CHAPTER 21.

Work-related lung diseases

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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^-, $HO^\cdot$, 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/m³ 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% ♀)	$TGF\text{-}\beta^{509TT}$	OR=2.5 (1.3-5.1)	
Controls (170, 36% ♀)	$TGF\text{-}\beta^{509TC}$	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.

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