CHAPTER 21.

Work-related lung diseases

Ainsley Weston

Summary

Work-related respiratory diseases affect people in every industrial sector, constituting approximately 60% of all disease and injury mortality and 70% of all occupational disease mortality. There are two basic types: interstitial lung diseases, that is the pneumoconioses (asbestosis, byssinosis, chronic beryllium disease, coal workers' pneumoconiosis (CWP), silicosis, flock workers' lung, and farmers' lung disease), and airways diseases, such as work-related or exacerbated asthma, chronic obstructive pulmonary disease and bronchiolitis obliterans (a disease that was recognized in the production of certain foods only 10 years ago). Common factors in the development of these diseases are exposures to dusts, metals, allergens and other toxins, which frequently cause oxidative damage. In response, the body reacts by activating primary immune response genes (i.e. cytokines that often lead to further oxidative damage), growth factors and tissue remodelling proteins. Frequently, complex imbalances in these processes contribute to the development of disease. For example, tissue matrix metalloproteases can cause the degradation of tissue, as in the development of CWP small profusions, but usually overexpression of matrix metalloproteases is controlled by serum protein inhibitors. Thus, disruption of such a balance can lead to adverse tissue damage. Susceptibility to these types of lung disease has been investigated largely through candidate gene studies, which have been characteristically small, often providing findings that have been difficult to corroborate. An important exception to this has been the finding that the *HLA-DPB1*^{E69} allele is closely associated with chronic beryllium disease and beryllium sensitivity. Although chronic beryllium disease is only caused by exposure to beryllium, inheritance of *HLA-DPB1*^{E69} carries an increased risk of between two- and 30-fold in beryllium exposed workers. Most, if not all, of these occupationally related diseases are preventable; therefore, it is disturbing that rates of CWP, for example, are again increasing in the United States in the 21st century.

Introduction

Excluding lung cancer, which is thought to account for 10 000–12 000 occupationally-related deaths annually in the United States (1), and infectious diseases like tuberculosis and histoplasmosis which may be work-related, several work-related lung diseases have been identified. These have been broadly divided into two types: interstitial lung diseases that are typified by the pneumoconioses (asbestosis, byssinosis, chronic beryllium disease, coal workers' pneumoconiosis, silicosis, flock workers' lung and farmers' lung disease), and airways diseases like asthma, chronic obstructive pulmonary disease (COPD) and bronchiolitis obliterans. Work-related respiratory diseases are a problem of major magnitude. They cut across all industrial sectors, constituting ~60% of all disease and injury mortality and ~70% of all occupational disease mortality (2).

Even though the capability has existed for many years to prevent pneumoconioses (e.g. silicosis, coal workers' pneumoconiosis (CWP) and asbestosis), they still cause or contribute to more than 2500 deaths per year in the United States (3). The threat of other interstitial lung diseases, such as chronic beryllium disease in beryllium metal extraction, production and processing, or hypersensitivity pneumonitis in those exposed to metal working fluids, are also important concerns in specific industries (4,5).

Airways diseases, such as asthma and COPD, are important occupational problems. In 2004, 11.4 million adults (aged ≥ 18) in the USA were estimated to have COPD (6). In the interval from 1997–1999, an estimated 7.4 million people in the United States (aged ≥ 15) reported an episode of asthma or asthma attack in the previous 12 months (7). A 2003 statement by the American Thoracic Society estimated that 15% of COPD and adult asthma cases were work-related, with a conservative annual estimated cost of nearly $7 billion in the USA alone (8).

An emerging area that thus far has not been explored in terms of molecular epidemiology is that of engineered nanotechnology. Nanoparticles and nanomaterials have diverse applications (e.g. drug delivery, electronics and cosmetics); however, their large surface area to volume and respirable nature suggest that they may pose a risk of lung disease. Studies in rodents have shown the potential of nanomaterials to cause oxidative stress, inflammation, and fibrosis (9).

In the last three decades, with the expansion of the emerging field of molecular epidemiology, several genetic susceptibility factors for work-related lung diseases and biomarkers of exposure and effect have been recognized. The majority of these findings took clues from physiological or pathobiological observations, and in some cases genetic linkage analysis, and applied them to candidate gene investigations in molecular epidemiological association studies. Though these types of studies may help to identify high risk subpopulations, their current utility is most valuable in understanding disease mechanisms and developing better laboratory models of disease.

Interstitial lung diseases

Asbestosis, asbestos-related lung cancer and mesothelioma

Several mineral fibres, including chrysotile, amosite, crocidolite, tremolite, actinolite and anthophyllite, are collectively known as asbestos. Asbestos mineral fibres are flame- and heat resistant, pliable, strong, refractory to corrosive chemicals, and provide insulation. Therefore asbestos has been used as a building material to insulate buildings from heat and protect against fire (it has been especially important in the shipbuilding industry), in fabric to make protective suits, as a brake liner (e.g. in automobiles and railroad rolling stock) and for engine gaskets, and in making filters (e.g. in the chemical industry).

Although known and used for its fire resistant properties as early as 3000 B.C., asbestos started to become widely used in the mid- to late-nineteenth century (10). Asbestos-associated fibrosis (asbestosis) was described in the 1920s, and mesothelioma (a very rare cancer of the mesothelium, an epithelial lining of the serous cavities: thorax and peritoneum) and lung cancer were linked to asbestos exposure in the 1960s (11). Thus, asbestosis, mesothelioma (almost exclusively associated with asbestos exposure) and asbestos-associated lung cancer are diseases frequently found in workers employed or formerly employed in construction, shipbuilding, mining, manufacturing and heat and frost insulation.

Fibrous particles generally have a large length to diameter aspect; asbestos fibres are generally considered to have a length to diameter ratio of at least 3:1. Respirable fibrous particles have an effective aerodynamic diameter that more closely resembles particle diameter than length. Thus, long, narrow fibres can reach the alveoli. Fibrous asbestos particles can exert their biological effects in several ways. Physiological attempts by the body to remove asbestos fibres from the deep lung may

result in "frustrated phagocytosis" by macrophages that engulf long, narrow fibres. These macrophages then disgorge digestive enzymes and other cytological materials potentially leading to inflammation, fibrosis and malignancy. It has also been proposed that the mineral fibres themselves can promote oxidative damage provoked by Fenton chemistry and the release of iron in the form of Fe^{3+} (12).

Several approaches have been taken to assess potential biomarkers of asbestosis. A major pathway is thought to be mediated through macromolecular and chromosomal damage resulting from reactive oxygen species (ROS) (e.g. O_2^-, $HO^{\bullet}$, ONOO, NO_2, NO_3) formed in the processes described above (13). Because fibrosis and inflammation are major components of the pathobiology of asbestosis, various procollagen genes and cytokine genes have been suggested as potential disease susceptibility markers. In addition, because asbestos exposure is a risk factor for lung cancer and mesothelioma, various tumour markers have been investigated.

Carboxyterminal propeptide of type 1 procollagen (PICP) is a marker for collagen synthesis; it is also associated with tissue and organ fibrosis (14). In this context it has been investigated as a marker for asbestosis. Levels of PICP in bronchoalveolar lavage fluid (BALF) and epithelial lining fluid (ELF) were found to be highest among asbestosis patients (n = 5), with ranges of greater than 7 µl/L to approximately 12 µl/L (mean = 9.8 ± 1.8 µl/L) and approximately 300–800 µl/L (mean = 489 ± 209) in BALF and ELF, respectively. Among 25 asbestos-exposed patients, pleural plaques levels were in the range of zero to less than 5 µl/L (mean = 0.6 ± 1.3 µl/L), and zero to 200 µl/L (mean = 51 ± 23 µl/L) in BALF and ELF, respectively. Among 12 persons with no X-ray evidence of abnormalities, only two were positive, and both of these had levels of PICP of less than 3 µl/L and 200 µl/L in BALF and ELF, respectively. Data for N-terminal propeptide of type 3 procollagen did not support it as a marker of asbestosis. These results are supportive of PICP as a biomarker for asbestosis; however, PICP has been associated with several other fibrotic and chronic inflammatory conditions (e.g. idiopathic fibrosing alveolitis (15), sarcoidosis (16) and myocardial fibrosis (17)). PICP has also been implicated in bone growth and bone metastasis (18). Thus, whereas PICP appears to be a good biomarker of asbestosis, it is not entirely specific.

Leukocyte glycoproteins (cluster of differentiation) CD66b and CD69 are antigens that signify leukocyte activation or hypersensitivity. Elevated levels of interleukins indicate increased inflammatory activity. Asbestos-exposed workers (n = 61 asbestos cement factory) and two groups of non-asbestos-exposed control workers (n = 48 "town" and n = 21 "factory") were evaluated for expression of multiple eosinophilic leukocyte cluster of differentiation marker expression by flow cytometry, as well as serum interleukin (IL) levels by immunoassay (19). A statistically significantly increased expression of markers CD69 and CD66b on eosinophils was found in blood samples collected from asbestos exposed workers. In addition, serum levels of the proinflammatory cytokines IL6 and IL8 were statistically significantly elevated (20). Although these findings reached statistical significance, they did not support the use of these biomarkers as robust screening tests. Furthermore, others have shown that CD69 can be induced in human peripheral blood mononuclear cells *in vivo* by silica, but not by chrysotile asbestos (20).

Asbestosis progression has been monitored by X-ray analysis; the radiographic changes (International Labour Office (ILO) classified) over 2–10 years were correlated with a large series of biomarkers: adenosine deaminase, α-1-antitrypsin, angiotensin-converting enzyme (ACE), β-2-microglobulin, β-*N*-acetylglucosaminidase, carcinoembryonic antigen (CEA), complement components (C3 and C4), erythrocyte sedimentation rate (ESR), ferritin, fibronectin, and lysozyme (21). Radiographic changes, which ranged from ILO 1/1 to ILO 2/2 (at an average of 0.4 minor ILO categories per year), were seen in 32 of 85 patients (OR = 1.54; 95% CI = 0.96–2.47). The only biomarkers that correlated with radiographic changes were fibronectin, ESR and ACE. The ranges of biomarker levels displayed overlap between the patient groups, and while the differences were statistically significant between those measured in patients who progressed compared to those who did not, they were relatively unimpressive (fibronectin OR = 1.01; 95% CI = 1.00–1.02; ESR OR = 1.05; 95% CI = 1.00–1.10; ACE OR = 1.10; 95% CI = 1.00–1.20) (21).

An important tumour marker that has been investigated in asbestos exposed groups is *p53*. Altered expression or overexpression of p53 can be detected in various ways: *p53* mutations can be detected in DNA from tumour tissue (22) or as exfoliated material in blood before a tumour is clinically detected (23), *p53* protein can be detected in blood if it is expressed at high enough levels, and *p53* autoantibodies can be detected. In a study of 115 compensable asbestosis cases, blood samples were drawn from 103 cases between 1980 and

1988. Autoantibodies for *p53* were assayed using an enzyme-linked immunosorbant assay (ELISA); 17 individuals were found to be positive. This cohort was followed for 20 years, and cancers developed in 49 people, among whom 13 were seropositive for *p53* autoantibodies (11 lung cancers, one mesothelioma and one lymphoma). The hazard ratio (HR) for cancer development in seropositive *p53* autoantibody asbestosis patients was determined to be statistically significant (HR = 5.5; 95% CI = 2.8–10.9) (24). Similar results have been obtained by others (25). These results, plus data that showed that both tumour and histologically normal tissue may test positive for *p53* expression, support the idea that *p53* changes are an early event in asbestos-associated lung cancer (25). Several reports have attempted to establish links between *p53* expression as measured in tumour tissue or serum, and *p53* mutations in DNA and autoantibodies (24,26,27). However, caution is recommended in consideration of such associations, as *p53* is both a tumour suppressor gene, and when mutated, an oncogene. Mechanisms that lead to detectable expression of *p53* can result from mutation or stabilization of wild-type *p53*. Mechanisms that lead to absence of detectable *p53* are normal expression of wild-type *p53*, and deletion of chromosome p17.13, which may be in the presence or absence of a *p53* mutation.

A panel of markers was evaluated as a "fuzzy classifier" in both lung cancer patients (n = 216) and asbestosis patients (n = 76). This panel consisted of CEA, neuron specific enolase, squamous cell carcinoma antigen, cytokeratin fragment and C-reactive protein. This panel of markers had 95% specificity in distinguishing cancer cases from asbestosis patients; they were present in 70–98% (overall 92%) of cancer patients, but only 1.3% (1/76) of asbestosis cases (28,29). Other studies of asbestosis cases have found expression of CEA, but this appears to be a preclinical marker of asbestos-related lung cancer and mesothelioma (30,31). Similarly, soluble mesothelin-related protein was found to be higher in mesothelioma patients (n = 24) than asbestosis patients (n = 33) or healthy controls (n = 109; $P < 0.05$) (32).

