify candidate genes for further study in humans. Genome-wide association analysis may now be performed looking for up to 500000 SNP's at any one time to identify regions in linkage disequilibrium (LD) with features of COPD. This approach does, however, have limitations. Firstly, the SNP's should be as independent as

**Table 2: Some genetic polymorphisms relevant in COPD**

Gene/Protein	Polymorphism ID	Gene ID	Role	Function
MMP9	CR994492*	4318	C-1562T	Increases promoter activity
MMP1	rs1799750	4312	G-1607GG	Increases transcription
MMP12		4321	Asn357Ser	
TIMP2	rs2277698	7077	G853A	
			G-418C	
SERPINA3	rs4934	12	Ala-15Thr	Alters signal peptide
	rs17473		Pro227Ala	Reduces enzyme level
	rs1800463		Leu55Pro	Reduces enzyme level
GSTP1	rs947894	2950	Ile105Val	Increased enzyme activity
GSTM1	CG931302*	2944	Null	No enzyme activity
SOD3	CM941295*	6649	Arg213Gly	Increases enzyme level
EPHX1	rs1051740	2052	Tyr213His	Reduces enzyme activity
	rs2234922		His139Arg	Increases enzyme activity
HMOX1	CE000297*	3162	(GT) <sub>n</sub>	Alters transcription
TNF $\alpha$	rs1800629	7124	A-308G	Increases TNF $\alpha$ level
TGF $\beta$	rs1800469	7040	C-509T	Increases TGF $\beta$ level
	rs1982073		C613T	Increases TGF $\beta$ level
GC	rs4588	2638	Thr436Lys	Decreases conversion to MAF
	rs7041		Asp432Glu	
IL13		3596	C-1055T	Increases IL13 production
SFTPB	rs1130866	6439	Thr131Ile	
SFTPB	D2S388**		(CA) <sub>n</sub>	

**Polymorphism ID:** if listed in Entrez SNP [132], the reference SNP (rs) number is given.

\*indicates an accession number from the Human gene mutation database [133].

\*\* is searchable from PubMed, but not Entrez SNP.

**Gene ID:** from Entrez Gene [134].

**Role** This indicates the alteration and location of the nucleotide polymorphism (e.g. G853A, meaning a G→A substitution at position +853) or shows the change that results in the amino acid sequence (e.g. Ile105Val, meaning a change from Ile→Val at position 105 within the protein).

Microsatellites are denoted (xx).

possible from one another, to avoid the complication of LD between them. If this is not the case statistical corrections for multiple testing will not be valid, as the variables would be related. This means that haplotype tagged SNP's should be used, but even with these, the number needed to identify all common variants across the genome is uncertain with estimates ranging from 180000 to 600000 [129]. Secondly, statistical adjustments will be needed to account for multiple testing. Software to help with analysis of large genetic datasets is available from industry[130] and academia[131] and is necessary to handle the huge amounts of data that a genome-wide study would generate. Thirdly the potential costs of such studies could be prohibitive. Finally, the number of areas being investigated will raise the potential for false positive results, so confirmation of any positive results in multiple independent populations should be sought.

As more genes are identified we may be able to characterise patients with COPD more accurately and target therapies to those subgroups most likely to benefit.

### Competing interests

The author(s) declare that they have no competing interests.

### Authors' contributions

AMW drafted the manuscript. Both authors read and approved the final manuscript.

### References

1. Celli BR, MacNee W: **Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper.** *Eur Respir J* 2004, **23(6)**:932-946.
2. ATS: **Chronic bronchitis, asthma and pulmonary emphysema: a statement by the committee on diagnostic standards for non-tuberculous respiratory diseases.** *Am Rev Resp Dis* 1962, **85**:762-768.
3. Snider GL, Kleinman J, Thurlbeck WM, Bengali ZK: **The definition of emphysema: a report of the National Heart, Lung and Blood Institute, Division of Lung Diseases Workshop.** *Am Rev Resp Dis* 1985, **132**:182-185.
4. Agusti AG: **Systemic effects of chronic obstructive pulmonary disease.** *Proc Am Thorac Soc* 2005, **2(4)**:367-70; discussion 371-2.
5. ATS: **Cigarette smoking and health.** *Am J Respir Crit Care Med* 1996, **153(2)**:861-865.
6. Burrows B, Knudson RJ, Cline MG, Lebowitz MD: **Quantitative relationship between cigarette smoking and ventilatory function.** *Am Rev Resp Dis* 1977, **115**:195-205.
7. Coultas DB, Hanis CL, Howard CA, Skipper BJ, Samet JM: **Heritability of ventilatory function in smoking and nonsmoking New Mexico Hispanics.** *Am Rev Respir Dis* 1991, **144(4)**:770-775.

