Genetics and pulmonary medicine • 9

Series editors: J Britton, J Hopkin

Molecular genetics of chronic obstructive pulmonary disease

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Chronic obstructive pulmonary disease (COPD) is a major cause of ill health and is increasing in many parts of the world. It is one of the commonest causes of death and the only common cause of death which is increasing. COPD is characterised by a slowly progressive irreversible airflow obstruction that is due to a loss of lung elasticity resulting from parenchymal destruction and peripheral airflow obstruction. Cigarette smoking is currently a causal factor in more than 90% of patients in westernised societies, so environmental factors are clearly very important in the disease.[1] However, in Caucasians only 10–20% of chronic heavy cigarette smokers develop symptomatic COPD, suggesting that genetic factors are likely to be important in determining which cigarette smokers are at risk from developing airflow obstruction. Furthermore, some patients develop airflow obstruction at an earlier age, again suggesting that genetic factors may determine the progression of COPD. Patients who have a genetic deficiency in the anti-protease α_1-antitrypsin (α_1-AT) have a very high risk of developing emphysema at an early age if they smoke, indicating the importance of genetic factors in some patients with COPD. Despite the clinical importance of COPD, relatively few studies have searched for genetic factors using modern molecular genetic techniques.

Ethnic differences

There may also be differences in the prevalence of COPD in different ethnic groups, but these are difficult to separate from lifestyle factors. For example, the prevalence of COPD is apparently low in China and this cannot be entirely accounted for by a lower tobacco consumption.[2] Anecdotally, COPD is uncommon in Chinese living in the USA which suggests that there may be genetic differences in the factors that protect against COPD. In Hawaii, the prevalence of COPD in Japanese-Americans smoking more than 20 cigarettes daily was 7.9% compared with 16.7% in a matched Caucasian-American group.[3] More studies are needed in different ethnic groups, particularly those living overseas, in order to explore these ethnic differences. Differences in the prevalence of COPD in different ethnic groups are likely to be accounted for by the differing frequencies of genes relevant to pathogenesis, so that exploration of these differences at a molecular level may be informative. For example, the ZZ phenotype of α_1-AT does not occur in black subjects and is very rare in Asians, and abnormalities in cystic fibrosis transmembrane regulator (CFTR) do not occur in the Japanese population. These differences in gene frequency between different racial groups make comparisons between different populations difficult and may account for some of the reported differences in the association between gene polymorphisms and COPD in different studies.

Family studies

Several studies have shown an increased prevalence of COPD within families. Case control studies have demonstrated an increased prevalence of COPD in relatives of patients with COPD which cannot be accounted for by known risk factors such as smoking.[4–7] Regressive models established to search for genetic factors in patients with COPD suggest major gene effects compared with families without pulmonary diseases.[8] In the Framingham study segregation analysis of over 5000 subjects from over 1000 families suggested that, after correction for smoking, polygenic gene effects and other environmental factors determine forced expiratory volume in one second (FEV_1).[9] A recent study showed that lung function was reduced in first degree relatives of patients with early onset COPD only if they currently or previously smoked, with an increased odds ratio of approximately 3.[10] Genetic influences determine pulmonary function and there is a closer similarity in spirometric measurements between monozygotic (identical) than dizygotic twins.[11] Twin studies in smokers have shown that there is a high risk in monozygotic twins that both will develop airflow obstruction, whereas in dizygotic twins who smoke,[12] even if raised apart,[13] this is not the case.

Which genes are important?

COPD is a complex disease which is still poorly understood at a molecular level. Many inflammatory cells, mediators, and enzymes are involved, but their relative importance is not yet clear. There is likely to be a complex interplay between genetic and environmental factors and many different genes will be involved. COPD may include two components: an inflammation and fibrosis of peripheral airways (chronic obstructive bronchiolitis) and an

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inflammatory-destructive process in the lung parenchyma leading to loss of elastic recoil and emphysema.[14–16] The importance of airway versus parenchymal effects is debated, but it may differ in different patients and there may be different genetic factors involved.