Osteopontin is a glycoprotein expressed in several malignancies (e.g. lung, gastric, colorectal, breast and ovarian, as well as mesothelioma and melanoma) (33,34). Osteopontin interacts with the integrin receptor and the CD44 receptor to mediate cell matrix interactions and cell signalling. Although it has been identified as a potentially valuable serum marker for mesothelioma, its expression appears to be associated with asbestos exposure. An ELISA test was used to determine serum osteopontin levels in 76 mesothelioma patients, 69 patients with asbestos-related non-malignant pulmonary disease, and 45 controls (no known asbestos exposure). The lowest serum osteopontin levels were found in the control group (20 ± 4 ng/ml) and the highest levels in mesothelioma patients (133 ± 10 ng/ml); the levels in the asbestos-related non-malignant pulmonary disease patient group were 30 ± 3 ng/ml. Interestingly, osteopontin levels in this last group increased with the onset of fibrosis. In addition, levels of osteopontin were higher in those study participants with greater duration of asbestos exposure (0–9 years, 16 ng/ml versus ≥10 years 34 ng/ml; $P = 0.02$) (33).

In summary, since asbestosis itself is a risk factor for lung cancer and pleural mesothelioma it is difficult to disentangle specific biomarkers of asbestosis from biomarkers of asbestos-related lung cancer and mesothelioma. In addition, more robust biomarkers of asbestosis tend to be biomarkers of other conditions where the underlying pathobiology involves chronic inflammation and fibrosis.

Berylliosis

The elemental metal beryllium was discovered in 1798, isolated in 1828, and became an important strategic commodity in 1923 when a patent for a copper-aluminum-beryllium alloy was filed (35). Beryllium has a wide range of interesting properties that have made this metal important in the manufacture of a host of products. It is light, with an atomic weight of 9.012, strong, and has a high melting point (1560°K). It is a neutron moderator and is X-ray transparent. It is non-sparking, corrosion resistant, and acts as an anti-galling agent. It has excellent heat and electrical conductivity, formability, castability and dimensional stability. With these properties it is invaluable in the aerospace, telecommunications, biomedical, defence and automotive industries (36).[1]

In the 1940s, exposure to beryllium in the fluorescent lamp industry was recognized as a respiratory hazard with the emergence of acute chemical pneumonitis (acute beryllium disease (ABD)) (37,38). In addition, extraction and primary production of beryllium metal was also associated with dermatitis, reversible pneumonitis and lung granulomas. In 1949, the Atomic

[1] This reference contains a more detailed listing of specific applications. See also: http://www.berylliumproducts.com/

Energy Commission introduced an occupational exposure limit for beryllium of 2 µg/m^3 and ABD disappeared. However, chronic beryllium disease (CBD), which is characterized by a cell-mediated immunologic (type 2) hypersensitivity and lung granulomas, remains problematic today (4).

Immunological sensitization to beryllium, which is generally considered to precede CBD, was originally recognized in the 1950s when beryllium salts were applied to the skin with a patch (39). Patch testing is not considered to be a viable procedure for diagnosis of beryllium sensitization, since it requires beryllium exposure itself, albeit through the skin (40,41). In 1987, an *in vitro* test for beryllium sensitization (BeS) was developed in which peripheral blood lymphocytes from beryllium sensitized individuals displayed beryllium specific proliferation (42). This beryllium lymphocyte proliferation test (BeLPT), though not perfect (43), has proved to be an important tool for occupational health screening and medical surveillance in the beryllium industry (44).

Latency in CBD is obscure; workers who are found to be positive for BeS are referred for bronchoalveolar lavage, to seek evidence of sensitized T-lymphocytes in the lung, and/or lung biopsy, to seek evidence of granulomas formation (4). Workers found to be BeS, through medical surveillance or screening, often have asymptomatic CBD. In other cases of BeS, clinical CBD has only developed decades later (4). These issues concerning latency have provoked debate over the value of using the BeLPT in medical surveillance, because early diagnosis provides no information on which to base treatment options. Moreover, there is no evidence to support the notion that a BeS worker can avoid CBD by leaving the industry, and having a positive BeLPT absent CBD might be an unwelcome source of anxiety.

The benefits of medical surveillance using the BeLPT are that evidence of BeS can support claims under the Energy Employees Occupational Illness Compensation Program Act of 2000 (20 CFR Part 30), help set priorities for disease prevention, and provide confirmation of the efficacy of intervention (4,45).

Together with the BeLPT, a genetic marker of BeS and CBD risk have also been described. In 1989, the BeLPT was used to show that the proliferative response in peripheral blood lymphocytes from a BeS individual could be inhibited in the presence of antibodies elicited against the major histocompatibility complex two molecule, HLA-DPβ1. This finding led to seven molecular epidemiologic association studies that unequivocally demonstrated that the genetic marker *HLA-DPβ1^{E69}* (a DNA sequence that codes for a glutamic acid residue at position 69 of the β chain of the HLA-DP molecule, an antigen presenting entity located on the surface of T-cells, macrophages, and Langerhans cells) is a risk factor for BeS and CBD (46–53).

The identification of a genetic marker closely associated with risk/susceptibility to CBD in the presence of occupational exposure raises serious ethical, legal and social issues. Indeed, a major United States beryllium producer briefly used an anonymous toll-free telephone line to introduce prospective employees to the possibility of undergoing an industry-sponsored genetic test for *HLA-DPβ1^{E69}* and pre-employment counselling. This programme was discontinued because of a hiring freeze and was not revived. However, it is reasonable to note that it has been shown that the positive predictive value (PPV) of *HLA-DPβ1^{E69}* is poor (around 10%), because the frequency of this marker in the population is high (~0.2 for the allele and 0.3–0.5 for carrier frequency) (54).

More recent refinements to these studies have provided evidence that not all *HLA-DPβ1^{E69}* alleles are equal with respect to CBD susceptibility. The *HLA-DPβ1* gene represents a family of at least 150 alleles having more than 40 single nucleotide polymorphisms (SNPs) in the hypervariable region (55). Consequently, there are 50 *HLA-DPβ1^{E69}* alleles, 5 *HLA-DPβ1^{R69}* alleles and 95 *HLA-DPβ1^{K69}* alleles. Among *HLA-DPβ1^{E69}* alleles, there appears to be a hierarchy of risk which ranges from approximately two- to 20-fold (36,56–58). Most recently these data have been used to shape the design of a transgenic mouse model. Moreover, scrutiny of specific genotypes is likely to reveal genetic biomarkers that have PPVs close to unity.

Coal workers' pneumoconiosis (black lung disease)

Coal workers' pneumoconiosis (CWP) is an interstitial lung disease that is caused by over-exposure to coal mine dust. In the United States, before the Coal Mine Health and Safety Act of 1969 (42 CFR Part 37), coal mine dust levels were as high as six to eight milligrams per cubic metre. The Act dictated that dust levels be capped at two milligrams per cubic metre. At that time, between 30 and 35% of miners developed CWP. As coal mine dust levels dropped to reported levels in the range of one milligram per cubic metre, the percentage of miners developing CWP dropped to approximately 5%. Diagnosis of

CWP is made by the observation of radiographic changes according to the ILO's classification system. In simple pneumoconiosis these changes are described as small opacities (graded, with increasing progression, as 1/0, 1/1, 1/2, 2/1, 2/2, 2/3, 3/2, 3/3; where 0/0 or 0/- reflects a normal x-radiogram, and 0/1 is no disease but stage 1 was considered), and in progressive massive fibrosis (PMF or macular CWP) these are described as large opacities (graded, with increasing progression, as A, B, C). CWP, a chronic inflammatory and fibrotic disease, is characterized by shortness of breath, cough, and deterioration of pulmonary function, all of which become progressively worse with increasing radiographic stage (59).

There is some blurring of distinction between CWP and silicosis in that both show characteristic small opacities on X-ray examination, and coal mine dust is often contaminated with crystalline silica, which is the more toxic component. It appears that oxygen free radical damage can be attributed to coal mine dust exposure from both ferrous iron, in the absence of silica, and silica itself (60,61). Apart from drawing a distinction between these two diseases, another challenge that faces the epidemiology of CWP is exposure assessment. One study that considered five strategies for exposure assessment found that using job and mine led to the most homogeneous exposure categories and most contrast between groups, although that method was the least precise (62).

It has been possible to determine measures of inflammatory response among miners (e.g. alveolar macrophages), polymorphonuclear leukocytes (PMNs), and the antioxidant superoxide dismutase (SOD). One small study of 20 coal miners and 16 control subjects (non-miners) was able to demonstrate a correlation between cumulative exposure to quartz, estimated from work histories and mine air sampling data, and PMNs in bronchoalveolar lavage ($P < 0.0001$), SOD ($P < 0.01$), and radiographic category ($P < 0.0001$) (63). However, a SOD promoter region polymorphism ($SOD^{9Val/Ala}$) was not associated with progression to PMF (n = 700 National Coal Workers Autopsy Study (NCWAS)) (64).

It has been shown that *TNF-α*, pulmonary surfactant protein A and phospholipids are increased in bronchoalveolar lavage fluids in response to coal mine dust, that *TNF-α* levels fall in response to cessation of exposure, and that these biomarkers increase with increasing radiographic evidence of disease progression (65). However, here as in most molecular epidemiologic studies of biomarkers of exposure and effect of coal mine dust exposures, the number of participants was small (n = 48).

Remodeling of extracellular matrix is also a critical event in the progression of fibrotic diseases. A small study of coal miners from Zonguldak, an old coal port on the Turkish Black Sea coast, found that serum pro-matrix metalloproteinase-3 (proMMP-3, also known as Stromelysin 1) was elevated in CWP (n = 44 CWP, 24 ILO 0/0, 0/1, and 17 surface worker controls) (61). In addition, among the CWP group, increasing serum proMMP-3 levels were detected with disease progression or severity measured x-radiographically ($P < 0.01$).

Observations that coal mine dust exposure can induce macrophages and monocytes to secrete cytokines, chemokines, and growth factors *in vivo* and *in vitro*, has led to the development of hypotheses implicating polymorphisms in members of these gene types in susceptibility to CWP and disease progression (66). The promoter region *TNF-α* G/A transversion polymorphism at positions −238 and −308, with respect to the ATG translation signal, has been investigated in numerous studies of diseases that involve inflammation and fibrosis (67). In a study of 78 coal miners and 56 controls (healthy members of a non-mining Belgian population), evidence of an association between the minor variant (A) of the −308 polymorphism and development of CWP was obtained by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP) (*NcoI*) (68). Miners inheriting a *TNF-α-308* A-variant (or 2) allele were three times more likely to develop CWP (OR = 3.0; 95% CI = 1.0–9.0, $\chi^2 = 4.1$, $P < 0.05$). In this study there was no association between inheritance of the minor variant (A or 2-allele) of the *TNF-α-238* polymorphism and CWP. When peripheral blood monocytes from 66 retired miners were exposed to coal mine dust *in vitro*, levels of *TNF-α* release were stimulated five- to 10-fold irrespective of genotype.

Eighty CWP patients and 54 healthy volunteers were recruited at a hospital in the Republic of Korea. Peripheral blood mononuclear cells were harvested to provide DNA, and a segment of the TNF-α promoter region −331 to +14 was amplified to determine the identity of the *TNF-α-308* G/A polymorphism (NcoI digestion) (69). The data showed that the frequency of the minor variant (A or *TNF2*) was over-represented in CWP patients by more than two-fold (F = 0.102 versus 0.206; $\chi^2 = 5.121$, $P = 0.024$). Moreover, when simple CWP (n = 41) was compared to cases of PMF (n = 39), the frequency of the minor variant was higher in PMF than

in simple CWP (F = 0.282 versus 0.134; $\chi^2 = 5.517$, $P = 0.019$).

A study of 259 unrelated coal miners in France investigated an association between inheritance of the *TNF-α-308* A-variant and CWP. There were 99 cases of CWP (80 active and 19 retired), and 152 without x-radiographic abnormalities for which genotyping data were presented (total n = 212), but no direct association was found (70). However, an interaction was observed in coal mine dust overexposed miners with disease between the *TNF-α-308* A-variant and erythrocyte glutathione peroxidase levels (OR = 2.5; 95% CI = 0.7–9.3; n = 61). In the same population, genotypes (n = 210) were also obtained for the biallelic A/G transversion polymorphism at nucleotide +252 (intron 1) of the lymphotoxin α gene (*LTα*, formerly known as TNF-β). In this case, again there was no difference in allelic distributions by disease status at the inception of the study (*LTα* A-allele frequencies of 0.277 and 0.367, and *LTα* A-homozygosity of 7% and 13% for radiologically normal and CWP groups respectively). However, after five years of follow-up, the CWP group constituted 33.6% of the remaining study population (n = 202), an increase of 5%. At that time, the *LTα* A-allele frequencies were 0.254 and 0.433, and *LTα* A-homozygosity was 4% and 16% for radiologically normal and CWP groups, respectively, which were borderline significant ($P = 0.07$).