8. Redline S, Tishler PV, Rosner B, Lewitter FI, Vandenberg M, Weiss ST, Speizer FE: **Genotypic and phenotypic similarities in pulmonary function among family members of adult monozygotic and dizygotic twins.** *Am J Epidemiol* 1989, **129(4)**:827-836.
9. Lewitter FI, Tager IB, McGue M, Tishler PV, Speizer FE: **Genetic and environmental determinants of level of pulmonary function.** *Am J Epidemiol* 1984, **120(4)**:518-530.
10. 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(3)**:336-342.
11. Fletcher C, Peto R: **The natural history of chronic airflow obstruction.** *Br Med J* 1977, **1(6077)**:1645-1648.
12. Hartl DG, Clark AG: **Principles of population genetics.** Sunderland, MA, Sinauer Associates; 1997.
13. Cordell HJ: **Epistasis: what it means, what it doesn't mean, and statistical methods to detect it in humans.** *Hum Mol Genet* 2002, **11(20)**:2463-2468.
14. Baierl A, Bogdan M, Frommlet F, Futschik A: **On locating multiple interacting quantitative trait loci in intercross designs.** *Genetics* 2006, **173(3)**:1693-1703.
15. Stockley RA: **Neutrophils and protease/antiprotease imbalance.** *Am J Respir Crit Care Med* 1999, **160(5 Pt 2)**:S49-52.
16. Shapiro SD, Senior RM: **Matrix metalloproteinases. Matrix degradation and more.** *Am J Respir Cell Mol Biol* 1999, **20(6)**:1100-1102.
17. Eriksson SS: **Studies in alpha 1 antitrypsin deficiency.** *Acta Med Scand* 1965, **177 (Suppl.)**:432.
18. Sloan B, Abrams WR, Meranze DR, Kimbel P, Weinbaum G: **Emphysema induced in vitro and in vivo in dogs by a purified elastase from homologous leukocytes.** *Am Rev Respir Dis* 1981, **124(3)**:295-301.
19. Foronjy R, D'Armiento J: **The role of collagenase in emphysema.** *Respir Res* 2001, **2(6)**:348-352.
20. MacNee W: **Oxidants/antioxidants and COPD.** *Chest* 2000, **117(5 Suppl 1)**:303S-17S.
21. O'Donnell R, Breen D, Wilson S, Djukanovic R: **Inflammatory cells in the airways in COPD.** *Thorax* 2006, **61(5)**:448-454.
22. Strachan T, Read AP: **Human Molecular Genetics.** Oxford, Garland Science Publishers; 2003.
23. Wang DG, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES: **Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome.** *Science* 1998, **280(5366)**:1077-1082.
24. Carlson CS, Eberle MA, Kruglyak L, Nickerson DA: **Mapping complex disease loci in whole-genome association studies.** *Nature* 2004, **429(6990)**:446-452.
25. Sachidanandam R, Weissman D, Schmidt SC, Kakol JM, Stein LD, Marth G, Sherry S, Mullikin JC, Mortimore BJ, Willey DL, Hunt SE, Cole CG, Coggill PC, Rice CM, Ning Z, Rogers J, Bentley DR, Kwok PY, Mardis ER, Yeh RT, Schultz B, Cook L, Davenport R, Dante M, Fulton L, Hillier L, Waterston RH, McPherson JD, Gilman B, Schaffner S, Van Etten WJ, Reich D, Higgins J, Daly MJ, Blumenstiel B, Baldwin J, Stange-Thomann N, Zody MC, Linton L, Lander ES, Altshuler D: **A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms.** *Nature* 2001, **409(6822)**:928-933.
26. The International HapMap Consortium: **A Haplotype Map of the Human Genome.** *Nature* 2005, **437**:1299-1320.
27. **HapMap** [<http://www.hapmap.org/>].
28. Hersh CP, Demeo DL, Lange C, Litonjua AA, Reilly JJ, Kwiatkowski D, Laird N, Sylvia JS, Sparrow D, Speizer FE, Weiss ST, Silverman EK: **Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations.** *Am J Respir Cell Mol Biol* 2005, **33(1)**:71-78.
29. Risch NJ: **Searching for genetic determinants in the new millennium.** *Nature* 2000, **405(6788)**:847-856.
30. Thompson EA: **Linkage analysis.** In *Handbook of statistical Genetics* Edited by: Balding DJ, Bishop M, Cannings C. Chichester, Wiley; 2001.
31. Toivonen HT, Onkamo P, Vasko K, Ollikainen V, Sevón P, Mannila H, Herr M, Kere J: **Data mining applied to linkage disequilibrium mapping.** *Am J Hum Genet* 2000, **67(1)**:133-145.