INFLAMMATORY CELLS

Bronchoalveolar lavage (BAL) in patients with COPD shows that there are increased numbers of neutrophils in the BAL fluid. Bronchial biopsy specimens have demonstrated an infiltration with mononuclear cells and CD8+ (cytotoxic) T lymphocytes rather than neutrophils, suggesting that neutrophils may transit rapidly from the circulation into the airway lumen.[17] CD8+ T cells are also prominent in the peripheral airways that are the major site of airflow obstruction.[18] Biopsy specimens from ex-smokers show a similar inflammatory process, suggesting that inflammation may persist in the airway once established.[19] Induced sputum samples from patients with COPD have a predominance of activated neutrophils, even in ex-smokers, in sharp contrast to the increased levels of eosinophils characteristic of asthma.[20 21] The role of neutrophils in the lumen of the airways in COPD is not yet established, but it is likely that the release of enzymes such as neutrophil elastase and matrix metalloproteinases (MMP) may contribute to the pathophysiology of the disease. Macrophages may play an important role in driving the inflammatory process in COPD and may release neutrophil chemotactic factors as well as proteolytic enzymes. Macrophage numbers are increased by 5–10 times in the BAL fluid of patients with COPD and are concentrated in the centriacinar zones where emphysema is most marked. Furthermore, the numbers of macrophages and T lymphocytes, but not the numbers of neutrophils, in the alveolar wall correlate with the amount of parenchymal destruction.[14] Macrophages may be responsible for the continued proteolytic activity in the lungs of patients with emphysema.

INFLAMMATORY MEDIATORS

The mechanism of the neutrophilic inflammation in COPD is not yet certain but it is likely that neutrophil chemotactic factors are released into the airways from activated macrophages and possibly from epithelial cells and CD8+ T lymphocytes. Interleukin (IL)-8 is selectively chemoattractant to neutrophils and is present in high concentrations in induced sputum from patients with COPD.[20] IL-8 may be secreted by macrophages, neutrophils, and by airway epithelial cells.[22] TNF-α, also present in high concentrations in the sputum of patients with COPD, may activate the transcription factor nuclear factor-κB (NF-κB) which switches on the transcription of the IL-8 gene.[23] Leukotriene(LT)B_4 is also a potent chemotactic agent for neutrophils in the airways and is increased in the sputum of patients with COPD.[24] Alveolar macrophages from patients with α_1-AT deficiency secrete greater amounts of LTB_4.[25] LTB_4 is synthesised from arachidonic acid via the enzyme 5'-lipoxygenase (5-LO) and LTA_4 hydrolase. Recent studies have shown that polymorphism in the promoter region of 5-LO that interferes with the binding of transcription factors may determine enzyme expression,[26] and this could theoretically alter the amount of LTB_4 secreted and thus the degree of neutrophilic inflammation.

Oxidative stress is increased in COPD and may be an important determinant of disease severity and progression.[27] Several enzymes regulate the formation of reactive oxygen species and the synthesis of endogenous antioxidants. All of these enzymes could show genetic polymorphisms resulting in alterations in oxidative stress response.

PROTEASES

There is compelling evidence in COPD for an imbalance between proteases that digest elastin (and other structural proteins in lung parenchyma) and antiproteases that protect against this.[28] Many enzymes with elastase activity and endogenous inhibitors of these enzymes have now been identified in COPD. Neutrophil elastase, a neutral serine protease, is a major constituent of lung elastolytic activity and also potently stimulates mucus secretion. In addition, neutrophil elastase induces IL-8 release from epithelial cells and therefore may perpetuate the inflammatory state. Neutrophil elastase is not the only proteolytic enzyme secreted by neutrophils. Cathepsin G and proteinase 3 have elastolytic activity and may need to be inhibited together with neutrophil elastase. Cathepsins B, L and S are also released from macrophages.