Among 246 Chinese (124 CWP patients and 122 controls), the frequency of the *TNF-α-308* A-variant was found to be 0.0635 and 0.0205, respectively ($P = 0.036$). However, when a similar analysis was performed for the *TNF-α-238* or the *TNF-α-376* polymorphisms, no associations were found (71). Another study of 674 Chinese (234 CWP patients and 450 coal worker controls) was less conclusive, finding no difference between CWP and controls (F = 0.1034 versus 0.1091, respectively), but finding an elevated frequency of the *TNF-α-308* A-variant among workers with advanced disease (0.2000) (72).

Polymorphisms in the chemokine receptor genes *CCR5* and *CX3CR1* and interleukin 6 and 18 (*IL6, IL18*) have been implicated in the development of CWP (73–75), as has the urokinase-plasminogen activator *PLAU (P141L)* (76). Elevated levels of serum, urine and bronchioalveolar lavage fluid neoptrin, a marker of cell mediated immune activation, have been reported in both simple CWP and PMF (77). In addition, proMMP-3 was found to be higher in miners with more advanced disease (61). In the NCWAS, multiple polymorphisms in a variety of cytokines, growth factors and matrix metalloproteinase genes were evaluated for associations with PMF (78), but only the polygenotype $VEGF^{+405C}/ICAM\text{-}1^{+241A}/IL\text{-}6^{-174G}$ appeared to have a positive relationship with disease (OR = 3.4; 95% CI = 1.3–8.8, n = 700) (78).

Silicosis

Silicosis is a problematic occupational lung disease; exposure to silica (quartz and cristobalite) causes an inflammatory fibrosing response that can result in interstitial disease (silicosis) or lung cancer (79). The primary origin of the tissue damage leading to these conditions is oxidative, thus fresh fractured silica is much more potent than aged materials (80). Therefore, silicosis has some commonality with both asbestosis and CWP. Occupations that incur prodigious risk are silica sand blasting and coal mining – especially roof-bolters. Indeed, in recent years, as coal seams have become thinner, there is need to cut more siliceous rock to extract the coal, which involves greater hazard of silicosis. An emerging area of concern is roadway repair and demolition, which generates airborne silica dust. Despite these problems, deaths from silicosis in the USA have fallen from more than 1060 in 1968 to less than 170 in 2005 (81).

Dosimetric methods for the assessment of silica are generally problematic. Methods have been developed that can detect silica in blood, urine, lung tissue, lymph nodes, and bronchoalveolar lavage cells, and range from chemical staining to a variety of electron microscopy techniques. However, measures of crystalline silica have not proved to be useful in establishing any kind of dose–response relationship with silicosis, and these methods are not recommended for routine laboratory use (79).

Exposure to silica, like asbestos and coal mine dust, results in oxidative damage (80). This primary damage, mediated by *MIP-2, TNF-α,* IL-β, and *TGF-β*, is central to our current basic understanding of the pathobiology of silicosis (82–84). Because various environmental and occupational exposures, as well as infections and chronic conditions, trigger oxidative stress, using measures of oxidative damage would be too non-specific to be a useful biomarker of silica exposure; indeed, few studies have assessed this possibility. However, 8-hydroxydeoxy-guanosine (8-OHdG) has been measured in leukocyte DNA and urine of quartz exposed workers (n = 42) and silicotics (n = 63) (85). The data from this study showed no difference in either 8-OHdG in leukocyte DNA and 8-OHdG exfoliated in urine between healthy workers and

silicotics. There was, however, an inverse relationship between urinary 8-OHdG and DNA-adducts in silicotics, suggesting impaired nucleotide and/or base excision DNA-repair of 8-OHdG, which may be a factor associated with lung cancer susceptibility in silicosis patients (86). In another study of silicosis patients (n = 46, with 27 controls), serum heme oxygenase-1 (heme-HO-1) levels were found to be elevated in silicosis patients compared to controls; serum heme-HO-1 was inversely correlated with serum 8-OHdG levels, but positively correlated with measures of pulmonary function (87). Taken together, the results of these studies (85,87) suggest that both nucleotide/base excision repair activity and antioxidant activity may play a role in protection against the adverse lung function effects in silicosis.

In addition to oxyradical damage, several potential biomarkers associated with oxidative stress have been investigated. A comprehensive review concluded that several factors may potentially be reliable biomarkers of early effects of exposure to crystalline silica (79). These include generation of reactive oxygen species from alveolar macrophages, activation of NFκB, total radical trapping antioxidant capacity, serum isoprostane and glutathione levels, antioxidant enzyme activities (glutathione peroxidase and superoxide dismutase), DNA damage in lymphocytes (measured by the comet assay), neoptrin (2-amino-6-[1, 2, 3-trihydroxypropyl]-1H-pteridin-4-one, a purine nucleotide derivative) (88), and clara cell 16 (CC16) (a protein secreted by non-ciliated cells unique to bronchioles).

More recent studies have investigated these markers further. Increased lipid peroxidation, resulting in isoprostane production, has been measured in urine and exhaled breath, and has been found to be elevated in silicosis patients (P = 0.0001, n = 85) (89,90); however, this marker of oxidative stress is not specific for silica exposure (91). Plasma erythrocyte glutathione levels were decreased among cement manufacturing workers (n = 48) compared to controls (n = 28); conversely, plasma malondialdehyde levels were elevated (92). These data indicate an adverse shift in oxidative balance in cement workers that is likely associated with exposure to silica. In addition, all objective measures of pulmonary function were depressed in the cement worker group.

Among 90 silica-exposed workers (3 groups of 30 each; silicotics phase I, silicosis phase 0+, and non-silicotics phase 0) compared with healthy controls, serum CC16 levels were reduced in all silica exposed workers (P < 0.0001) (93). In the same study, surfactant protein D was increased in silicotics (phase I). In an autopsy study of 29 Canadian hard rock miners, there was a correlation between the amount of silica in the lungs and lymph nodes, the X-ray classification (ILO), and the amount of hydroxyvaline in the lung tissues (94).

Just as in CWP, *TNF-α* promoter region SNPs have been implicated in silicosis. In 2001, it was reported that among 489 study subjects (325 silicotics and 164 controls) silicotics were one and a half- to two-fold more likely to have inherited the minor *TNF-α-238* A-variant (OR = 1.56; 95% CI = 1.0–2.5) and the minor *TNF-α-308* A-variant (OR = 2.35; 95% CI = 1.4–3.6) than controls (95,96). The same study also implicated the minor *IL-1RA+2018* allele (OR = 2.12; 95% CI = 1.3–3.5); however, there were no associations with IL1α and IL1β polymorphisms that were investigated. The association of silicosis with the minor *IL-1RA+2018* allele was confirmed in 212 Chinese silica-exposed workers (75 cases and 137 controls) (97). The association was confirmed between the minor A-variants of *TNF-α-308* and *TNF-α-238* and silicosis in 241 South African miners (121 silicosis cases and 120 controls) (98). This study further implicated the minor A-variant of the *TNF-α-376* promoter region polymorphism. Other proinflammatory cytokines that have been linked to silicosis include CD25+ and CD69+ (99).

The tumour suppressor and prooncogene *p53* has an important role in programmed cell death (apoptosis) and DNA-repair mechanisms (100). Silica has been shown to cause *p53* transactivation through both induction of *p53* protein expression and *p53* protein phosphorylation *in vitro* and *in vivo* (101). It was observed that most apoptotic cells in mice instilled with fresh fractured silica were macrophages. Although it was not investigated in this study, different polymorphic variants of *p53* have been implicated in carcinogenesis (102).

Silicosis patients frequently have associated autoimmune disease disorders (103). These appear to be mediated through the Fas or CD95 pathway. Fas is an important component of the TNF receptor pathway that triggers apoptosis upon ligand binding. Numerous studies have reported elevated Fas levels and variant Fas transcripts in bronchioalveolar lavage fluid and peripheral blood mononuclear cells of silicosis patients (79,104,105). Moreover, serum soluble Fas ligand (sFas) is elevated in silicosis patients and in systemic lupus erythematosus patients (106).

Airways diseases

Asthma

Occupational asthma, or work-exacerbated asthma, is a widespread constriction or obstruction of the airways due to exposure to an irritant present in the workplace that may occur through an allergic or non-allergic mechanism. Work-related asthma was recognized by Hippocrates (460–370 BCE) and associated with occupations involving work with metals, textiles and animals, including fish (107). Today work-related asthma is commonly encountered in isocyanate production, in healthcare workers who use natural rubber latex gloves (although this is becoming less of a problem due to the substitution of other materials), and among office workers due to poor indoor environmental quality (108–110). It is estimated that between 15 and 30% of asthmatics have new-onset adult asthma or work exacerbated asthma. Thus, over two million workers in the United States suffer from work-related or work exacerbated asthma (7). Despite these facts and statistics that suggest a major occupational disease that has been known for more than 2000 years, asthmagens remain difficult to identify, and the connection of asthma with materials or conditions in the workplace may be hard to establish.

Asthma has long been recognized to have both an environmental and a genetic component in addition to being a recognized multigenic disease. A large number of genetic linkage studies, molecular genetic studies, and molecular epidemiology association studies of asthma have been conducted. Examples of fifteen molecular epidemiology association studies or candidate gene studies are given in Table 21.1 (111–125). These studies have focused on: major histocompatibility genes (*HLA-DR, HLA-DQ, HLA-DP*), chemical detoxication genes (*GSTM1, GSTT1, GSTP1, GSTM3*), cytokines (*CD13, CD14, IL4, IL10, IL12b, IL13, IL18, TNF-α*), oxyradical associated pathways (*PTGS2*), proteinase inhibitors (*PAI* or *SERPINE2*), growth factors (*TGF-β*), chemokines (*RANTES*) and related receptors (*CCR3, FCER1B*).

In addition to these studies, linkage studies have implicated genes on chromosomes 5q and 11q. These regions of the genome code are for atopy-related genes, cytokine genes, and the β-2-adrenoceptor gene (or β-2-adrenergic receptor *ADRB2*) (126). These studies have led to the conclusion that asthma is a multigenic disease with an environmental component.

Multiple studies have implicated the *ADRB2*; the product of this gene is present on smooth muscle cells in pulmonary airways. Polymorphisms in this receptor may dispose individuals to be susceptible to nocturnal asthma (127). A meta-analysis suggests that the $ADRB2^{G16}$adrenoceptor glycine 16 allele is associated with nocturnal asthma (OR = 2.2; 95% CI = 1.6–3.1), and that β-2-adrenoceptor glutamic acid 27 ($ADRB2^{E16}$) is not an asthma risk factor (OR = 1.0; 95% CI = 0.7–1.4).

A transmembrane protein, *ADAM33* (also known as MMP33), is a disintegrin and metalloprotease (endopeptidase) that has also been implicated in bronchial hyperresponsiveness. Matrix metalloproteases are normally involved with the structural modeling of tissues, like the lung, therefore disruption of their normal function, either through lack of proteinase inhibition or chronic inflammatory processes, may result in adverse pathology. In a study of 652 nuclear families, a haplotype of 16 *ADAM33* SNPs was associated with susceptibility to asthma ($P < 0.006$); however, no single polymorphism alone was found to have a statistically significant association (128). All of these data contribute to asthma—a complex multigenic disease that has an environmental trigger.

With the advent of the HapMap, a collection of millions of SNP markers arrayed across the genome, genome-wide association studies (GWAS) have become popular. These studies are unfettered by formal hypotheses, and multiplex SNP analysis is used to interrogate the entire genome simultaneously. For asthma, the following chromosomal regions have been found to contain markers that have P-values for association as low as 0.0000000001. They are: 1q32, 2q12, 5q12, 5q22, 5q33, 6q23, 8p21, 9q21, 17q21 and 20pter-p12 (129,130). These GWAS studies have confirmed the involvement of various genes in asthma, while others have suggested new candidates. Examples of genes that have been confirmed by GWAS include: *IL4, IL5, IL13, CD14, ADRB2, HLA-DQB1* and *HLA-DRB1* (131). New candidate genes that have been suggested by GWAS include: *ORMDL3* (a transmembrane protein of unknown function that is associated with the endoplasmic reticulum) (132), *ADRA1B* (an adrenergic receptor distinct from *ADRB2*), *PRNP* (a prion related protein found on chromosome 20p), *DPP10* (adipeptidyl peptidase (130), *PDE4D* (a protein involved in the regulation of smooth muscle) (133), *IL3, TLE4* (a transcription corepressor that in part regulates *PAX5*, a transcription factor), *IL1R1, IL33, WDR36* (a gene involved in the synthesis of ribosomes), *MYB* (a transcription factor) and *CHI3L1* (a chitinase-3-like protein) (129).