32. Mira MT, Alcais A, Nguyen VT, Moraes MO, Di Flumeri C, Vu HT, Mai CP, Nguyen TH, Nguyen NB, Pham XK, Sarno EN, Alter A, Montpetit A, Moraes ME, Moraes JR, Dore C, Gallant CJ, Lepage P, Verner A, Van De Vosse E, Hudson TJ, Abel L, Schurr E: **Susceptibility to leprosy is associated with PARK2 and PACRG.** *Nature* 2004, **427(6975)**:636-640.
33. Morton NE: **Sequential tests for the detection of linkage.** *Am J Hum Genet* 1955, **7(3)**:277-318.
34. Lander E, Kruglyak L: **Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results.** *Nat Genet* 1995, **11(3)**:241-247.
35. Demeo DL, Mariani TJ, Lange C, Srisuma S, Litonjua AA, Celedon JC, Lake SL, Reilly JJ, Chapman HA, Mechem BH, Haley KJ, Sylvia JS, Sparrow D, Spira AE, Beane J, Pinto-Plata V, Speizer FE, Shapiro SD, Weiss ST, Silverman EK: **The SERPINE2 Gene Is Associated with Chronic Obstructive Pulmonary Disease.** *Am J Hum Genet* 2006, **78(2)**:253-264.
36. Palmer LJ, Celedon JC, Chapman HA, Speizer FE, Weiss ST, Silverman EK: **Genome-wide linkage analysis of bronchodilator responsiveness and post-bronchodilator spirometric phenotypes in chronic obstructive pulmonary disease.** *Hum Mol Genet* 2003, **12(10)**:1199-1210.
37. Silverman EK, Mosley JD, Palmer LJ, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, Province MA, Rao DC, Reilly JJ, Ginns LC, Speizer FE, Weiss ST: **Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes.** *Hum Mol Genet* 2002, **11(6)**:623-632.
38. Silverman EK, Palmer LJ, Mosley JD, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, Province MA, Rao DC, Reilly JJ, Ginns LC, Speizer FE, Weiss ST: **Genome-wide linkage analysis of quantitative spirometric phenotypes in severe early-onset chronic obstructive pulmonary disease.** *Am J Hum Genet* 2002, **70(5)**:1229-1239.
39. DeMeo DL, Celedon JC, Lange C, Reilly JJ, Chapman HA, Sylvia JS, Speizer FE, Weiss ST, Silverman EK: **Genome-wide linkage of forced mid-expiratory flow in chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med* 2004, **170(12)**:1294-1301.
40. Beeh KM, Kornmann O, Buhl R, Culpitt SV, Giermycz MA, Barnes PJ: **Neutrophil chemotactic activity of sputum from patients with COPD: role of interleukin 8 and leukotriene B4.** *Chest* 2003, **123(4)**:1240-1247.
41. Barnes PJ, Shapiro SD, Pauwels RA: **Chronic obstructive pulmonary disease: molecular and cellular mechanisms.** *Eur Respir J* 2003, **22(4)**:672-688.
42. Dery O, Corvera CU, Steinhoff M, Bunnett NW: **Proteinase-activated receptors: novel mechanisms of signaling by serine proteases.** *Am J Physiol* 1998, **274(6 Pt 1)**:C1429-52.
43. Fagerhol MK, Laurell CB: **The Pi system-inherited variants of serum alpha 1-antitrypsin.** *Prog Med Genet* 1970, **7**:96-111.
44. Luisetti M, Seersholm N: **Alpha 1-antitrypsin deficiency. I: epidemiology of alpha 1-antitrypsin deficiency.** *Thorax* 2004, **59(2)**:164-169.
45. Needham M, Stockley RA: **Alpha 1-antitrypsin deficiency. 3: Clinical manifestations and natural history.** *Thorax* 2004, **59(5)**:441-445.
46. DeMeo DL, Silverman EK: **Alpha 1-antitrypsin deficiency. 2: genetic aspects of alpha(1)-antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk.** *Thorax* 2004, **59(3)**:259-264.
47. Chappell S, Daly L, Morgan K, Guetta Baranes T, Roca J, Rabinovich R, Millar A, Donnelly SC, Keatings V, MacNee W, Stolk J, Hiemstra P, Miniati M, Monti S, O'Connor CM, Kalsheker N: **Cryptic haplotypes of SERPINA1 confer susceptibility to chronic obstructive pulmonary disease.** *Hum Mutat* 2006, **27(1)**:103-109.
48. Silverman EK, Province MA, Campbell EJ, Pierce JA, Rao DC: **Variability of pulmonary function in alpha-1-antitrypsin deficiency: residual family resemblance beyond the effect of the Pi locus.** *Hum Hered* 1990, **40(6)**:340-355.
49. Novoradovsky A, Brantly ML, Wacławiw MA, Chaudhary PP, Ihara H, Qi L, Eissa NT, Barnes PM, Gabriele KM, Ehrmantraut ME, Rogliani P, Moss J: **Endothelial nitric oxide synthase as a potential susceptibility gene in the pathogenesis of emphysema in alpha 1-antitrypsin deficiency.** *Am J Respir Cell Mol Biol* 1999, **20(3)**:441-447.