Matrix metalloproteinases (MMP) are a group of over 20 closely related endopeptidases that are capable of degrading all of the components of the extracellular matrix of lung parenchyma including elastin, collagen, proteoglycans, laminin, and fibronectin. They are produced by neutrophils, alveolar macrophages, and airway epithelial cells.[29] Increased levels of collagenase (MMP-1) and gelatinase B (MMP-9) have been detected in BAL fluid of patients with emphysema.[30] Lavaged macrophages from patients with emphysema express more MMP-9 and MMP-1 than cells from control subjects, suggesting that these cells, rather than neutrophils, may be the major cellular source.[31] Alveolar macrophages also express a unique MMP, macrophage metalloelastase (MMP-12).[32] MMP-12 knock-out mice do not develop emphysema and do not show the expected increases in lung macrophages after long term exposure to cigarette smoke.[33]

ANTIPROTEASES

It is very likely that defective production of antiproteases may be important in the development of COPD. α_1-AT is the major antiprotease that neutralises neutrophil elastase activity. The association of inherited α_1-AT deficiency with early onset emphysema suggested that genetic factors may be an important determinant of antiprotease balance. Other serum protease inhibitors (serpins) such as elafin may also be important in counteracting elastolytic activity in the lung. Elafin, an

elastase specific inhibitor, is found in BAL fluid and is synthesised by epithelial cells in response to inflammatory stimuli.[34] Secretory leukoprotease inhibitor (SLPI) is a 12 kDa serpin that appears to be a major inhibitor of elastase activity in the airways. It is secreted by epithelial cells.[34] Recombinant human SLPI is more effective at inhibiting neutrophil mediated proteolysis in vitro than α_1-AT.[35] Tissue inhibitors of metalloproteinases (TIMP) are endogenous inhibitors of MMPs and four TIMPs have now been characterised.

The recognition that many genes must be involved in the inflammatory response and the enzymatic destruction of the lung parenchyma suggests that there are many sites where differences in gene expression of particular proteins might influence the pathophysiology of COPD.

How are COPD genes identified?

There are several approaches to identifying the genes that may influence the development of COPD.

POSITIONAL CLONING

Positional cloning has been used in large numbers of affected families to identify the association between inheritance of various diseases with inheritance of genetic markers in known chromosomal locations that are co-inherited. However, while this approach has been successful in studying diseases that follow a simple Mendelian pattern of inheritance, its chances of success are much less in complex polygenic diseases where there is an important influence of environmental factors. It is difficult to match families for amount and duration of cigarette smoking and difficult to find patients where causal factors other than cigarette smoking are identified. A further problem with COPD is that most patients are not diagnosed until late in life so that extensive family studies are not possible. There are no published studies in COPD using the positional cloning approach.

CANDIDATE GENE APPROACH

A more promising approach has been to select genes that are likely to be involved in the pathogenesis of COPD and then to study polymorphisms (variations) in these candidate genes and relate these to disease severity. This involves an understanding of the disease process at a cellular and molecular level, and this research has been slow to develop. However, it is clear that there are many possible genes that could contribute to the occurrence, severity, and progression of COPD. Each polymorphism may impart only a small relative risk of COPD and it is likely that it is the coincidence of many polymorphisms that will be important in pathophysiology. The use of small high density synthetic oligonucleotide arrays bound to a solid surface (gene chips) with more than 10 000 different probes now makes it possible to screen for multiple gene polymorphisms in parallel.[36 37] The drawback of this approach is that it is only possible to look at genes that are known, whereas unknown genes may be more important.

WHOLE GENOME SCREENS

The increasing availability of genetic markers (particularly microsatellites that contain nucleotide repeat sequences) that are closely interspersed throughout the genome makes it possible to search the whole genome for associations with affected individuals. This strategy may reveal several areas in chromosomes where there are genes of interest, but the technique is not able to localise individual genes. In atopic asthma six regions of potential linkages were found in one UK study.[38] In a US study more and different linkages were found in different ethnic groups, indicating the differences between different populations.[39] This approach has not yet been reported in COPD.