Table 21.1. Genetic epidemiology association studies of asthma

Study and Subjects (n)	Allele(s)	Association†	Reference
Paris, France	*HLA-DR4*	P<0.0004	(111)
Cases (56, 62% ♀)	*HLA-DR7*	P<0.05	
Controls (39, 62% ♀)	*HLA-DQB1*0103* *HLA-DQB1*0302*	P<0.002 P<0.01	
Helsinki, Finland‡	*NAT1§*	OR=2.5 (1.3-4.9)	(112)
Cases (109, 22% ♀)	*GSTM1 + NAT1*	OR=4.5 (1.8-11.6)	
Controls (73, 12% ♀)	*GSTM1 + NAT2* *NAT1 + NAT2*	OR=3.1 (1.1-8.8) OR=4.2 (1.5-11.6)	
Cincinnati, OH, USA	*CD14^{159T}*	P=0.03	(113)
Cases (175)	*CD14^{159TT}*	OR=2.3 (0.9-5.8)	
Controls (61)	*CD14^{159TT**}*	OR=3.1 (1.1-9.1)	
Taichung, Taiwan, China	*IL10^{627AA}*	OR=3.6 (1.2-10.4)	(114)
Cases (117, 48% ♀)	*IL10^{627AC}*	OR=4.8 (1.7-13.9)	
Controls (47, 64% ♀)			
SE Anatolia, Turkey			(115)
Cases (210, 74% ♀)	*GSTP1^{105val}*	OR=0.3 (0.1-0.6)	
Controls (265, 69% ♀)			
Tokyo, Japan			(116)
Japanese (210)	*CCR3^{51C}*	OR=1.4 (0.7-2.7)	
Controls (181)			
British (142)		OR=2.4 (1.3-4.3)	
Controls (92)			
San Diego, CA, USA			(117)
Cases (236)	*TNF-α-308* A	OR=1.9 (1.0-3.3)	
Controls (275)		OR=1.7 (1.0-2.9)††	
Osaka, Japan			(118)
Cases (479)	*IL18^{105A}*	P<0.01	
Controls (85)			
Sapporo, Japan			(119)
Cases (298)	*RANTES^{-28G}*	OR=2.0 (1.4-3.0)	
Controls (311)			
Boston, MA, USA			(120)
Cases (527, 51% ♀)	*TGF-β^{509TT}*	OR=2.5 (1.3-5.1)	
Controls (170, 36% ♀)	*TGF-β^{509TC}*	OR=1.3 (0.9-1.8)	
Vancouver, Canada	*HLA-DRB1*0101*	OR=0.3 (0.1-0.8)	(121)
Cases (56, 2% ♀)	*HLA-DQB1*0603*	OR=2.9 (1.0-8.2)	
Controls (63, 0% ♀)	*HLA-DQB1*0302*	OR=4.9 (1.3-18.6)	
Helsinki, Finland			(122)
Cases (42)	*GSTM1^{null}*	OR=1.9 (1.0-3.5)	
Controls (56)	*GSTM3^{MnlI+}*, *GSTP1^{313val}*, GSTT1^{null}	*Not significant*	

Amsterdam, Netherlands			(123)
Cases (101)	*IL13*$^{-1055TT}$	P<0.002	
Controls (107)			
Hong Kong SAR, China			(124)
Cases (299)	*PTGS2*^{8473C}	OR=1.5 (1.0-2.3)	
Controls (175)			
Sapporo, Japan			(125)
Cases (374)	*PAI-1*^{5G}/ *FCER1B*$^{109T/654C}$	OR=0.2 (0.1-0.5)	
Controls (374)			

†Statistics given as either P-values or odds ratios (OR) with 95% confidence intervals in parentheses
‡Isocyanate workers
§ Slow acetylator phenotype. Risk of *NAT2* alone not significant (OR=1.4; 95% CI=0.7-2.6)
** Nonatopy only (n=47)
††European-Americans only (n=169 cases, 170 controls)
‡‡Statistics given as either P-values or odds ratios (OR) with 95% confidence intervals in parentheses

To address the multigenic nature of asthma, a statistical modeling attempt has been made to elucidate asthma risk. Sixteen alleles, most conveying susceptibility, but some with evidence of protection, were used as a basis of the model (134). A similar model has been used to predict overall risk of breast cancer (135). The model revealed a broad spectrum of potential risk and may help to more clearly identify susceptible populations; however, it will be challenging to integrate an environmental component. As noted in the section on berylliosis, this may be accomplished through an understanding of gene–environment interaction at the molecular level using the tools of computational chemistry (58).

Bronchiolitis obliterans

Bronchiolitis obliterans syndrome (BOS) is a fibroproliferative process that causes intraluminal obstruction of the smallest airways, the bronchioles. This condition can be caused by exposure to toxic chemicals (e.g. diacetyl in artificial butter flavoring, responsible for popcorn workers' lung), it can occur following transplant surgery (notably bone marrow, lung, or heart and lung) and as the result of infection (136–139). Only a few studies exist that have looked for biomarkers of susceptibility, exposure and effect.

The first study of six lung transplant recipients evaluated transcripts of platelet-derived growth factor (PDGF)-β and *TGF-β1* in bronchoalveolar lavage cells. Slightly elevated levels of both growth factors were found in BOS patients compared to controls, and the PDGF-β increase was associated with lung function decrement (140). Another study of 93 lung transplant recipients evaluated SNPs in *TNF-α, TGF-β, IL-6, INF-γ,* and *IL-10*. Both of the high expression variants of *IL-6*$^{-174G}$ and *INF-γ*$^{+874T}$ were found to be correlated with BOS ($P < 0.05$ and 0.04 respectively). In addition, onset of BOS was more rapid in patients carrying these variants (141). A third study extended these data by examining the frequency of the same alleles in a cohort of 78 lung transplant recipients. This study was able to confirm that *IL-6*$^{-174G}$ was associated with earlier onset BOS ($P < 0.04$) and a decreased overall survival ($P < 0.05$) (142).

A novel receptor gene, *NOD2/CARD15*, can interact with *NFκB* to trigger an inflammatory response. Three SNPs in this gene (*Arg702Trp, Gly908Arg,* and *Leu1007finsC*) were investigated in a cohort of 427 donor-recipient pairs involved in allogenic stem cell transplantation. The cumulative incidence of BOS rose in donor recipient pairs with a minor variant of this gene (F = 0.187 versus F = 0.013 (those without mutation), $P < 0.001$); donor variants alone were significantly associated with the complication of BOS (F = 0.132, $P < 0.04$) (143).

Chronic obstructive pulmonary disease

Chronic obstructive pulmonary disease (COPD) results in shortness of breath (dyspnea) due to thickening of the airways of the lung. This is an inflammatory condition, which in contrast to asthma is irreversible, and is caused by toxic exposure to tobacco smoke, dust and/or gases. COPD may be an occupational hazard caused by exposures to dusts and gases in the textile industry, coal and other mining industries, construction industry (silica), services industry (secondhand smoke), and damp non-industrial indoor environments (volatile organic compounds) (6).

COPD is a leading cause of morbidity and mortality in the United States and worldwide (6). In 2003, 10.7 million United States adults were estimated to have COPD,

although close to 24 million adults had evidence of impaired lung function, indicating underdiagnosis of COPD in the United States (144). The economic burden in the United States is approximately US$37.2 billion, which includes health care expenditures of US$20.9 billion in direct costs, US$7.4 billion in indirect morbidity costs, and US$8.9 billion in indirect mortality costs (145). Although smoking accounts for the majority of COPD cases, occupational factors associated with many industries are estimated to account for 19% of all cases and 31% among never smokers (144).

COPD is a complex, mutagenic disease that only affects a fraction of smokers (15–20%), therefore it has been reasoned that genetic predisposition and environmental factors are important in its development. A genetic factor that was implicated about 40 years ago was α-1-antitrypsin (α1AT), or rather its deficiency (146). Alpha-1-antitrypsin is a serum protease inhibitor (SERPIN). This family of glycoproteins prevents massive tissue damage from proteases released by host cells during inflammation.

Deficiency of *SERPINA1*, also known as α1AT (PiZ homozygotes), accounts for approximately 2% of COPD patients. Six *SERPINA1* 5 SNP haplotypes were shown to increase risk of COPD by six- to 50-fold (147). In contrast, there was no such association with *SERPINA3* even after an initial study had yielded positive results (141,147). *SERPIN1A* deficiency has also been implicated in liver disease. Another serum protease inhibitor, *SERPINE2*, was implicated in COPD by linkage analysis of 127 probands and 949 total individuals in a family-based study (148).

Several matrix metalloprotease molecules have been implicated in COPD using a linkage strategy. They are: MMP1 or interstitial collagenase, MMP2 or gelatinase-A, MMP8 or neutrophil collagenase, MMP9 or gelatinase-B, and macrophage mellatoelastase. The allele $MMP1^{-1607G}$ was found to be associated with lung function decline ($P = 0.02$ for allele frequency between 284 patients with rapid decline and 306 with no decline) (149). In addition, this group found evidence that the $MMP12^{357Ser}$ allele was also associated with lung function decline. In several other epidemiological association studies, $MMP9^{-1562T}$ was found to be associated with COPD diagnosed with conventional computed tomography (CT) scans (150), spirometry (151) or high-resolution CT scans (152). Two further studies also implicated *MMP9* alleles, $MMP9^{279Arg}$ that modifies substrate binding (153), and a promoter region polymorphism *MMP9–82G* (154). A large study using Boston, USA early-onset COPD study subjects set out to confirm COPD associations with SNPs of 12 genes, including MMP1, MMP9 (short tandem repeats, not −1562T), and *TIMP2* (155). The association between $TIMP2^{853A}$ and COPD ($P < 0.0001$), originally reported in Japanese subjects (85 cases, 40 controls), was found to be of marginal significance in the Boston population ($P = 0.08$) (155,156). Associations previously reported for $MMP1^{-1607G}$ and the short tandem repeats in *MMP9* were not confirmed. A contemporary study has also implicated multiple SNPs in *ADAM33* (157).

As with asthma and pneumoconioses, which are driven to some extent by oxidative damage, cytokines have been implicated in COPD. Several studies have examined the influence of SNPs in *TNF-α* (158–165). Most of these studies were null, and a meta-analysis that included several of them confirmed this. Other cytokine genes that were investigated for COPD-associated SNPs include: *LTα* (159,164), *IL6* and *IL10* (159), and *IL13* (162); of these the *IL10–1082G* was associated with COPD (OR = 2.6; 95% CI = 1.5–4.4) (159). In a recent study of 374 active firefighters with at least five serial lung function tests, $TNF\text{-}^{\alpha\text{-}238}$ was found to be associated with a more rapid rate of FEV1 decline (166).

Several polymorphisms in xenobiotic metabolizing genes have received some attention. It is reasonable to assume that some of these genes could at least contribute to oxidative damage since induction of, for example, cytochrome P450s leads to redox cycling and the formation of oxygen free radicals (167). The isoleucine/valine polymorphism in residue 462 of *CYP1A1*, previously considered to be involved in gene induction (168), was investigated in patients recruited at the University of Edinburgh Medical School, Scotland (36 cases, 281 controls). An association was found between inheritance of the $CYP1A1^{462val}$ and COPD (OR = 2.3; 95% CI = 1.0–5.2) (169). Other xenobiotic metabolism genes that have been investigated are *GSTM1, GSTP1, GSTT1* and *EPHX1* (165,170-172). With the exceptions of epoxide hydrolase (EPHX1) and *GSTP1*, none have shown a positive association that could be confirmed (155). In the case of *EPHX1*, there is an histidine/arginine polymorphism in residue *139*, $EPHX1^{139Arg}$, which was found to be associated with COPD ($P = 0.02$) (155). In the case of *GSTP1*, there is an isoleucine/valine polymorphism in residue 105; $GSTP1^{105Val}$ was found to be associated with COPD ($P = 0.05$) (155).

More recently, GWAS technology has also been applied to analysis of

genetic factors in COPD. Using this strategy, involvement of several of the above implicated genes has been confirmed, including *SERPINE2* (at 2q33–2q37), *EPHX1* (at 1q42) and *GSTP1* (at 1p13) (173,174). These and other GWAS have implicated additional genes: *SFTPB* (a pulmonary surfactant protein at 2p11), *ADRB1* (at 5q32), *TGF-β* (at 19q13) (175), and *FAM13A* (involved in hypoxia response through signal transduction in human lung epithelial cells at 4q22) (176). In addition, GWAS studies of COPD have also identified an association with *CHRNA* sub-units 3 and 5 (an α-nicotinic acetylcholine receptor, located at chromosome 15q25) (177) and *ADAM33* (the metalloprotease located at chromosome 20p13) (178). For both asthma and COPD, it can be seen from the GWAS approach that there is some genetic overlap in these airways diseases.