50. Hart CM: **Nitric oxide in adult lung disease.** *Chest* 1999, **115(5)**:1407-1417.
51. D'Armiento J, Dalal SS, Okada Y, Berg RA, Chada K: **Collagenase expression in the lungs of transgenic mice causes pulmonary emphysema.** *Cell* 1992, **71(6)**:955-961.
52. Hautamaki RD, Kobayashi DK, Senior RM, Shapiro SD: **Requirement for macrophage elastase for cigarette smoke-induced emphysema in mice.** *Science* 1997, **277(5334)**:2002-2004.
53. Finlay GA, Russell KJ, McMahon KJ, D'Arcy E M, Masterson JB, FitzGerald MX, O'Connor CM: **Elevated levels of matrix metalloproteinases in bronchoalveolar lavage fluid of emphysematous patients.** *Thorax* 1997, **52(6)**:502-506.
54. Zhang B, Ye S, Herrmann SM, Eriksson P, de Maat M, Evans A, Arveiler D, Luc G, Cambien F, Hamsten A, Watkins H, Henney AM: **Functional polymorphism in the regulatory region of gelatinase B gene in relation to severity of coronary atherosclerosis.** *Circulation* 1999, **99(14)**:1788-1794.
55. Zhou M, Huang SG, Wan HY, Li B, Deng WW, Li M: **Genetic polymorphism in matrix metalloproteinase-9 and the susceptibility to chronic obstructive pulmonary disease in Han population of south China.** *Chin Med J (Engl)* 2004, **117(10)**:1481-1484.
56. Minematsu N, Nakamura H, Tateno H, Nakajima T, Yamaguchi K: **Genetic polymorphism in matrix metalloproteinase-9 and pulmonary emphysema.** *Biochem Biophys Res Commun* 2001, **289(1)**:116-119.
57. Ito I, Nagai S, Handa T, Muro S, Hirai T, Tsukino M, Mishima M: **Matrix metalloproteinase-9 promoter polymorphism associated with upper lung dominant emphysema.** *Am J Respir Crit Care Med* 2005, **172(11)**:1378-1382.
58. Rutter JL, Mitchell TI, Buttice G, Meyers J, Gusella JF, Ozelius LJ, Brinckerhoff CE: **A single nucleotide polymorphism in the matrix metalloproteinase-1 promoter creates an Ets binding site and augments transcription.** *Cancer Res* 1998, **58(23)**:5321-5325.
59. Joos L, He JQ, Shepherdson MB, Connett JE, Anthonisen NR, Pare PD, Sandford AJ: **The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function.** *Hum Mol Genet* 2002, **11(5)**:569-576.
60. Howard EW, Bullen EC, Banda MJ: **Preferential inhibition of 72- and 92-kDa gelatinases by tissue inhibitor of metalloproteinases-2.** *J Biol Chem* 1991, **266(20)**:13070-13075.
61. Ohnishi K, Takagi M, Kurokawa Y, Satomi S, Kontinen YT: **Matrix metalloproteinase-mediated extracellular matrix protein degradation in human pulmonary emphysema.** *Lab Invest* 1998, **78(9)**:1077-1087.
62. Hirano K, Sakamoto T, Uchida Y, Morishima Y, Masuyama K, Ishii Y, Nomura A, Ohtsuka M, Sekizawa K: **Tissue inhibitor of metalloproteinases-2 gene polymorphisms in chronic obstructive pulmonary disease.** *Eur Respir J* 2001, **18(5)**:748-752.
63. Poller W, Faber JP, Scholz S, Weidinger S, Bartholome K, Olek K, Eriksson S: **Mis-sense mutation of alpha 1-antichymotrypsin gene associated with chronic lung disease.** *Lancet* 1992, **339(8808)**:1538.
64. Poller W, Faber JP, Weidinger S, Tief K, Scholz S, Fischer M, Olek K, Kirchgesser M, Heidtmann HH: **A leucine-to-proline substitution causes a defective alpha 1-antichymotrypsin allele associated with familial obstructive lung disease.** *Genomics* 1993, **17(3)**:740-743.
65. Ishii T, Matsuse T, Teramoto S, Matsui H, Hosoi T, Fukuchi Y, Ouchi Y: **Association between alpha-1-antichymotrypsin polymorphism and susceptibility to chronic obstructive pulmonary disease.** *Eur J Clin Invest* 2000, **30(6)**:543-548.
66. Benetazzo MG, Gile LS, Bombieri C, Malerba G, Massobrio M, Pignatti PF, Luisetti M: **alpha 1-antitrypsin TAQ I polymorphism and alpha 1-antichymotrypsin mutations in patients with obstructive pulmonary disease.** *Respir Med* 1999, **93(9)**:648-654.
67. Hunninghake GW, Crystal RG: **Cigarette smoking and lung destruction. Accumulation of neutrophils in the lungs of cigarette smokers.** *Am Rev Respir Dis* 1983, **128(5)**:833-838.
68. Young RP, Hopkins R, Black PN, Eddy C, Wu L, Gamble GD, Mills GD, Garrett JE, Eaton TE, Rees MI: **Functional variants of antioxidant genes in smokers with COPD and in those with normal lung function.** *Thorax* 2006, **61(5)**:394-399.