FINDING NEW GENES

To detect unknown genes that may be important other approaches have to be used. The technique of mRNA differential display compares the expression of mRNA in cells from affected patients with cells from appropriate control subjects. Differences will be due to known or unknown genes which may then be sequenced.[40] Many genes are expressed at the mRNA level but may not be translated into proteins so other techniques may be needed to identify important proteins. These may be discovered by a complementary technique called proteomics which, using two-dimensional high resolution electrophoresis to separate proteins in cells or secretions, compares expression from diseases and control cells.[41] Novel proteins can then be sequenced by mass spectroscopy. These approaches are currently being applied to the study of COPD to identify novel therapeutic targets, but may also reveal differences in gene expression in COPD.

ANIMAL MODELS

Animal models of COPD have been difficult to develop. Guinea pigs chronically exposed to cigarette smoke develop a form of emphysema but it does not have the characteristics of COPD.[42] Transgenic and knock-out mice have been useful in studying the genetics of some diseases. However, there are no satisfactory models of COPD and no models that show variable susceptibility to cigarette smoke. Mice that are exposed chronically to high doses of cigarette smoke develop emphysema but are protected when there is a deletion of the MMP-12 gene.[33]

Candidate genes

Several genes have been studied in relation to COPD based on our understanding of its pathophysiology.[43 44]

GENE POLYMORPHISMS

Polymorphisms (variations) have been described in many genes. This may involve a substitution of a single nucleotide base in the coding region of a gene which results in coding for a different amino acid so that protein structure and function may be altered. Polymorphisms may also occur in the 5'-promoter regions of genes and, if this occurs at the site of binding of a transcription factor, this may result in

increased or decreased expression of the gene, or the substitution may allow binding of new transcription factors resulting in altered control of gene expression. Repeat sequences of nucleotide bases may also occur and some people have increased numbers of these repeats so that this can affect transcriptional control of the gene. Finally, there may be mutations in the 3'-untranslated region of the gene and this may affect the stability of mRNA and thus the amount of protein translated. These polymorphisms may be associated with altered susceptibility to disease, to disease severity, and to the response to treatment. It is likely that the coincidence of multiple polymorphisms will be an important mechanism for determining differences in the phenotypic expression of polygenic diseases.

α_1-ANTITRYPSIN

The observation that patients with low levels of α_1-AT (or α_1-protease inhibitor) develop early emphysema, particularly if they smoke, was the first to identify a genetic defect that could lead to COPD.[45] α_1-AT is an acute phase protein synthesised predominantly in the liver, but also by alveolar macrophages, and provides the major defence against neutrophil elastase. Over 75 variants of α_1-AT have now been identified.[46] The Z variant of α_1-AT (^{342}Glu→Lys) and patients homozygous for this (ZZ) have levels of α_1-AT that are only about 10% of normal and develop severe emphysema. However, these patients account for only a small proportion (1–2%) of all patients with emphysema. The ZZ phenotype is seen predominately in Northern European populations and is rare in Southern Europeans, Asians and black populations. There has been considerable debate about whether the many variants of α_1-AT or heterozygotes that produce lesser reductions in circulating α_1-AT predispose to COPD. For example, the S variant (^{264}Glu→Val) is found in more than 25% of Southern Europeans and is associated with circulating α_1-AT concentrations of about 60% of normal, yet it is not associated with COPD.[47] The SZ phenotype has been associated with an increased risk of COPD but with lesser degrees of airflow obstruction than the ZZ phenotype,[48] although in a recent study from Southern Europe no association between SZ phenotype and COPD was found.[49] The relatively common MZ phenotype, which is also associated with levels of about 60%, is not apparently associated with an increased risk of COPD, nor is there any association between the MZ genotype and reduced lung function in a general population in most studies. A polymorphism in the 3'-promoter region of the α_1-AT gene which was associated with normal levels of circulating α_1-AT was found in 17% of patients with COPD compared with only 5% in the general population.[50] This polymorphism was associated with decreased binding of a transcription factor and decreased gene expression. The most likely transcription factor is nuclear factor of IL-6 (NF-IL6 or C/EBPβ) which is activated by IL-6 and which is known to increase expression of α_1-AT. This suggests that the acute phase response protein α_1-AT may not increase normally in response to an increased production of proteases. These findings were not replicated in another study which found a similar prevalence in patients with COPD and controls (~10%), and there was no association with loss of elastic recoil.[51] Another rare polymorphism in the 3'-promoter region of α_1-AT associated with normal circulating levels has been associated with early onset COPD and was reported in three of 70 patients with COPD but in none of 52 controls.[52]

