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Chronic Obstructive Pulmonary Disease

Mechanisms of Disease Development and Prevention Strategies with Antioxidants

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I. Introduction

Chronic obstructive pulmonary disease (COPD) is characterized by the progressive decline of lung function and the presence of air flow obstruction due to chronic bronchitis or emphysema. The American Thoracic Society (1) defines COPD as a "disease state characterized by air flow limitation that is not fully reversible and is usually progressive. It is associated with an abnormal inflammatory response of the lung to noxious particles or gases." According to the Global Initiative for Chronic Obstructive Lung Disease (GOLD) (2), COPD is defined as a disease state characterized by a limited air flow that is not fully reversible. They also state that air flow limitations are progressive and associated with an abnormal inflammatory response in the lungs to noxious particles and gases.

There is a continued increase in the prevalence of COPD morbidity and mortality in industrialized and developing countries during the last few decades. The World Health Organization (WHO) predicts that COPD will rank as the third most common cause of death on a worldwide basis by the year 2020. In North America, COPD has become the fourth cause of death and afflicts more than 15 million Americans (4). This increased

prevalence in COPD is suspected to be caused by the alarming increase in environmental pollution, increasing automobile use, diesel use, industrialization, and cigarette smoking. These factors, along with indoor air pollution from cooking and other sources, are implicated with a dramatic increased incidence of COPD morbidity and mortality, especially in developing countries.

II. Prevalence

Based on published and unpublished estimates, the worldwide prevalence of COPD was estimated at 9.34 per 1000 in men and 7.33 per 1000 in women (2). In the United States, it is estimated that more than 14 million people have COPD, with a high (14%) incidence in smokers compared to a low (3%) incidence in nonsmokers (3). Most epidemiological studies in developed countries have documented that COPD prevalence is greater in men than women and is associated with cigarette use. However, the recent increased cigarette consumption in women is speculated to have an impact on this trend such that the prevalence is similar to that in males. Current estimates in the United States between 1988 and 1994 document that the prevalence of COPD varied markedly by smoking status, with the highest prevalence in current smokers followed by ex-smokers and never-smokers (3). In addition to smoking, there is considerable epidemiological evidence showing that occupational exposure to certain specific agents can lead to the development of COPD. According to a recent statement on COPD, a value of 15% is considered a reasonable estimate of the occupational contribution to the population burden of COPD (5).

III. Risk Factors

Cigarette smoking, α_1-antitrypsin (α_1-AT) deficiency and environmental/occupational exposures are the three most important risk factors involved in the development of COPD. Additional risk factors such as indoor air pollution, age, sex, diet, socio-economic status, birth weight, childhood respiratory infections, recurrent bronchopulmonary infections, airway hyperresponsiveness, and genetic factors are also important. The precise mechanisms involved in the development of COPD by these risk factors are not well understood. Even with smoking and α_1-AT deficiency, which carry the highest risk, it is not possible to predict which persons with the proposed risk factors will actually develop COPD.

A. Cigarette Smoking

Smoking is the single most important etiological factor identified conclusively as a major risk factor in the development of COPD. However, only 10–20% of smokers develop clinically significant COPD, whereas roughly half never develop any clinically significant physiological deficit (6,7). There is overwhelming evidence that tobacco smoking is the most important etiological factor (8,9). A dose–response relationship between the number of cigarettes smoked and corresponding accelerated decline in ventilatory function has been well documented (2,3). Increased exposure to tobacco smoke heightens the risk of developing COPD (Fig. 1) (6,9). Pipe and cigar smokers have a lower morbidity than cigarette smokers (6). According to British Thoracic Society estimates, most patients with COPD have at least a 20-pack-year smoking history (10).

Smokers with COPD develop an accelerated decline of ventilatory function in a progressive manner. A decline of around 50 mL/year in FEV_1 is seen in smokers, which is much higher than the average value of 30 mL/year decline observed in nonsmokers. There is considerable variation in decline in forced expiratory volume in 1 s (FEV_1) with some smokers showing very rapid rates of decline (11). A man who has smoked one pack daily for 30 years will have an FEV_1 that is 270 mL less than that of a nonsmoker. Annual mortality per 100,000 persons from COPD is 10 for those who never smoked and 225 for smokers using more than 25 cigarettes per day (12).