Summary

The interstitial lung diseases asbestosis, silicosis and CWP have in common exposure to dusts and fibres that induce oxygen free radical damage. These exposures tend to stimulate inflammation and fibrosis, at least in part mediated through the *TNF-α* pathway. In silicosis and CWP this probably influenced the choice of SNP biomarkers that have been examined, and there is a preponderance of evidence to suggest that the promoter region polymorphism of *TNF-α* is implicated in susceptibility and severity of these diseases; this has not been the case for CBD. While most molecular epidemiology has focused on the major histocompatibility complex type 2 molecules, and especially the *HLA-DPB1* gene, there are several studies concerning the *TNF-α* promoter regions in CBD, but none of them have provided support for implication of this gene (49,178,179).

The studies on berylliosis provide an interesting example of a susceptibility marker for several reasons. First, the *HLA-DPB1*^{E69} allele has been shown to be associated with CBD and beryllium sensitization in at least three sufficiently-sized, well-characterized study populations (51–53) and several smaller studies, and essentially all of the studies agree. Second, it is a marker that could be used for pre-employment screening, but the positive predictive value is only about 7–14% (54). (This is a cautionary note: despite the strong and uncontested association with disease, it would not make good economic or ethical sense to use beryllium for testing, as exposure to it is what drives disease.) Third, if similar markers could be found for asthma, it may be possible to learn about asthmagens through computational chemical modelling (57,58).

In the case of the airways diseases, asthma and COPD, it is clear that aberrant tissue remodeling is a major contributory factor to pathology (180). Imbalances in matrix metalloproteases and serum protease inhibitors (SERPINs) in the presence of inflammation, which are associated to some extent with genetic polymorphisms, appear to be critical factors. These findings have prompted therapeutic targeting of matrix metalloproteases through the use of inhibitors for the treatment of COPD (181).

In terms of occupational diseases, molecular epidemiological studies of bronchiolitis obliterans, byssinosis and flock workers' lung have not yet been developed. Byssinosis, or brown lung disease, was highly prevalent in the United States in the early 1970s, but numbers have declined due to implementation of the Cotton Dust standard (29 CFR Part 1910) and migrations of textile work to Asia. Thus, research in this area would now be confined to populations in India, China and other parts of Asia. A similar situation is evolving for flock workers.

Many of the molecular epidemiological association studies reported on here are small, and the variation in the quality of participant characterization is considerable. Many of the control populations are convenience samples, and less-than-appropriate samples that come from expired units from blood banks. This has led to considerable disparity across the field of molecular epidemiology with respect to the soundness of specific associations. One study, using a well-characterized molecular epidemiologic case–control population to attempt to verify previous reports for 15 alleles in COPD, is a model and an approach that should be adopted if meaningful associations are to be established (155).

Disclaimer: The findings and conclusions in this chapter are those of the author and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

References

1. Steenland K, Loomis D, Shy C, Simonsen N (1996). Review of occupational lung carcinogens. *Am J Ind Med,* 29:474–490.doi:10.1002/(SICI)1097-0274(199605)29:5<474::AID-AJIM6>3.0.CO;2-M PMID:8732921

2. Steenland K, Burnett C, Lalich N *et al.* (2003). Dying for work: The magnitude of US mortality from selected causes of death associated with occupation. *Am J Ind Med,* 43:461–482.doi:10.1002/ajim.10216 PMID:12704620

3. National Institute for Occupational Safety and Health. All pneumoconioses and related exposures. Section 6. In: Work-related lung disease surveillance report 2002. U.S. Department of Health and Human Services. Public Health Service. Centers for Disease Control and Prevention. Number 2003–111; Cincinnati (OH); 2003 p. 125–56.

4. Kreiss K, Day GA, Schuler CR (2007). Beryllium: a modern industrial hazard. *Annu Rev Public Health,* 28:259–277.doi:10.1146/annurev.publhealth.28.021406.144011 PMID:17094767

5. U.S. Department of Health and Human Services. Criteria for a recommended standard: occupational exposure to metalworking fluids. Public Health Service. Centers for Disease Control and Prevention. National Institute for Occupational Safety and Health Number, 98–102; Cincinnati (OH);1998.

6. American Lung Association. Chronic obstructive pulmonary disease (COPD) fact sheet. 2008. Available from URL: http://www.lungusa.org/site/apps/nlnet/content3.aspx?c=dvLUK9O0E&b=2058829&content_id={EE451F66-996B-4C23-874D-BF66586196FF}&notoc=1.

7. Sama SR, Milton DK, Hunt PR *et al.* (2006). Case-by-case assessment of adult-onset asthma attributable to occupational exposures among members of a health maintenance organization. *J Occup Environ Med,* 48:400–407.doi:10.1097/01.jom.0000199437.33100.cf PMID:16607195

8. Balmes J, Becklake M, Blanc P *et al.*; Environmental and Occupational Health Assembly, American Thoracic Society (2003). American Thoracic Society Statement: Occupational contribution to the burden of airway disease. *Am J Respir Crit Care Med,* 167:787–797.doi:10.1164/rccm.167.5.787 PMID:12598220

9. Shvedova AA, Kisin ER, Mercer R *et al.* (2005). Unusual inflammatory and fibrogenic pulmonary responses to single-walled carbon nanotubes in mice. *Am J Physiol Lung Cell Mol Physiol,* 289:L698–L708.doi:10.1152/ajplung.00084.2005 PMID:15951334

10. Barbalace RC. A brief history of asbestos use and associated health risks. 2004. Available from URL: http://environmentalchemistry.com/yogi/environmental/asbestoshistory2004.html.

11. Williams PRD, Phelka AD, Paustenbach DJ (2007). A review of historical exposures to asbestos among skilled craftsmen (1940–2006). *J Toxicol Environ Health B Crit Rev,* 10:319–377. PMID:17687724

12. Luster MI, Simeonova PP (1998). Asbestos induces inflammatory cytokines in the lung through redox sensitive transcription factors. *Toxicol Lett,* 102-103:271–275.doi:10.1016/S0378-4274(98)00321-X PMID:10022265

13. Bhattacharya K, Dopp E, Kakkar P *et al.* (2005). Biomarkers in risk assessment of asbestos exposure. *Mutat Res,* 579:6–21. PMID:16112146

14. Lammi L, Ryhänen L, Lakari E *et al.* (1999). Carboxyterminal propeptide of type I procollagen in ELF: elevation in asbestosis, but not in pleural plaque disease. *Eur Respir J,* 14:560–564.doi:10.1034/j.1399-3003.1999.14c13.x PMID:10543275

15. Lammi L, Ryhänen L, Lakari E *et al.* (1999). Type III and type I procollagen markers in fibrosing alveolitis. *Am J Respir Crit Care Med,* 159:818–823. PMID:10051256

16. Lammi L, Kinnula V, Lähde S *et al.* (1997). Propeptide levels of type III and type I procollagen in the serum and bronchoalveolar lavage fluid of patients with pulmonary sarcoidosis. *Eur Respir J,* 10:2725–2730.doi:10.1183/09031936.97.10122725 PMID:9493651

17. Querejeta R, Varo N, López B *et al.* (2000). Serum carboxy-terminal propeptide of procollagen type I is a marker of myocardial fibrosis in hypertensive heart disease. *Circulation*, 101:1729–1735. PMID:10758057

18. Wallace JD, Cuneo RC, Lundberg PA *et al.* (2000). Responses of markers of bone and collagen turnover to exercise, growth hormone (GH) administration, and GH withdrawal in trained adult males. *J Clin Endocrinol Metab,* 85:124–133.doi:10.1210/jc.85.1.124 PMID:10634375

19. Ilavská S, Jahnová E, Tulinská J *et al.* (2005). Immunological monitoring in workers occupationally exposed to asbestos. *Toxicology,* 206:299–308.doi:10.1016/j.tox.2004.09.004 PMID:15588921

20. Wu P, Hyodoh F, Hatayama T *et al.* (2005). Induction of CD69 antigen expression in peripheral blood mononuclear cells on exposure to silica, but not by asbestos/chrysotile-A. *Immunol Lett,* 98:145–152.doi:10.1016/j.imlet.2004.11.005 PMID:15790520

21. Oksa P, Huuskonen MS, Järvisalo J *et al.* (1998). Follow-up of asbestosis patients and predictors for radiographic progression. *Int Arch Occup Environ Health,* 71:465–471.doi:10.1007/s004200050307 PMID:9826079

22. Greenblatt MS, Bennett WP, Hollstein M, Harris CC (1994). Mutations in the p53 tumor suppressor gene: clues to cancer etiology and molecular pathogenesis. *Cancer Res,* 54:4855–4878. PMID:8069852

23. Chen JG, Kuang SY, Egner PA *et al.* (2007). Acceleration to death from liver cancer in people with hepatitis B viral mutations detected in plasma by mass spectrometry. *Cancer Epidemiol Biomarkers Prev,* 16:1213–1218.doi:10.1158/1055-9965.EPI-06-0905 PMID:17548687

24. Husgafvel-Pursiainen K, Kannio A, Oksa P *et al.* (1997). Mutations, tissue accumulations, and serum levels of p53 in patients with occupational cancers from asbestos and silica exposure. *Environ Mol Mutagen,* 30:224–230.doi:10.1002/(SICI)1098-2280(1997)30:2<224::AID-EM15>3.0.CO;2-F PMID:9329647

25. Li Y, Karjalainen A, Koskinen H *et al.* (2005). p53 autoantibodies predict subsequent development of cancer. *Int J Cancer,* 114:157–160.doi:10.1002/ijc.20715 PMID:15523685

26. Rusin M, Butkiewicz D, Malusecka E *et al.* (1999). Molecular epidemiological study of non-small-cell lung cancer from an environmentally polluted region of Poland. *Br J Cancer,* 80:1445–1452.doi:10.1038/sj.bjc.6690542 PMID:10424749

27. Trivers GE, De Benedetti VM, Cawley HL *et al.* (1996). Anti-p53 antibodies in sera from patients with chronic obstructive pulmonary disease can predate a diagnosis of cancer. *Clin Cancer Res,* 2:1767–1775. PMID:9816128

28. Schneider J, Bitterlich N, Kotschy-Lang N *et al.* (2007). A fuzzy-classifier using a marker panel for the detection of lung cancers in asbestosis patients. *Anticancer Res,* 27 4A;1869–1877. PMID:17649786

29. Shitrit D, Zingerman B, Shitrit AB *et al.* (2005). Diagnostic value of CYFRA 21–1, CEA, CA 19–9, CA 15–3, and CA 125 assays in pleural effusions: analysis of 116 cases and review of the literature. *Oncologist,* 10:501–507.doi:10.1634/theoncologist.10-7-501 PMID:16079317

30. Krajewska B, Lutz W, Piłacik B (1996). [Exposure to asbestos and levels of selected tumor biomarkers]. *Med Pr,* 47:89–96. PMID:8657007

31. Eyden BP, Banik S, Harris M (1996). Malignant epithelial mesothelioma of the peritoneum: observations on a problem case. *Ultrastruct Pathol,* 20:337–344.doi:10.3109/01913129609016334 PMID:8837340

32. Di Serio F, Fontana A, Loizzi M *et al.* (2007). Mesothelin family proteins and diagnosis of mesothelioma: analytical evaluation of an automated immunoassay and preliminary clinical results. *Clin Chem Lab Med,* 45:634–638.doi:10.1515/CCLM.2007.112 PMID:17484626

33. Pass HI, Lott D, Lonardo F *et al.* (2005). Asbestos exposure, pleural mesothelioma, and serum osteopontin levels. *N Engl J Med,* 353:1564–1573.doi:10.1056/NEJMoa051185 PMID:16221779

34. Cullen MR (2005). Serum osteopontin levels–is it time to screen asbestos-exposed workers for pleural mesothelioma? *N Engl J Med,* 353:1617–1618.doi:10.1056/NEJMe058176 PMID:16221786

35. Kolanz ME (2001). Introduction to beryllium: uses, regulatory history, and disease. *Appl Occup Environ Hyg,* 16:559–567.doi:10.1080/10473220119088 PMID:11370935

36. Weston A, Snyder J, McCanlies EC *et al.* (2005). Immunogenetic factors in beryllium sensitization and chronic beryllium disease. *Mutat Res,* 592:68–78. PMID:16054169

37. No authors listed (1984). Archives of the Cleveland Clinic Quarterly 1943: Chemical pneumonia in workers extracting beryllium oxide. Report of three cases. By H.S. VanOrdstrand, Robert Hughes and Morris G. Carmody. *Cleve Clin Q,* 51:431–439. PMID:6380823

38. DeNARDI JM, Van Ordstrand HS, Carmody MG (1949). Acute dermatitis and pneumonitis in beryllium workers; review of 406 cases in 8-year period with follow-up on recoveries. *Ohio Med,* 45:567–575. PMID:18127900

39. Curtis GH (1951). Cutaneous hypersensitivity due to beryllium; a study of thirteen cases. *AMA Arch Derm Syphilol,* 64:470–482. PMID:14867858