69. Mannervik B: **The isoenzymes of glutathione transferase.** *Adv Enzymol Relat Areas Mol Biol* 1985, **57**:357-417.
70. He JQ, Ruan J, Connett JE, Anthonisen NR, Pare PD, Sandford AJ: **Antioxidant gene polymorphisms and susceptibility to a rapid decline in lung function in smokers.** *Am J Respir Crit Care Med* 2002, **166(3)**:323-328.
71. Ishii T, Matsuse T, Teramoto S, Matsui H, Miyao M, Hosoi T, Takahashi H, Fukuchi Y, Ouchi Y: **Glutathione S-transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease.** *Thorax* 1999, **54(8)**:693-696.
72. He JQ, Connett JE, Anthonisen NR, Pare PD, Sandford AJ: **Glutathione S-transferase variants and their interaction with smoking on lung function.** *Am J Respir Crit Care Med* 2004, **170(4)**:388-394.
73. Ali-Osman F, Akande O, Antoun G, Mao JX, Buolamwini J: **Molecular cloning, characterization, and expression in Escherichia coli of full-length cDNAs of three human glutathione S-transferase Pi gene variants. Evidence for differential catalytic activity of the encoded proteins.** *J Biol Chem* 1997, **272(15)**:10004-10012.
74. Sundberg K, Johansson AS, Stenberg G, Widersten M, Seidel A, Mannervik B, Jernstrom B: **Differences in the catalytic efficiencies of allelic variants of glutathione transferase P1-I towards carcinogenic diol epoxides of polycyclic aromatic hydrocarbons.** *Carcinogenesis* 1998, **19(3)**:433-436.
75. Gilliland FD, Gauderman WJ, Vora H, Rappaport E, Dubeau L: **Effects of glutathione-S-transferase M1, T1, and P1 on childhood lung function growth.** *Am J Respir Crit Care Med* 2002, **166(5)**:710-716.
76. Harrison DJ, Cantlay AM, Rae F, Lamb D, Smith CA: **Frequency of glutathione S-transferase M1 deletion in smokers with emphysema and lung cancer.** *Hum Exp Toxicol* 1997, **16(7)**:356-360.
77. Baranova H, Perriot J, Albuissou E, Ivaschenko T, Baranov VS, Hemery B, Mouraire P, Riou N, Malet P: **Peculiarities of the GSTM1 0/0 genotype in French heavy smokers with various types of chronic bronchitis.** *Hum Genet* 1997, **99(6)**:822-826.
78. Ryberg D, Skaug V, Hewer A, Phillips DH, Harries LW, Wolf CR, Ogreid D, Ulvik A, Vu P, Haugen A: **Genotypes of glutathione transferase M1 and P1 and their significance for lung DNA adduct levels and cancer risk.** *Carcinogenesis* 1997, **18(7)**:1285-1289.
79. Yim JJ, Park GY, Lee CT, Kim YW, Han SK, Shim YS, Yoo CG: **Genetic susceptibility to chronic obstructive pulmonary disease in Koreans: combined analysis of polymorphic genotypes for microsomal epoxide hydrolase and glutathione S-transferase M1 and T1.** *Thorax* 2000, **55(2)**:121-125.
80. Oury TD, Chang LY, Marklund SL, Day BJ, Crapo JD: **Immunocytochemical localization of extracellular superoxide dismutase in human lung.** *Lab Invest* 1994, **70(6)**:889-898.
81. Folz RJ, Abushama AM, Suliman HB: **Extracellular superoxide dismutase in the airways of transgenic mice reduces inflammation and attenuates lung toxicity following hyperoxia.** *J Clin Invest* 1999, **103(7)**:1055-1066.
82. Bowler RP, Nicks M, Tran K, Tanner G, Chang LY, Young SK, Worthen GS: **Extracellular superoxide dismutase attenuates lipopolysaccharide-induced neutrophilic inflammation.** *Am J Respir Cell Mol Biol* 2004, **31(4)**:432-439.
83. Juul K, Tybjaerg-Hansen A, Marklund S, Lange P, Nordestgaard BG: **Genetically increased antioxidative protection and decreased chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med* 2006, **173(8)**:858-864.
84. Bartsch H, Petruzzelli S, De Flora S, Hietanen E, Camus AM, Castegnaro M, Alexandrov K, Rojas M, Saracci R, Giuntini C: **Carcinogen metabolism in human lung tissues and the effect of tobacco smoking: results from a case-control multicenter study on lung cancer patients.** *Environ Health Perspect* 1992, **98**:119-124.
85. Tingle MD, Pirmohamed M, Templeton E, Wilson AS, Madden S, Kitteringham NR, Park BK: **An investigation of the formation of cytotoxic, genotoxic, protein-reactive and stable metabolites from naphthalene by human liver microsomes.** *Biochem Pharmacol* 1993, **46(9)**:1529-1538.
86. Hassett C, Aicher L, Sidhu JS, Omiecinski CJ: **Human microsomal epoxide hydrolase: genetic polymorphism and functional expression in vitro of amino acid variants.** *Hum Mol Genet* 1994, **3(3)**:421-428.