Cessation of smoking leads to reduction in the accelerated decline in FEV_1. This was clearly shown in a lung health study, where the decline in FEV_1 slowed in those smokers who quit smoking (Fig. 2) (13). However,

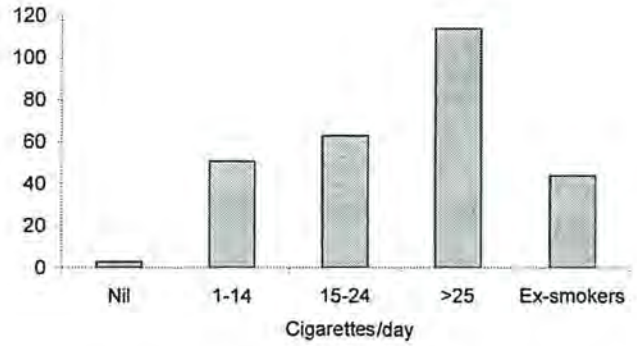

Figure 1 Death rates due to bronchitis in British male doctors per 100,000 according to smoking habit. (Data from Ref. 8.)

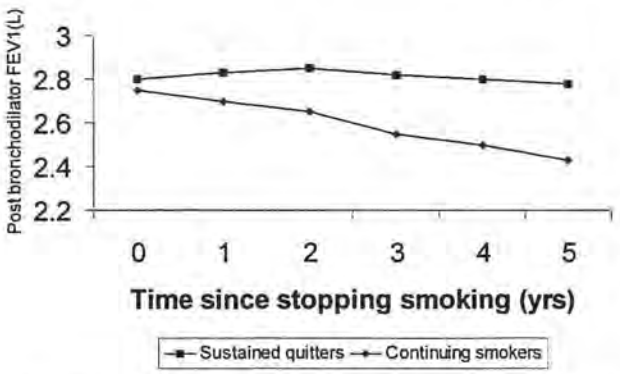

Figure 2 Mean forced expiratory volume in 1 s (FEV_1) after bronchodilation in subjects in the lung health study who were sustained quitters and those who continued to smoke. (From Ref. 13.)

the relationship between amount of smoking and risk of COPD is quite unpredictable on an individual basis, and there is wide variation in the annual rate of decline among smokers with the same smoking history (14). This is further exemplified by the fact that the rate of decline of FEV_1 is much larger in the group of smokers who have demonstrated an increased susceptibility to the effects of smoking on ventilatory function, than in others who have normal or near-normal ventilatory function (13). Other confounding factors include the extent to which cigarette smoke is inhaled and the tar, nicotine, and other constituents present in the cigarette smoke (15). It is still not possible to predict which of these persons will be among the approximately 15–20% of smokers who will go on to develop COPD clinically.

B. Passive Smoking

Passive cigarette smoke exposure measurements and clinical correlation of apparent pulmonary changes with exposure to secondary smoke are faced with many methodological difficulties. The complexities of these investigations and published studies on passive smoking as a risk factor for asthma and COPD are comprehensively reviewed by Coultas (16). A relationship between passive smoking and the development of chronic air flow obstruction using case-control studies (17,18) and a cohort (17) study has been observed, but the relationship was not powerful enough to demonstrate statistical significance. However, in a study of 91,540 nonsmoking Japanese housewives whose husbands were heavy smokers,

the risk of death from emphysema or asthma was 29% higher than that of women whose husbands were nonsmokers (19). A nationwide population-based survey conducted in the United States suggests that approximately 3–5% of lifelong nonsmokers may be affected with COPD (20). However, the effect of passive smoking on lung function was not established in these studies.

C. Air Pollution

Several studies during the period of 1950–1970 have demonstrated evidence incriminating air pollution as an etiological factor in COPD, including the famous London smog of December 1952 (21–25). An increase in symptoms of chronic bronchitis and higher prevalence of emphysema in autopsy studies in areas with greater air pollution provide additional corroborative evidence of air pollution toxicity. A decline in smoke and sulfur dioxide levels, leading to less pollution in some studies, deduced to be related to a decrease in morbidity and mortality further substantiates the same. In 1992 a WHO expert committee on air pollution concluded that high concentrations of sulfur dioxide ($150\,\mu g/m^3$) or similar concentrations of particulate air pollution measured as black smoke were associated with increased morbidity in terms of symptoms and hospital admissions in adults with COPD (26). In a study of six U.S. cities, fine particulate air pollution was found to be a more critical contributing factor to increased mortality than a more complex mixture of pollutants (27). There is no direct correlation showing an association between ozone and deaths from respiratory diseases (28). However, it is likely that particulate air pollution associated with ozone may exacerbate or even cause death in COPD patients. Recent findings reinforce the deleterious role of environmental particulate air pollution and suggest that other gaseous pollutants may have only an additive and not a multiplicative effect on COPD (29). Fine particulate air pollution and not gases is associated with the risk of death in patients with COPD (29). In a community-based study of older adults with chronic respiratory illness, an association between environmental nitrogen dioxide levels and short-term effects on symptoms and pulmonary function was reported (30). In addition, laboratory-based experimental studies provided validation for these community-based human studies (30).