40. Tinkle SS, Antonini JM, Rich BA *et al.* (2003). Skin as a route of exposure and sensitization in chronic beryllium disease. *Environ Health Perspect,* 111:1202–1208. doi:10.1289/ehp.5999 PMID:12842774

41. Day GA, Stefaniak AB, Weston A, Tinkle SS (2006). Beryllium exposure: dermal and immunological considerations. *Int Arch Occup Environ Health,* 79:161–164.doi:10.1007/s00420-005-0024-0 PMID:16231190

42. Kreiss K, Newman LS, Mroz MM, Campbell PA (1989). Screening blood test identifies subclinical beryllium disease. *J Occup Med,* 31:603–608.doi:10.1097/00043764-198907000-00011 PMID:2788726

43. Deubner DC, Goodman M, Iannuzzi J (2001). Variability, predictive value, and uses of the beryllium blood lymphocyte proliferation test (BLPT): preliminary analysis of the ongoing workforce survey. *Appl Occup Environ Hyg,* 16:521–526.doi:10.1080/104732201750169598 PMID:11370932

44. Kreiss K, Wasserman S, Mroz MM, Newman LS (1993). Beryllium disease screening in the ceramics industry. Blood lymphocyte test performance and exposure-disease relations. *J Occup Med,* 35:267–274. PMID:8455096

45. Cummings KJ, Deubner DC, Day GA *et al.* (2007). Enhanced preventive programme at a beryllium oxide ceramics facility reduces beryllium sensitisation among new workers. *Occup Environ Med,* 64:134–140.doi:10.1136/oem.2006.027987 PMID:17043076

46. Richeldi L, Sorrentino R, Saltini C (1993). HLA-DPB1 glutamate 69: a genetic marker of beryllium disease. *Science,* 262:242–244. doi:10.1126/science.8105536 PMID:8105536

47. Richeldi L, Kreiss K, Mroz MM *et al.* (1997). Interaction of genetic and exposure factors in the prevalence of berylliosis. *Am J Ind Med,* 32:337–340.doi:10.1002/(SICI)1097-0274(199710)32:4<337::AID-AJIM3>3.0.CO;2-R PMID:9258386

48. Wang Z, White PS, Petrovic M *et al.* (1999). Differential susceptibilities to chronic beryllium disease contributed by different Glu69 HLA-DPB1 and -DPA1 alleles. *J Immunol,* 163:1647–1653. PMID:10415070

49. Saltini C, Richeldi L, Losi M *et al.* (2001). Major histocompatibility locus genetic markers of beryllium sensitization and disease. *Eur Respir J,* 18:677–684.doi:10.1183/09031936.01.00106201 PMID:11716174

50. Wang Z, Farris GM, Newman LS *et al.* (2001). Beryllium sensitivity is linked to HLA-DP genotype. *Toxicology,* 165:27–38. doi:10.1016/S0300-483X(01)00410-3 PMID:11551429

51. Rossman MD, Stubbs J, Lee CW *et al.* (2002). Human leukocyte antigen Class II amino acid epitopes: susceptibility and progression markers for beryllium hypersensitivity. *Am J Respir Crit Care Med,* 165:788–794. PMID:11897645

52. Maier LA, McGrath DS, Sato H *et al.* (2003). Influence of MHC class II in susceptibility to beryllium sensitization and chronic beryllium disease. *J Immunol,* 171:6910–6918. PMID:14662898

53. McCanlies EC, Ensey JS, Schuler CR *et al.* (2004). The association between HLA-DPB1Glu69 and chronic beryllium disease and beryllium sensitization. *Am J Ind Med,* 46:95–103.doi:10.1002/ajim.20045 PMID:15273960

54. Weston A, Ensey J, Kreiss K *et al.* (2002). Racial differences in prevalence of a supratypic HLA-genetic marker immaterial to pre-employment testing for susceptibility to chronic beryllium disease. *Am J Ind Med,* 41:457–465.doi:10.1002/ajim.10072 PMID:12173370

55. European Bioinformatics Institute. IMGT/HLA database. 2005. Available from URL: http://www.ebi.ac.uk/imgt/hla/allele.html.

56. McCanlies EC, Kreiss K, Andrew M, Weston A (2003). HLA-DPB1 and chronic beryllium disease: a HuGE review. *Am J Epidemiol,* 157:388–398.doi:10.1093/aje/kwg001 PMID:12615603

57. Snyder JA, Weston A, Tinkle SS, Demchuk E (2003). Electrostatic potential on human leukocyte antigen: implications for putative mechanism of chronic beryllium disease. *Environ Health Perspect,* 111:1827–1834.doi:10.1289/ehp.6327 PMID:14630515

58. Snyder JA, Demchuk E, McCanlies EC *et al.* (2008). Impact of negatively charged patches on the surface of MHC class II antigen-presenting proteins on risk of chronic beryllium disease. *J R Soc Interface,* 5:749–758.doi:10.1098/rsif.2007.1223 PMID:17956852

59. Wang XR, Christiani DC (2000). Respiratory symptoms and functional status in workers exposed to silica, asbestos, and coal mine dusts. *J Occup Environ Med,* 42:1076–1084.doi:10.1097/00043764-200011000-00009 PMID:11094786

60. Huang C, Li J, Zhang Q, Huang X (2002). Role of bioavailable iron in coal dust-induced activation of activator protein-1 and nuclear factor of activated T cells: difference between Pennsylvania and Utah coal dusts. *Am J Respir Cell Mol Biol,* 27:568–574. PMID:12397016

61. Altin R, Kart L, Tekin I *et al.* (2004). The presence of promatrix metalloproteinase-3 and its relation with different categories of coal workers' pneumoconiosis. *Mediators Inflamm,* 13:105–109.doi:10.1080/09629350410001688549 PMID:15203551

62. Heederik D, Attfield M (2000). Characterization of dust exposure for the study of chronic occupational lung disease: a comparison of different exposure assessment strategies. *Am J Epidemiol,* 151:982–990. PMID:10853637

63. Kuempel ED, Attfield MD, Vallyathan V *et al.* (2003). Pulmonary inflammation and crystalline silica in respirable coal mine dust: dose-response. *J Biosci,* 28:61–69. doi:10.1007/BF02970133 PMID:12682426

64. Yucesoy B, Johnson VJ, Kashon ML *et al.* (2005). Lack of association between antioxidant gene polymorphisms and progressive massive fibrosis in coal miners. *Thorax*, 60:492–495. doi:10.1136/thx.2004.029090 PMID:15923250

65. Xing JC, Chen WH, Han WH *et al.* (2006). Changes of tumor necrosis factor, surfactant protein A, and phospholipids in bronchoalveolar lavage fluid in the development and progression of coal workers' pneumoconiosis. *Biomed Environ Sci,* 19: 124–129. PMID:16827183

66. Borm PJ, Schins RP (2001). Genotype and phenotype in susceptibility to coal workers' pneumoconiosis. the use of cytokines in perspective. *Eur Respir J Suppl,* 32:127s–133s. PMID:11816820

67. Wilson AG, di Giovine FS, Duff GW (1995). Genetics of tumour necrosis factor-alpha in autoimmune, infectious, and neoplastic diseases. *J Inflamm,* 45:1–12. PMID:7583349

68. Zhai R, Jetten M, Schins RP *et al.* (1998). Polymorphisms in the promoter of the tumor necrosis factor-alpha gene in coal miners. *Am J Ind Med,* 34:318–324.doi:10.1002/(SICI)1097-0274(199810)34:4<318::AID-AJIM4>3.0.CO;2-O PMID:9750937

69. Kim KA, Cho YY, Cho JS *et al.* (2002). Tumor necrosis factor-alpha gene promoter polymorphism in coal workers' pneumoconiosis. *Mol Cell Biochem,* 234-235:205–209.doi:10.1023/A:1015914409661 PMID:12162435

70. Nadif R, Jedlicka A, Mintz M *et al.* (2003). Effect of TNF and LTA polymorphisms on biological markers of response to oxidative stimuli in coal miners: a model of gene-environment interaction. Tumour necrosis factor and lymphotoxin alpha. *J Med Genet,* 40:96–103.doi:10.1136/jmg.40.2.96 PMID:12566517

71. Wang XT, Ohtsuka Y, Kimura K *et al.* (2005). Antithetical effect of tumor necrosis factor-alphagene polymorphism on coal workers' pneumoconiosis (CWP). *Am J Ind Med,* 48:24–29.doi:10.1002/ajim.20180 PMID:15940715

72. Li L, Yu C, Qi F *et al.* (2004). [Potential effect of tumor necrosis factor-alpha and its receptor II gene polymorphisms on the pathogenesis of coal worker's pneumoconiosis]. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*, 22:241–244. PMID:15355698

73. Zhai R, Liu G, Ge X *et al.* (2002). Serum levels of tumor necrosis factor-alpha (TNF-alpha), interleukin 6 (IL-6), and their soluble receptors in coal workers' pneumoconiosis. *Respir Med,* 96:829–834.doi:10.1053/rmed.2002.1367 PMID:12412984

74. Nadif R, Mintz M, Rivas-Fuentes S *et al.* (2006). Polymorphisms in chemokine and chemokine receptor genes and the development of coal workers' pneumoconiosis. *Cytokine*, 33:171–178.doi:10.1016/j.cyto.2006.01.001 PMID:16524739

75. Nadif R, Mintz M, Marzec J *et al.* (2006). IL18 and IL18R1 polymorphisms, lung CT and fibrosis: A longitudinal study in coal miners. *Eur Respir J,* 28:1100–1105.doi:10.1183/09031936.00031506 PMID:16971411

76. Chang LC, Tseng JC, Hua CC *et al.* (2006). Gene polymorphisms of fibrinolytic enzymes in coal workers' pneumoconiosis. *Arch Environ Occup Health,* 61:61–66.doi:10.3200/AEOH.61.2.61-66 PMID:17649957

77. Ulker OC, Yucesoy B, Durucu M, Karakaya A (2007). Neopterin as a marker for immune system activation in coal workers' pneumoconiosis. *Toxicol Ind Health,* 23:155–160.doi:10.1177/0748233707083527 PMID:18220157

78. Yucesoy B, Johnson VJ, Kissling GE *et al.* (2008). Genetic susceptibility to progressive massive fibrosis in coal miners. *Eur Respir J,* 31:1177–1182.doi:10. 1183/09031936.00075107 PMID:18256065

79. Gulumian M, Borm PJ, Vallyathan V *et al.* (2006). Mechanistically identified suitable biomarkers of exposure, effect, and susceptibility for silicosis and coal-worker's pneumoconiosis: a comprehensive review. *J Toxicol Environ Health B Crit Rev,* 9:357–395.doi:10.1080/15287390500196537 PMID:16990219

80. Castranova V (2004). Signaling pathways controlling the production of inflammatory mediators in response to crystalline silica exposure: role of reactive oxygen/nitrogen species. *Free Radic Biol Med,* 37:916–925. doi:10.1016/j.freeradbiomed.2004.05.032 PMID:15336307

81. U.S. Department of Health and Human Services. Work-related lung disease surveillance report: silicosis and related exposures. Public Health Service. National Institute for Occupational Safety and Health. Centers for Disease Control and Prevention. *DHHS (NIOSH)* Number 2003–111. Cincinnati (OH); 2002. p. 51–86.