87. Hosagrahara VP, Rettie AE, Hassett C, Omiecinski CJ: **Functional analysis of human microsomal epoxide hydrolase genetic variants.** *Chem Biol Interact* 2004, **150(2)**:149-159.
88. Raaka S, Hassett C, Omiecinski CJ: **Human microsomal epoxide hydrolase: 5'-flanking region genetic polymorphisms.** *Carcinogenesis* 1998, **19(3)**:387-393.
89. Smith CA, Harrison DJ: **Association between polymorphism in gene for microsomal epoxide hydrolase and susceptibility to emphysema.** *Lancet* 1997, **350(9078)**:630-633.
90. Yoshikawa M, Hiyaama K, Ishioka S, Maeda H, Maeda A, Yamakido M: **Microsomal epoxide hydrolase genotypes and chronic obstructive pulmonary disease in Japanese.** *Int J Mol Med* 2000, **5(1)**:49-53.
91. Sandford AJ, Chagani T, Weir TD, Connett JE, Anthonisen NR, Pare PD: **Susceptibility genes for rapid decline of lung function in the lung health study.** *Am J Respir Crit Care Med* 2001, **163(2)**:469-473.
92. Hersh CP, Demeo DL, Lazarus R, Celedon JC, Raby BA, Benditt JO, Criner G, Make B, Martinez FJ, Scanlon PD, Sciurba FC, Utz JP, Reilly JJ, Silverman EK: **Genetic association analysis of functional impairment in chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med* 2006, **173(9)**:977-984.
93. Tenhunen R, Marver HS, Schmid R: **The enzymatic conversion of heme to bilirubin by microsomal heme oxygenase.** *Proc Natl Acad Sci U S A* 1968, **61(2)**:748-755.
94. Maestrelli P, El Messelmani AH, De Fina O, Nowicki Y, Saetta M, Mapp C, Fabbri LM: **Increased expression of heme oxygenase (HO)-1 in alveolar spaces and HO-2 in alveolar walls of smokers.** *Am J Respir Crit Care Med* 2001, **164(8 Pt 1)**:1508-1513.
95. Okinaga S, Takahashi K, Takeda K, Yoshizawa M, Fujita H, Sasaki H, Shibahara S: **Regulation of human heme oxygenase-1 gene expression under thermal stress.** *Blood* 1996, **87(12)**:5074-5084.
96. Yamada N, Yamaya M, Okinaga S, Nakayama K, Sekizawa K, Shibahara S, Sasaki H: **Microsatellite polymorphism in the heme oxygenase-1 gene promoter is associated with susceptibility to emphysema.** *Am J Hum Genet* 2000, **66(1)**:187-195.
97. Guenegou A, Leynaert B, Benessiano J, Pin I, Demoly P, Neukirch F, Boczkowski J, Aubier M: **Association of lung function decline with the heme oxygenase-1 gene promoter microsatellite polymorphism in a general population sample. Results from the European Community Respiratory Health Survey (ECRHS), France.** *J Med Genet* 2006, **43(8)**:e43.
98. Sevenoaks MJ, Stockley RA: **Chronic Obstructive Pulmonary Disease, inflammation and co-morbidity--a common inflammatory phenotype?** *Respir Res* 2006, **7**:70.
99. Wouters EF: **Local and systemic inflammation in chronic obstructive pulmonary disease.** *Proc Am Thorac Soc* 2005, **2(1)**:26-33.
100. Churg A, Wang RD, Tai H, Wang X, Xie C, Wright JL: **Tumor necrosis factor-alpha drives 70% of cigarette smoke-induced emphysema in the mouse.** *Am J Respir Crit Care Med* 2004, **170(5)**:492-498.
101. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW: **Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation.** *Proc Natl Acad Sci U S A* 1997, **94(7)**:3195-3199.
102. Huang SL, Su CH, Chang SC: **Tumor necrosis factor-alpha gene polymorphism in chronic bronchitis.** *Am J Respir Crit Care Med* 1997, **156(5)**:1436-1439.
103. Sakao S, Tatsumi K, Igari H, Shino Y, Shirasawa H, Kuriyama T: **Association of tumor necrosis factor alpha gene promoter polymorphism with the presence of chronic obstructive pulmonary disease.** *Am J Respir Crit Care Med* 2001, **163(2)**:420-422.
104. Sakao S, Tatsumi K, Igari H, Watanabe R, Shino Y, Shirasawa H, Kuriyama T: **Association of tumor necrosis factor-alpha gene promoter polymorphism with low attenuation areas on high-resolution CT in patients with COPD.** *Chest* 2002, **122(2)**:416-420.
105. Wilson AG, de Vries N, Pociot F, di Giovine FS, van der Putte LB, Duff GW: **An allelic polymorphism within the human tumor necrosis factor alpha promoter region is strongly associated with HLA A1, B8, and DR3 alleles.** *J Exp Med* 1993, **177(2)**:557-560.
106. Blobel GC, Schiemann WP, Lodish HF: **Role of transforming growth factor beta in human disease.** *N Engl J Med* 2000, **342(18)**:1350-1358.