D. Occupation

Occupational exposure has been recognized as one of the major risk factors for development of COPD (5). Several longitudinal and cross-sectional epidemiological studies show a decline in FEV_1 in miners (31–34). The risk of disability from loss of ventilatory function (defined as mean loss in FEV_1)

is less than 5% among nonsmoking miners with low cumulative exposure to coal dust but increases to 20% in those with heavy cumulative coal dust exposures. Smoking enhances the effect of increasing dust exposure retention levels and augments the risk of developing COPD (35–37). In addition, welding fumes exposure has been shown to increase the chances of developing COPD (38).

Epidemiological and clinical studies have demonstrated that exposure to silica dust can lead to the development of COPD even in the absence of silicosis (39–42). In a study of South African gold miners with an average underground mining exposure of 24 years, it was shown that age-, height-, and smoking-adjusted decline in FEV_1 and FEV_1/FVC were associated with dust exposure in both smokers and nonsmokers (40–42). The potential of silica dust to cause pathological changes in the lung, which may lead to the development of COPD, was reviewed in a recent publication (43). The ability of occupational agents to cause COPD is confounded by smoking. In a recent study, 517 never-smokers with occupational exposure to gases, dusts, or fumes underwent pulmonary function tests and were evaluated retrospectively for pulmonary disorders (44). Results of the study showed that there was a statistically significant association between a history of occupational exposure and obstructive ventilatory defect in pulmonary function (44).

In a recent ad hoc committee ATS statement on occupational contribution to the burden of airway disease, it was reported that a value of 15% was a reasonable estimate of the occupational contribution to the population burden of COPD (5). Although studies evaluated ranged in reported population attributable risk from 12% to 34%, the committee concluded, due to the lack of standardization of a definition for COPD, that a 15% attribution to occupation is justified (5).

E. Chronic Bronchopulmonary Infection

Several studies have failed to demonstrate a strong association between the annual rate of decline in FEV_1 and recurrent bronchopulmonary infection (45,46); however, these studies have been done only in smokers with mildly impaired lung function. In subjects with established COPD, an accelerated decline in FEV_1 with lower respiratory tract infection has been observed (47). The role of infection may be more critical in producing acute exacerbations, and cigarette smoke probably predisposes to infection. In a large community study in Copenhagen, Lange and coworkers have found a fourfold increased relative risk of fatality from COPD with infection (mucus hypersecretion) if the FEV_1 was 40% of predicted as compared to a group with a FEV_1 80% of the predicted value (48).

Childhood respiratory infections do have some association with chronic respiratory morbidity and impaired respiratory function in adults (49). But it is unclear whether these lung infections in early life cause lung damage or reflect an underlying susceptibility to lower respiratory infection (50). A cohort of children born in 1946 were reported to have cough and sputum production between the ages of 20 and 36 years. These complaints were more commonly reported in those with a history of chest illness in childhood (51,52).

F. Growth and Nutrition

The association between childhood respiratory illness and ventilatory impairment in adulthood is probably multifactorial. Factors like socioeconomic status, greater exposure to passive smoking, poor diet, and housing in areas of high environmental pollution during childhood may contribute to COPD in adulthood. A correlation has been reported between consumption of fresh fruit in the diet and maintained ventilatory function (53). A low intake of vitamin C and low levels of plasma ascorbic acid were associated with lower pulmonary function in the US National Health and Nutrition Examination Survey (54). Similarly, mortality from chronic respiratory diseases correlated inversely with birth weight and weight at one year of age (55).

G. Atopy and Airway Hyperresponsiveness

Airway hyperresponsiveness (AHR) in smokers is probably acquired rather than constitutional. Several possible mechanisms for increased AHR in smokers with COPD are (a) geometrical factors related to increased thickening of airway walls producing narrow airways, (b) more central deposition of inhaled aerosols as a result of airway obstruction, (c) loss of airway wall support due to a loss of alveolar walls in emphysema, and (d) increased airway wall permeability resulting in airway wall edema (56). However, there is no evidence of increased prevalence of positive skin tests to common aeroallergens in smokers (57). Similarly, the relationship between increased IgE levels, age and pack years smoked, and the fact that the AHR severity declines following cessation of smoking again suggests an acquired rather than constitutional cause for raised IgE in smokers (58).