82. Driscoll KE (2000). TNFalpha and MIP-2: role in particle-induced inflammation and regulation by oxidative stress. *Toxicol Lett,* 112-113:177–183.doi:10.1016/S0378-4274(99)00282-9 PMID:10720729

83. Rimal B, Greenberg AK, Rom WN (2005). Basic pathogenetic mechanisms in silicosis: current understanding. *Curr Opin Pulm Med,* 11:169–173.doi:10.1097/01.mcp.0000152998.11335.24 PMID:15699791

84. Barrett EG, Johnston C, Oberdörster G, Finkelstein JN (1999). Silica-induced chemokine expression in alveolar type II cells is mediated by TNF-alpha-induced oxidant stress. *Am J Physiol,* 276:L979–L988. PMID:10362723

85. Pilger A, Germadnik D, Schaffer A *et al.* (2000). 8-Hydroxydeoxyguanosine in leukocyte DNA and urine of quartz-exposed workers and patients with silicosis. *Int Arch Occup Environ Health,* 73:305–310. doi:10.1007/s004200000117 PMID:10963413

86. Dusinská M, Dzupinková Z, Wsólová L *et al.* (2006). Possible involvement of XPA in repair of oxidative DNA damage deduced from analysis of damage, repair and genotype in a human population study. *Mutagenesis*, 21:205–211.doi:10.1093/mutage/gel016 PMID:16613913

87. Sato T, Takeno M, Honma K *et al.* (2006). Heme oxygenase-1, a potential biomarker of chronic silicosis, attenuates silica-induced lung injury. *Am J Respir Crit Care Med,* 174:906–914.doi:10.1164/rccm.200508-1237OC PMID:16858012

88. Altindag ZZ, Baydar T, Isimer A, Sahin G (2003). Neopterin as a new biomarker for the evaluation of occupational exposure to silica. *Int Arch Occup Environ Health,* 76:318–322. PMID:12768284

89. Pelclová D, Fenclová Z, Kacer P *et al.* (2007). 8-isoprostane and leukotrienes in exhaled breath condensate in Czech subjects with silicosis. *Ind Health,* 45:766–774. doi:10.2486/indhealth.45.766 PMID:18212471

90. Saenger AK, Laha TJ, Edenfield MJ, Sadrzadeh SM (2007). Quantification of urinary 8-iso-PGF2alpha using liquid chromatography-tandem mass spectrometry and association with elevated troponin levels. *Clin Biochem,* 40:1297–1304.doi:10.1016/j.clinbiochem.2007.07.023 PMID:17854792

91. Nourooz-Zadeh J, Cooper MB, Ziegler D, Betteridge DJ (2005). Urinary 8-epi-PGF2alpha and its endogenous beta-oxidation products (2,3-dinor and 2,3-dinor-5,6-dihydro) as biomarkers of total body oxidative stress. *Biochem Biophys Res Commun,* 330:731–736.doi:10.1016/j.bbrc.2005.03.024 PMID:15809058

92. Orman A, Kahraman A, Cakar H *et al.* (2005). Plasma malondialdehyde and erythrocyte glutathione levels in workers with cement dust-exposure [corrected]. *Toxicology,* 207:15–20.doi:10.1016/j.tox.2004.07.021 PMID:15590118

93. Wang SX, Liu P, Wei MT *et al.* (2007). Roles of serum clara cell protein 16 and surfactant protein-D in the early diagnosis and progression of silicosis. *J Occup Environ Med,* 49:834–839.doi:10.1097/JOM.0b013e318124a927 PMID:17693780

94. Verma DK, Ritchie AC, Muir DC (2008). Dust content of lungs and its relationships to pathology, radiology and occupational exposure in Ontario hardrock miners. *Am J Ind Med,* 51:524–531.doi:10.1002/ajim.20589 PMID:18459150

95. Yucesoy B, Vallyathan V, Landsittel DP *et al.* (2001). Association of tumor necrosis factor-alpha and interleukin-1 gene polymorphisms with silicosis. *Toxicol Appl Pharmacol,* 172:75–82.doi:10.1006/taap.2001.9124 PMID:11264025

96. Yucesoy B, Vallyathan V, Landsittel DP *et al.* (2001). Polymorphisms of the IL-1 gene complex in coal miners with silicosis. *Am J Ind Med,* 39:286–291.doi:10.1002/1097-0274(200103)39:3<286::AID-AJIM1016>3.0.CO;2-7 PMID:11241561

97. Wang DJ, Yang YL, Xia QJ *et al.* (2006). [Study on association of interleukin-1 receptor antagonist(RA) gene + 2018 locus mutation with silicosis]. *Wei Sheng Yan Jiu,* 35:693–696. PMID:17290743

98. Corbett EL, Mozzato-Chamay N, Butterworth AE *et al.* (2002). Polymorphisms in the tumor necrosis factor-alpha gene promoter may predispose to severe silicosis in black South African miners. *Am J Respir Crit Care Med*, 165:690–693. PMID:11874815

99. Carlsten C, de Roos AJ, Kaufman JD *et al.* (2007). Cell markers, cytokines, and immune parameters in cement mason apprentices. *Arthritis Rheum,* 57:147–153.doi:10.1002/art.22483 PMID:17266079

100. Hussain SP, Harris CC (2006). p53 biological network: at the crossroads of the cellular-stress response pathway and molecular carcinogenesis. *J Nippon Med Sch,* 73:54–64. doi:10.1272/jnms.73.54 PMID:16641528

101. Wang L, Bowman L, Lu Y *et al.* (2005). Essential role of p53 in silica-induced apoptosis. *Am J Physiol Lung Cell Mol Physiol,* 288:L488–L496.doi:10.1152/ajplung.00123.2003 PMID:15557088

102. Weston A, Wolff MS, Morabia A (1998). True extended haplotypes of p53: indicators of breast cancer risk. *Cancer Genet Cytogenet,* 102:153–154. PMID:9546072

103. Otsuki T, Maeda M, Murakami S *et al.* (2007). Immunological effects of silica and asbestos. *Cell Mol Immunol,* 4:261–268. PMID:17764616

104. Otsuki T, Miura Y, Nishimura Y *et al.* (2006). Alterations of Fas and Fas-related molecules in patients with silicosis. *Exp Biol Med (Maywood),* 231:522–533. PMID:16636300

105. Hamzaoui A, Ammar J, Graïri H, Hamzaoui K (2003). Expression of Fas antigen and Fas ligand in bronchoalveolar lavage from silicosis patients. *Mediators Inflamm,* 12:209–214.doi:10.1080/09629350310001599648 PMID:14514471

106. Tomokuni A, Otsuki T, Isozaki Y *et al.* (1999). Serum levels of soluble Fas ligand in patients with silicosis. *Clin Exp Immunol,* 118:441–444.doi:10.1046/j.1365-2249.1999.01083.x PMID:10594565

107. Pepys J, Bernstein IL. Historical aspects of occupational asthma. In: Bernstein IL, Chan-Yeung M, Malo J-L, Bernstein DI, editors. Asthma in the workplace. 3rd ed. New York (NY): Taylor & Francis; 2006. p. 9–35.

108. Bello D, Herrick CA, Smith TJ *et al.* (2007). Skin exposure to isocyanates: reasons for concern. *Environ Health Perspect,* 115:328–335.doi:10.1289/ehp.9557 PMID:17431479

109. Straus DC, Cooley JD, Wong WC, Jumper CA (2003). Studies on the role of fungi in Sick Building Syndrome. *Arch Environ Health,* 58:475–478.doi:10.3200/AEOH.58.8.475-478 PMID:15259426

110. Mirabelli MC, Zock JP, Plana E *et al.* (2007). Occupational risk factors for asthma among nurses and related healthcare professionals in an international study. *Occup Environ Med,* 64:474–479.doi:10.1136/oem.2006.031203 PMID:17332135

111. Aron Y, Desmazes-Dufeu N, Matran R *et al.* (1996). Evidence of a strong, positive association between atopy and the HLA class II alleles DR4 and DR7. *Clin Exp Allergy,* 26:821–828.doi:10.1111/j.1365-2222.1996.tb00614.x PMID:8842557

112. Wikman H, Piirilä P, Rosenberg C *et al.* (2002). N-Acetyltransferase genotypes as modifiers of diisocyanate exposure-associated asthma risk. *Pharmacogenetics*, 12:227–233. doi:10.1097/00008571-200204000-00007 PMID:11927838

113. Woo JG, Assa'ad A, Heizer AB *et al.* (2003). The -159 C–>T polymorphism of CD14 is associated with nonatopic asthma and food allergy. *J Allergy Clin Immunol,* 112:438–444. doi:10.1067/mai.2003.1634 PMID:12897754

114. Hang LW, Hsia TC, Chen WC *et al.* (2003). Interleukin-10 gene -627 allele variants, not interleukin-I beta gene and receptor antagonist gene polymorphisms, are associated with atopic bronchial asthma. *J Clin Lab Anal,* 17:168–173.doi:10.1002/jcla.10088 PMID:12938145

115. Aynacioglu AS, Nacak M, Filiz A *et al.* (2004). Protective role of glutathione S-transferase P1 (GSTP1) Val105Val genotype in patients with bronchial asthma. *Br J Clin Pharmacol,* 57:213–217.doi:10.1046/j.1365-2125.2003.01975.x PMID:14748821

116. Fukunaga K, Asano K, Mao XQ *et al.* (2001). Genetic polymorphisms of CC chemokine receptor 3 in Japanese and British asthmatics. *Eur Respir J,* 17:59–63.doi:10.1183/09031936.01.17100590 PMID:11307756

117. Witte JS, Palmer LJ, O'Connor RD *et al.* (2002). Relation between tumour necrosis factor polymorphism TNFalpha-308 and risk of asthma. *Eur J Hum Genet,* 10:82–85. doi:10.1038/sj.ejhg.5200746 PMID:11896460

118. Higa S, Hirano T, Mayumi M *et al.* (2003). Association between interleukin-18 gene polymorphism 105A/C and asthma. *Clin Exp Allergy,* 33:1097–1102.doi:10.1046/j.1365-2222.2003.01739.x PMID:12911784

119. Hizawa N, Yamaguchi E, Konno S *et al.* (2002). A functional polymorphism in the RANTES gene promoter is associated with the development of late-onset asthma. *Am J Respir Crit Care Med,* 166:686–690.doi:10.1164/rccm.200202-090OC PMID:12204866

120. Silverman ES, Palmer LJ, Subramaniam V *et al.* (2004). Transforming growth factor-beta1 promoter polymorphism C-509T is associated with asthma. *Am J Respir Crit Care Med,* 169:214–219.doi:10.1164/rccm.200307-973OC PMID:14597484

121. Horne C, Quintana PJ, Keown PA *et al.* (2000). Distribution of DRB1 and DQB1 HLA class II alleles in occupational asthma due to western red cedar. *Eur Respir J,* 15:911–914.doi:10.1034/j.1399-3003.2000.15e17.x PMID:10853858

122. Piirilä P, Wikman H, Luukkonen R *et al.* (2001). Glutathione S-transferase genotypes and allergic responses to diisocyanate exposure. *Pharmacogenetics*, 11:437–445. doi:10.1097/00008571-200107000-00007 PMID:11470996

123. van der Pouw Kraan TC, van Veen A, Boeije LC *et al.* (1999). An IL-13 promoter polymorphism associated with increased risk of allergic asthma. *Genes Immun,* 1:61–65. doi:10.1038/sj.gene.6363630 PMID:11197307

124. Chan IH, Tang NL, Leung TF *et al.* (2007). Association of prostaglandin-endoperoxide synthase 2 gene polymorphisms with asthma and atopy in Chinese children. *Allergy*, 62:802–809.doi:10.1111/j.1398-9995.2007.01400.x PMID:17573729

125. Hizawa N, Maeda Y, Konno S *et al.* (2006). Genetic polymorphisms at FCER1B and PAI-1 and asthma susceptibility. *Clin Exp Allergy,* 36:872–876.doi:10.1111/j.1365-2222.2006.02413.x PMID:16839401

126. Thakkinstian A, McEvoy M, Minelli C *et al.* (2005). Systematic review and meta-analysis of the association between β2-adrenoceptor polymorphisms and asthma: a HuGE review. *Am J Epidemiol,* 162:201–211.doi:10.1093/aje/kwi184 PMID:15987731

127. Contopoulos-Ioannidis DG, Manoli EN, Ioannidis JP (2005). Meta-analysis of the association of beta2-adrenergic receptor polymorphisms with asthma phenotypes. *J Allergy Clin Immunol,* 115:963–972.doi:10.1016/j.jaci.2004.12.1119 PMID:15867853

128. Raby BA, Silverman EK, Kwiatkowski DJ *et al.* (2004). ADAM33 polymorphisms and phenotype associations in childhood asthma. *J Allergy Clin Immunol,* 113:1071–1078.doi:10.1016/j.jaci.2004.03.035 PMID:15208587

129. Kabesch M (2010). Novel asthma-associated genes from genome-wide association studies: what is their significance? *Chest*, 137:909–915.doi:10.1378/chest.09-1554 PMID:20371526

130. Mathias RA, Grant AV, Rafaels N *et al.* (2010). A genome-wide association study on African-ancestry populations for asthma. *J Allergy Clin Immunol,* 125:336–346,e4. doi:10.1016/j.jaci.2009.08.031 PMID:19910028

131. Li X, Howard TD, Zheng SL *et al.* (2010). Genome-wide association study of asthma identifies RAD50-IL13 and HLA-DR/DQ regions. *J Allergy Clin Immunol,* 125:328–335, e11.doi:10.1016/j.jaci.2009.11.018 PMID:20159242

132. Willis-Owen SA, Cookson WO, Moffatt MF (2009). Genome-wide association studies in the genetics of asthma. *Curr Allergy Asthma Rep,* 9:3–9.doi:10.1007/s11882-009-0001-x PMID:19063818

133. Himes BE, Hunninghake GM, Baurley JW *et al.* (2009). Genome-wide association analysis identifies PDE4D as an asthma-susceptibility gene. *Am J Hum Genet,* 84:581–593.doi:10.1016/j.ajhg.2009.04.006 PMID:19426955

134. Demchuk E, Yucesoy B, Johnson VJ *et al.* (2007). A statistical model for assessing genetic susceptibility as a risk factor in multifactorial diseases: lessons from occupational asthma. *Environ Health Perspect,* 115:231–234.doi:10.1289/ehp.8870 PMID:17384770