OTHER ANTIPROTEASES

The association of emphysema with genetic defects in α_1-AT prompted a search for genetic abnormalities of other proteases that may be involved in lung destruction. Alpha$_1$-antichymotrypsin (α_1-ACT) is another serpin which is secreted by the liver and alveolar macrophages. Two mutations of the α_1-ACT gene have been described in a German population that are associated with reduced circulating α_1-ACT levels and COPD. One mutation (^{227}Pro→Ala) was found in four of 100 patients with COPD but in no controls, and the other (^{55}Leu→Pro) in three of 200 patients with COPD and no controls.[53 54] One of the patients with the second mutation was a member of a family in which three members were affected by early onset COPD. Women who were heterozygous for α_1-ACT with lower plasma levels had a higher residual volume than control subjects.[55] This association between α_1-ACT deficiency and COPD has not been replicated in a Canadian population, however.[56]

Alpha$_2$-macroglobulin is a broad spectrum macroglobulin that is also synthesised in liver and in alveolar macrophages. Several polymorphisms of the α_2-macroglobulin gene have been described, but a common variant (^{1000}Val→Ile) is not associated with COPD and one patient with COPD had a substitution (^{972}Cys→Tyr) which was not associated with decreased serum α_2-macroglobulin levels.[57]

SLPI is the major antiprotease secreted into the airways and is derived largely from epithelial cells. No polymorphisms of the SLPI gene were found in a small number of patients with early onset COPD.[58] Elafin is another antiprotease and at least two polymorphisms have been described[59] but it is not known whether they are more common in COPD. TIMPs may play an important role in protecting against proteolytic damage from the increased MMP expression in COPD. So far no polymorphisms of TIMP genes have been reported in COPD.

PROTEASES

Polymorphisms of neutrophil elastase (NE) have not been reported, but expression of the NE gene is transient and occurs only during neutrophil maturation. A polymorphism in the coding region of the related serine protease cathepsin G has been reported but is not associated with COPD.[60] MMPs may play a critical role in the progression of emphysema and it is possible that gene polymorphisms may be an important determinant of lung destruction. Polymorphisms in the promoter regions of

some MMP genes have been described[61] but this has not yet been examined in COPD.

OXIDATIVE STRESS

The increased oxidative stress observed in patients with COPD is due to exogenous oxidants from cigarette smoking, but also from reactive oxygen species generated endogenously.[27] Several enzymes are involved in the generation of reactive oxygen species, and several antioxidants counteract their effects in the airways. Genetic polymorphisms of these enzymes and proteins may therefore affect the degree of oxidative stress that occurs in response to activating stimuli. Extracellular superoxide dismutase (EC-SOD) degrades superoxide anions and is the major extracellular antioxidant in the lungs. A polymorphism of the EC-SOD gene (^{213}Arg→Gly) which occurs in approximately 2% of the general population affects its binding to heparin so that circulating levels are increased 10-fold since it is not bound to proteoglycans in the tissue interstitium.[62 63] However, this polymorphism has not yet been associated with COPD. Polymorphisms of glutathione-S-transferase have also been described. A partial deletion of the μ class of this family GSTM1 is found in 50% of the population and this could increase oxidative stress.[64] This deletion was more frequent in patients with emphysema (65%) than in normal controls (53%), giving an odds ratio of 2.1.[65]