107. Morris DG, Huang X, Kaminski N, Wang Y, Shapiro SD, Dolganov G, Glick A, Sheppard D: **Loss of integrin alpha(v)beta6-mediated TGF-beta activation causes Mmp12-dependent emphysema.** *Nature* 2003, **422(6928)**:169-173.
108. Celedon JC, Lange C, Raby BA, Litonjua AA, Palmer LJ, DeMeo DL, Reilly JJ, Kwiatkowski DJ, Chapman HA, Laird N, Sylvia JS, Hernandez M, Speizer FE, Weiss ST, Silverman EK: **The transforming growth factor-beta1 (TGFB1) gene is associated with chronic obstructive pulmonary disease (COPD).** *Hum Mol Genet* 2004, **13(15)**:1649-1656.
109. Grainger DJ, Heathcote K, Chiano M, Snieder H, Kemp PR, Metcalfe JC, Carter ND, Spector TD: **Genetic control of the circulating concentration of transforming growth factor type beta1.** *Hum Mol Genet* 1999, **8(1)**:93-97.
110. Suthanthiran M, Li B, Song JO, Ding R, Sharma VK, Schwartz JE, August P: **Transforming growth factor-beta 1 hyperexpression in African-American hypertensives: A novel mediator of hypertension and/or target organ damage.** *Proc Natl Acad Sci U S A* 2000, **97(7)**:3479-3484.
111. Yamamoto N, Homma S: **Vitamin D3 binding protein (group-specific component) is a precursor for the macrophage-activating signal factor from lysophosphatidylcholine-treated lymphocytes.** *Proc Natl Acad Sci U S A* 1991, **88(19)**:8539-8543.
112. 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(1)**:364-369.
113. DiMartino SJ, Shah AB, Trujillo G, Kew RR: **Elastase controls the binding of the vitamin D-binding protein (Gc-globulin) to neutrophils: a potential role in the regulation of C5a chemotactic activity.** *J Immunol* 2001, **166(4)**:2688-2694.
114. Schellenberg D, Pare PD, Weir TD, Spinelli JJ, Walker BA, Sandford AJ: **Vitamin D binding protein variants and the risk of COPD.** *Am J Respir Crit Care Med* 1998, **157(3 Pt 1)**:957-961.
115. Horne SL, Cockcroft DW, Dosman JA: **Possible protective effect against chronic obstructive airways disease by the GC2 allele.** *Hum Hered* 1990, **40(3)**:173-176.
116. Viau M, Constans J, Debray H, Montreuil J: **Isolation and characterization of the O-glycan chain of the human vitamin-D binding protein.** *Biochem Biophys Res Commun* 1983, **117(1)**:324-331.
117. Ito I, Nagai S, Hoshino Y, Muro S, Hirai T, Tsukino M, Mishima M: **Risk and severity of COPD is associated with the group-specific component of serum globulin IF allele.** *Chest* 2004, **125(1)**:63-70.
118. Ishii T, Keicho N, Teramoto S, Azuma A, Kudoh S, Fukuchi Y, Ouchi Y, Matsuse T: **Association of Gc-globulin variation with susceptibility to COPD and diffuse panbronchiolitis.** *Eur Respir J* 2001, **18(5)**:753-757.
119. Zheng T, Zhu Z, Wang Z, Homer RJ, Ma B, Riese RJ Jr., Chapman HA Jr., Shapiro SD, Elias JA: **Inducible targeting of IL-13 to the adult lung causes matrix metalloproteinase- and cathepsin-dependent emphysema.** *J Clin Invest* 2000, **106(9)**:1081-1093.
120. van der Pouw Kraan TC, van Veen A, Boeijs LC, van Tuyl SA, de Groot ER, Stapel SO, Bakker A, Verweij CL, Aarden LA, van der Zee JS: **An IL-13 promoter polymorphism associated with increased risk of allergic asthma.** *Genes Immun* 1999, **1(1)**:61-65.
121. van der Pouw Kraan TC, Kucukaycan M, Bakker AM, Baggen JM, van der Zee JS, Dentener MA, Wouters EF, Verweij CL: **Chronic obstructive pulmonary disease is associated with the -1055 IL-13 promoter polymorphism.** *Genes Immun* 2002, **3(7)**:436-439.
122. Ingenito EP, Tsai LW, Majumdar A, Suki B: **On the role of surface tension in the pathophysiology of emphysema.** *Am J Respir Crit Care Med* 2005, **171(4)**:300-304.
123. Guo X, Lin HM, Lin Z, Montano M, Sansores R, Wang G, DiAngelo S, Pardo A, Selmán M, Floros J: **Surfactant protein gene A, B, and D marker alleles in chronic obstructive pulmonary disease of a Mexican population.** *Eur Respir J* 2001, **18(3)**:482-490.
124. Chappell S, Daly L, Morgan K, Baranes TG, Roca J, Rabinovich R, Millar A, Donnelly SC, Keatings V, MacNee WW, Stolk J, Hiemstra PS, Miniati M, Monti S, O'Connor CM, Kalsheker N: **The SERPINE2 gene and chronic obstructive pulmonary disease.** *Am J Hum Genet* 2006, **79(1)**:184-6; author reply 186-7.