135. Pharoah PD, Antoniou A, Bobrow M *et al.* (2002). Polygenic susceptibility to breast cancer and implications for prevention. *Nat Genet,* 31:33–36.doi:10.1038/ng853 PMID: 11984562

136. Sharples LD, Tamm M, McNeil K *et al.* (1996). Development of bronchiolitis obliterans syndrome in recipients of heart-lung transplantation–early risk factors. *Transplantation*, 61:560–566.doi:10.1097/00007890-199602270-00008 PMID:8610381

137. Kanwal R (2008). Bronchiolitis obliterans in workers exposed to flavoring chemicals. *Curr Opin Pulm Med,* 14:141–146.doi:10.1097/MCP.0b013e3282f52478 PMID:18303424

138. Hubbs AF, Goldsmith WT, Kashon ML *et al.* (2008). Respiratory toxicologic pathology of inhaled diacetyl in sprague-dawley rats. *Toxicol Pathol,* 36:330–344.doi:10.1177/0192623307312694 PMID:18474946

139. Epler GR (2001). Bronchiolitis obliterans organizing pneumonia. *Arch Intern Med,* 161:158–164.doi:10.1001/archinte.161.2.158 PMID:11176728

140. Bergmann M, Tiroke A, Schäfer H *et al.*; (1998). Gene expression of profibrotic mediators in bronchiolitis obliterans syndrome after lung transplantation. *Scand Cardiovasc J,* 32:97–103.doi:10.1080/14017439850140247 PMID:9636965

141. Lu KC, Jaramillo A, Lecha RL *et al.* (2002). Interleukin-6 and interferon-gamma gene polymorphisms in the development of bronchiolitis obliterans syndrome after lung transplantation. *Transplantation*, 74:1297–1302.doi:10.1097/00007890-200211150-00017 PMID:12451269

142. Snyder LD, Hartwig MG, Ganous T *et al.* (2006). Cytokine gene polymorphisms are not associated with bronchiolitis obliterans syndrome or survival after lung transplant. *J Heart Lung Transplant,* 25:1330–1335.doi:10.1016/j.healun.2006.07.001 PMID:17097497

143. Hildebrandt GC, Granell M, Urbano-Ispizua A *et al.* (2008). Recipient NOD2/CARD15 variants: a novel independent risk factor for the development of bronchiolitis obliterans after allogeneic stem cell transplantation. *Biol Blood Marrow Transplant,* 14:67–74.doi:10.1016/j.bbmt.2007.09.009 PMID:18158963

144. Mannino DM, Buist AS (2007). Global burden of COPD: risk factors, prevalence, and future trends. *Lancet*, 370:765–773. doi:10.1016/S0140-6736(07)61380-4 PMID:17765526

145. Hnizdo E, Sullivan PA, Bang KM, Wagner G (2002). Association between chronic obstructive pulmonary disease and employment by industry and occupation in the US population: a study of data from the Third National Health and Nutrition Examination Survey. *Am J Epidemiol,* 156:738–746.doi:10.1093/aje/kwf105 PMID:12370162

146. Sampsonas F, Karkoulias K, Kaparianos A, Spiropoulos K (2006). Genetics of chronic obstructive pulmonary disease, beyond a1-antitrypsin deficiency. *Curr Med Chem,* 13:2857–2873.doi:10.2174/092986706778521922 PMID:17073633

147. Chappell S, Daly L, Morgan K *et al.* (2006). Cryptic haplotypes of SERPINA1 confer susceptibility to chronic obstructive pulmonary disease. *Hum Mutat,* 27:103–109. doi:10.1002/humu.20275 PMID:16278826

148. Demeo DL, Mariani TJ, Lange C *et al.* (2006). The SERPINE2 gene is associated with chronic obstructive pulmonary disease. *Am J Hum Genet,* 78:253–264. doi:10.1086/499828 PMID:16358219

149. Joos L, He JQ, Shepherdson MB *et al.* (2002). The role of matrix metalloproteinase polymorphisms in the rate of decline in lung function. *Hum Mol Genet,* 11:569–576. doi:10.1093/hmg/11.5.569 PMID:11875051

150. Minematsu N, Nakamura H, Tateno H *et al.* (2001). Genetic polymorphism in matrix metalloproteinase-9 and pulmonary emphysema. *Biochem Biophys Res Commun,* 289:116–119.doi:10.1006/bbrc.2001.5936 PMID:11708786

151. Zhou M, Huang SG, Wan HY *et al.* (2004). Genetic polymorphism in matrix metalloproteinase-9 and the susceptibility to chronic obstructive pulmonary disease in Han population of south China. *Chin Med J (Engl),* 117:1481–1484. PMID:15498369

152. Ito I, Nagai S, Handa T *et al.* (2005). Matrix metalloproteinase-9 promoter polymorphism associated with upper lung dominant emphysema. *Am J Respir Crit Care Med,* 172:1378–1382.doi:10.1164/rccm.200506-953OC PMID:16126934

153. Tesfaigzi Y, Myers OB, Stidley CA *et al.* (2006). Genotypes in matrix metalloproteinase 9 are a risk factor for COPD. *Int J Chron Obstruct Pulmon Dis,* 1:267–278. PMID:18046864

154. Korytina GF, Akhmadishina LZ, Ianbaeva DG, Viktorova TV (2008). [Polymorphism in promoter regions of matrix metalloproteinases (MMP1, MMP9, and MMP12) in chronic obstructive pulmonary disease patients]. *Genetika*, 44:242–249. PMID:18619044

155. Hersh CP, Demeo DL, Lange C *et al.* (2005). Attempted replication of reported chronic obstructive pulmonary disease candidate gene associations. *Am J Respir Cell Mol Biol,* 33:71–78.doi:10.1165/rcmb.2005-0073OC PMID:15817713

156. Hirano K, Sakamoto T, Uchida Y *et al.* (2001). Tissue inhibitor of metalloproteinases-2 gene polymorphisms in chronic obstructive pulmonary disease. *Eur Respir J,* 18:748–752.doi:10.1183/09031936.01.00102101 PMID:11757622

157. van Diemen CC, Postma DS, Vonk JM *et al.* (2005). A disintegrin and metalloprotease 33 polymorphisms and lung function decline in the general population. *Am J Respir Crit Care Med,* 172:329–333.doi:10.1164/rccm.200411-1486OC PMID:15879414

158. Küçükaycan M, Van Krugten M, Pennings HJ *et al.* (2002). Tumor necrosis factor-alpha +489G/A gene polymorphism is associated with chronic obstructive pulmonary disease. *Respir Res,* 3:29.doi:10.1186/rr194 PMID:12537602

159. Seifart C, Dempfle A, Plagens A *et al.* (2005). TNF-alpha-, TNF-beta-, IL-6-, and IL-10-promoter polymorphisms in patients with chronic obstructive pulmonary disease. *Tissue Antigens,* 65:93–100.doi:10.1111/j.1399-0039.2005.00343.x PMID:15663746

160. Sakao S, Tatsumi K, Igari H *et al.* (2002). Association of tumor necrosis factor-alpha gene promoter polymorphism with low attenuation areas on high-resolution CT in patients with COPD. *Chest*, 122:416–420. doi:10.1378/chest.122.2.416 PMID:12171811

161. Chierakul N, Wongwisutikul P, Vejbaesya S, Chotvilaiwan K (2005). Tumor necrosis factor-alpha gene promoter polymorphism is not associated with smoking-related COPD in Thailand. *Respirology*, 10:36–39.doi:10.1111/j.1440-1843.2005.00626.x PMID:15691236

162. Jiang L, He B, Zhao MW *et al.* (2005). Association of gene polymorphisms of tumour necrosis factor-alpha and interleukin-13 with chronic obstructive pulmonary disease in Han nationality in Beijing. *Chin Med J (Engl),* 118:541–547. PMID:15820084

163. Gingo MR, Silveira LJ, Miller YE *et al.* (2008). Tumour necrosis factor gene polymorphisms are associated with COPD. *Eur Respir J,* 31:1005–1012.doi:10.1183/09031936.00100307 PMID:18256059

164. Ruse CE, Hill MC, Tobin M *et al.* (2007). Tumour necrosis factor gene complex polymorphisms in chronic obstructive pulmonary disease. *Respir Med*, 101:340–344.doi:10.1016/j.rmed.2006.05.017 PMID:16867312

165. Brøgger J, Steen VM, Eiken HG *et al.* (2006). Genetic association between COPD and polymorphisms in TNF, ADRB2 and EPHX1. *Eur Respir J,* 27:682–688.doi:10.1183/09031936.06.00057005 PMID:16585076

166. Yucesoy B, Kurzius-Spencer M, Johnson VJ *et al.* (2008). Association of cytokine gene polymorphisms with rate of decline in lung function. *J Occup Environ Med,* 50:642–648.doi:10.1097/JOM.0b013e31816515e1 PMID:18545091

167. Weston A, Harris CC. Chemical Carcinogenesis In: Holland JF, Frei E, Bast R *et al*., editors. Cancer Medicine, 7th ed. Ontario, Canada: B.C. Decker Inc.; 2006. p. 1–13.

168. Hayashi SI, Watanabe J, Nakachi K, Kawajiri K (1991). PCR detection of an A/G polymorphism within exon 7 of the CYP1A1 gene. *Nucleic Acids Res,* 19:4797.doi:10.1093/nar/19.17.4797 PMID:1891387

169. Cantlay AM, Lamb D, Gillooly M *et al.* (1995). Association between the CYP1A1 gene polymorphism and susceptibility to emphysema and lung cancer. *Clin Mol Pathol,* 48:M210–M214.doi:10.1136/mp.48.4.M210 PMID:16696009

170. Ishii T, Matsuse T, Teramoto S *et al.* (1999). Glutathione S-transferase P1 (GSTP1) polymorphism in patients with chronic obstructive pulmonary disease. *Thorax*, 54:693–696.doi:10.1136/thx.54.8.693 PMID:10413721

171. Cheng SL, Yu CJ, Chen CJ, Yang PC (2004). Genetic polymorphism of epoxide hydrolase and glutathione S-transferase in COPD. *Eur Respir J,* 23:818–824.doi:10.1183/09031936.04.00104904 PMID:15218992

172. Vibhuti A, Arif E, Deepak D *et al.* (2007). Genetic polymorphisms of GSTP1 and mEPHX correlate with oxidative stress markers and lung function in COPD. *Biochem Biophys Res Commun,* 359:136–142.doi:10.1016/j.bbrc.2007.05.076 PMID:17532303

173. Cha SI, Kang HG, Choi JE *et al.* (2009). SERPINE2 polymorphisms and chronic obstructive pulmonary disease. *J Korean Med Sci,* 24:1119–1125.doi:10.3346/jkms.2009.24.6.1119 PMID:19949669

174. Kim WJ, Hersh CP, DeMeo DL *et al.* (2009). Genetic association analysis of COPD candidate genes with bronchodilator responsiveness. *Respir Med,* 103:552–557.doi:10.1016/j.rmed.2008.10.025 PMID:19111454

175. Cho MH, Boutaoui N, Klanderman BJ *et al.* (2010). Variants in FAM13A are associated with chronic obstructive pulmonary disease. *Nat Genet,* 42:200–202.doi:10.1038/ng.535 PMID:20173748

176. Boezen HM (2009). Genome-wide association studies: what do they teach us about asthma and chronic obstructive pulmonary disease? *Proc Am Thorac Soc,* 6:701–703.doi:10.1513/pats.200907-058DP PMID:20008879

177. Wang X, Li L, Xiao J *et al.* (2009). Association of ADAM33 gene polymorphisms with COPD in a northeastern Chinese population. *BMC Med Genet*, 10:132.doi:10.1186/1471-2350-10-132 PMID:20003279

178. Sato H, Silveira L, Fingerlin T *et al.* (2007). TNF polymorphism and bronchoalveolar lavage cell TNF-alpha levels in chronic beryllium disease and beryllium sensitization. *J Allergy Clin Immunol,* 119:687–696.doi:10.1016/j.jaci.2006.10.028 PMID:17208287

179. McCanlies EC, Schuler CR, Kreiss K *et al.* (2007). TNF-alpha polymorphisms in chronic beryllium disease and beryllium sensitization. *J Occup Environ Med,* 49:446–452.doi:10.1097/JOM.0b013e31803b9499 PMID:17426528

180. Warburton D, Gauldie J, Bellusci S, Shi W (2006). Lung development and susceptibility to chronic obstructive pulmonary disease. *Proc Am Thorac Soc,* 3:668–672.doi:10.1513/pats.200605-122SF PMID:17065371

181. Daheshia M (2005). Therapeutic inhibition of matrix metalloproteinases for the treatment of chronic obstructive pulmonary disease (COPD). *Curr Med Res Opin,* 21:587–593.doi:10.1185/030079905X41417 PMID:15899108