DETOXIFYING ENZYMES

One possibility to account for an increased susceptibility to the effects of cigarette smoke may be genetic variations in the enzymes which detoxify cigarette smoke products and other inhaled substances. These enzymes include microsomal epoxide hydrolase (mEPHX) which plays an important role in the lung in metabolising highly reactive epoxide intermediates that may be formed in cigarette smoke. There are polymorphisms of the gene for mEPHX that confer different enzyme activity and there are two common alleles that confer fast and slow activity.[66] The slow metabolising form of the enzyme was found in a higher proportion of patients with emphysema (22%) and COPD (19%) than in control subjects (13%), giving an odds ratio of 4–5.[67]

There appears to be an association between lung cancer and COPD that is independent of increasing age and smoking history, suggesting that there may be a common genetic predisposition.[68 69] A member of the cytochrome P450 family encoded by the gene *CYP1A1* metabolises the enzymatic activation of polyaromatic hydrocarbons in tobacco smoke to carcinogens. A mutation of this gene (^{462}Iso→Val) which increases enzyme activity has been associated with an increased risk of lung cancer and emphysema.[70]

CYTOKINE GENES

It is likely that several cytokines are involved in the inflammatory component of COPD.[71] Increased concentrations of TNF-α are present in the sputum of patients with COPD[20] and this cytokine may have an amplifying effect on the inflammatory process by activating NF-κB and other transcription factors to increase the expression of inflammatory genes, including IL-8. A relatively common polymorphism (approximately 10% of the population) in the 5'-promoter region of the TNF-α gene (G→A at position –308) known as the TNF2 variant results in twofold increased transcription of the TNF-α gene on activation[72 73] and a doubling of TNF-α concentrations.[74] In a study from Taiwan the TNF2 polymorphism was found in 2.4% of a control group and 19% of patients with COPD giving an odds ratio of 11.1.[75] However, this has not been replicated in an Italian population where the frequency of TNF2 was similar between patients with COPD and normal subjects (~12%), indicating that there are likely to be differences between populations.[76]

IL-8 and other CXC chemokines may be important chemotactic stimuli for neutrophils in COPD and IL-8 concentrations in sputum are markedly increased in patients with COPD compared with smokers who do not have COPD.[20 77] Furthermore, the concentrations of IL-8 correlate with the reduction in FEV_1. Polymorphisms of the IL-8 gene or the receptors for IL-8 on neutrophils (CXCR1, CXCR2) have not yet been reported in COPD.[78]

IL-10 is a cytokine with marked anti-inflammatory effects. In the context of COPD IL-10 inhibits the expression of TNF-α, IL-8, and other CXC chemokines and MMPs while increasing the expression of TIMPs and increasing neutrophil apoptosis.[79–81] Polymorphisms of the IL-10 5'-promoter gene have been described that result in altered gene expression.[82] Whether these polymorphisms increase susceptibility to COPD has not been determined. However, in asthma and rheumatoid arthritis there is an association between these promoter polymorphisms and disease severity.[83 84] The reduction in IL-10 secretion from macrophages may result in increased inflammatory cytokines and MMP release in response to inhaled irritants such as cigarette smoke.

CYSTIC FIBROSIS TRANSMEMBRANE REGULATOR

Cystic fibrosis transmembrane regulator (CFTR) is a chloride channel that is expressed in epithelial cells and is functionally abnormal in patients with cystic fibrosis who have homozygous mutant alleles. The predominant mutation in cystic fibrosis is a deletion at position 508 (ΔF508) which is found in about 70% of patients. However, over 500 different mutants have now been identified but are not clearly related to the cystic fibrosis phenotype. There is debate about whether heterozygotes are associated with pulmonary diseases such as COPD and asthma. Initial studies using linkage analysis did not reveal any association between a cystic fibrosis locus marker and COPD.[85] In a survey of the most common CFTR mutations no association was found with chronic bronchitis in a Northern European population.[86] A study of more than 70 mutations of CFTR similarly found no association in 12 patients with COPD although there

was an association with bronchiectasis, as reported in previous studies.[87]