125. Scott RW, Bergman BL, Bajpai A, Hersh RT, Rodriguez H, Jones BN, Barreda C, Watts S, Baker JB: **Protease nexin. Properties and a modified purification procedure.** *J Biol Chem* 1985, **260(11):7029-7034.**
126. Baker JB, Low DA, Simmer RL, Cunningham DD: **Protease-nexin: a cellular component that links thrombin and plasminogen activator and mediates their binding to cells.** *Cell* 1980, **21(1):37-45.**
127. Ashitani J, Mukae H, Arimura Y, Matsukura S: **Elevated plasma pro-coagulant and fibrinolytic markers in patients with chronic obstructive pulmonary disease.** *Intern Med* 2002, **41(3):181-185.**
128. Guerassimov A, Hoshino Y, Takubo Y, Turcotte A, Yamamoto M, Ghezzi H, Triantafillopoulos A, Whittaker K, Hoidal JR, Cosio MG: **The development of emphysema in cigarette smoke-exposed mice is strain dependent.** *Am J Respir Crit Care Med* 2004, **170(9):974-980.**
129. Judson R, Salisbury B, Schneider J, Windemuth A, Stephens JC: **How many SNPs does a genome-wide haplotype map require?** *Pharmacogenomics* 2002, **3(3):379-391.**
130. **SAS Genetics** [<http://www.sas.com/industry/pharma/genetics/>]. .
131. **Haplo.stats** [<http://cran.r-project.org/doc/packages/haplo.stats.pdf>]. .
132. **Entrez SNP** [<http://www.ncbi.nlm.nih.gov/projects/SNP/>]. .
133. **The Human Gene Mutation Database** [<http://archive.uwcm.ac.uk/uwcm/mg/hgmd0.html>]. .
134. **Entrez Gene** [<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene>]. .

Publish with **BioMed Central** and every scientist can read your work free of charge

*"BioMed Central will be the most significant development for disseminating the results of biomedical research in our lifetime."*

Sir Paul Nurse, Cancer Research UK

Your research papers will be:

- available free of charge to the entire biomedical community
- peer reviewed and published immediately upon acceptance
- cited in PubMed and archived on PubMed Central
- yours — you keep the copyright

Submit your manuscript here:  
[http://www.biomedcentral.com/info/publishing\\_adv.asp](http://www.biomedcentral.com/info/publishing_adv.asp)