BLOOD GROUP ANTIGENS

Blood group antigens are glycosyltransferases involved in the formation of mucopolysaccharides by epithelial cells that may affect the adhesion of micro-organisms. Early studies identified an association between COPD and blood group A or the absence of group B,[68] and a five year longitudinal study showed that patients with blood group A had a more rapid decline in lung function than patients with other blood groups.[88] However, subsequent studies failed to confirm any association between blood group and COPD or lung function in other populations.[89–91] COPD has also been linked to blood group antigen secretor status which is determined by a dominant gene on chromosome 19q. Approximately 80% of the population secrete blood group antigens into the saliva and respiratory tract secretions where the glycoproteins may play some sort of protective role against infections. In some studies non-secretor status is associated with a higher risk of COPD and a greater fall in lung function with age[92–94] but this has not been seen in other studies.[90 95] The Lewis blood group has also been examined and one report has shown an association between Lewis non-secretor status and COPD.[96] In individuals who are Lewis negative and also group O non-secretors there is an increased risk of poor lung function, but also of asthma and wheeze.[97]

HLA STATUS

As for many other common diseases, there have been attempts to relate COPD to histocompatibility genes such as human leucocyte antigen (HLA) class I genes. Some association was reported between low FEV_1 values and HLA-B7 with a reduced frequency of HLA-Bw16.[94]

IMMUNOGLOBULIN DEFICIENCY

Deficiencies in immunoglobulins may be determined by genetic factors. Since low IgA levels have been associated with an increased frequency of respiratory infections, the association between low IgA and immunoglobulins has been investigated in patients with COPD. In a large population study there was some association between selective IgA deficiency and COPD,[98] and this association was also seen in patients with combined IgA and IgG_2 and IgG_3 deficiency[99] or with selective IgG_2 or IgG_3 deficiency.[100 101]

VITAMIN D BINDING PROTEIN

Vitamin D binding protein (VDBP) or group-specific component (Gc) globulin is secreted by the liver which, as well as binding circulating vitamin D, has several effects that may enhance neutrophilic inflammation. VDBP binds to endotoxins, enhances the chemotactic activity of complement factor 5a for neutrophil chemotaxis, and activates macrophages.[102] Polymorphisms of VDBP gene result in three major isotypes of this protein[103] and some of these have been associated with increased or decreased risks of COPD.[7 104] However, this was not confirmed in another study.[94] Recently one of these genotypes (Gc2) was associated with a reduced relative risk of COPD (odds ratio 0.17) but was not associated with any change in neutrophil chemotaxis.[105]

ATOPY

For many years there has been debate about the relationships between asthma and COPD, and whether atopy or airway hyperresponsiveness predispose to the development of COPD (the "Dutch hypothesis").[106] This question has not yet been resolved as airway narrowing causes airway hyperresponsiveness for purely geometric reasons. In the Lung Health Study in the USA methacholine reactivity was related to rate of decline in lung function[107] but it is uncertain whether this was secondary to cigarette smoking or due to pre-existing asthma and atopy. There are many genetic influences affecting atopy and airway hyperresponsiveness[108 109] but it is not certain whether these are relevant to the development of COPD in smokers.

Future directions

There is convincing evidence that several genes influence the development of COPD. In a complex polygenic disease such as COPD it is likely that multiple genes are operating and that the influence of each gene in isolation may be relatively weak. The susceptibility to develop COPD with smoking or other environmental factors is likely to depend on the coincidence of several gene polymorphisms that act together. COPD has several components including peripheral airway inflammation and parenchymal tissue destruction. There are therefore many possible candidate genes but so far few polymorphisms have been studied in relation to COPD. Using new techniques such as gene chip technology it is possible to investigate multiple gene polymorphisms in parallel and to study their frequency in cigarette smokers who are susceptible to an increased rate of decline in lung function and those who are not. A complementary approach is to search for new genes and proteins by mRNA differential display or proteomics. Identification of genetic markers that predict the rate of loss of lung function, or that are associated with responsiveness to different treatments as they are developed, may help in disease prevention and improved management in the future.[110]

I am very grateful to Professor Neil Pride for his helpful comments on the manuscript.

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