The role of AHR in the pathogenesis of airway obstruction remains unclear. The airway obstruction in middle-aged subjects may be of two types. The first is associated with asthmatic predisposition or associated allergic phenomenon (59), while the second type may represent an emphysematous type of COPD. AHR may result from structural changes as described above and does not have a significant pathogenic role in accelerated decline of respiratory function. Hence, whether AHR is an important

risk factor for the development of progressive airways obstruction in smokers still remains unanswered.

H. Genetic Factors

Despite chronic long-term smoking, only 15–20% of individuals develop COPD. Hence, the role of genetic factors that influence susceptibility to the detrimental effects of cigarette smoke and, therefore, to the development of COPD have been extensively studied (60).

COPD is known to cluster in families. However, this clustering of COPD may occur because family members share a similar environment, not because they have genetic risk factors in common (61). COPD is increased in the relatives of patients compared with the relatives of controls. This increased prevalence is not due to factors like age, gender, smoking history, etc., and is further substantiated by a decrease in prevalence of COPD and similarly in lung function with increasing genetic distance (62,63).

The studies of mono- and dizygotic twins provide a means to estimate the relative contributions of genes and environment to a trait. Similarly, segregation analysis is a technique that can provide information regarding the mode of inheritance of a trait (dominant or recessive inheritance, number of genes involved, etc.).

Pulmonary function has been investigated in families, and these studies have confirmed a significant genetic component to pulmonary function, which indicates several zones each with a small effect rather than a single major gene.

Identification of Susceptibility Genes: Linkage Analysis

Link analysis compares the inheritance of disease with inheritance of genetic markers in families with multiple affected family members. In a study of families with severe early onset of COPD, no linkage was found (64). Linkage analysis is difficult to use in most families with COPD because symptoms develop only after prolonged exposure to cigarette smoke. So parents of COPD patients are probably already dead and children are too young to manifest significant air flow obstruction.

Association Studies

Case-control studies and longitudinal cohort studies require unrelated individuals, thereby avoiding many problems with family studies. However, the major problem in case-control studies is the adequate matching of cases and controls. The most difficult factors to match are cigarette consumption

and ethnic origin. Because of unreliable cigarette consumption reports and mixture of ethnic groups, false-positive or negative results are often produced.

Susceptibility Genes

Several cell types, enzymes, and inflammatory mediators interact in a complex manner to influence the development of airway inflammation and parenchymal destruction in COPD. Hence, numerous genes (in conjunction with environmental factors) are likely to influence susceptibility to COPD. Moreover, expression of different gene combinations affects the heterogeneous, histopathological, and clinical profile of COPD seen among individuals.

The genes thus implicated are involved in antiproteolysis, metabolism of toxic substance in cigarette smoke, inflammatory response to smoking, and efficiency of mucociliary clearance in the lung. Some of the candidate genes implicated in the pathogenesis of COPD are listed in a schematic diagram showing their functional involvement (Fig. 3).

Some genes do not influence the pathogenesis of COPD directly but may modify the disease phenotype. These genes may modify the individual degree of addiction to nicotine. These genes influence whether one begins smoking, the amount smoked, and the ability to quit. Also, evidence of ventilatory response to hypoxia and hypercapnia being genetic in nature is present.

Severe α_1-Antitrypsin Deficiency

α_1-AT inhibits a broad range of proteases including neutrophil elastase, an important enzyme in COPD pathogenesis. Individuals with extremely low levels of α_1-AT have an increased prevalence of emphysema (65). Subsequently it was shown that α_1-AT deficiency follows a simple Mendelian pattern of inheritance and is usually associated with the Z isoform of α_1-AT. Individuals, homozygous for the Z mutation, have a very low level of circulating α_1-AT (less than 15% of normal) and have a clearly accelerated rate of decline in lung function even in the absence of smoking (66).

Hence, 22 genotype is a genetic risk factor for COPD, but the clinical course of disease in them is variable (67). This variability is not entirely attributable to cigarette smoke exposure, as the rate of decline of lung function in them is highly variable in nonsmokers who are 22 genotype (68). Other mutations may act as modifiers of the clinical course in 22 homozygotes. A polymorphism in the endothelial nitric oxide synthase gene was recently shown to contribute to the development of COPD in 22 genotype individuals (69).

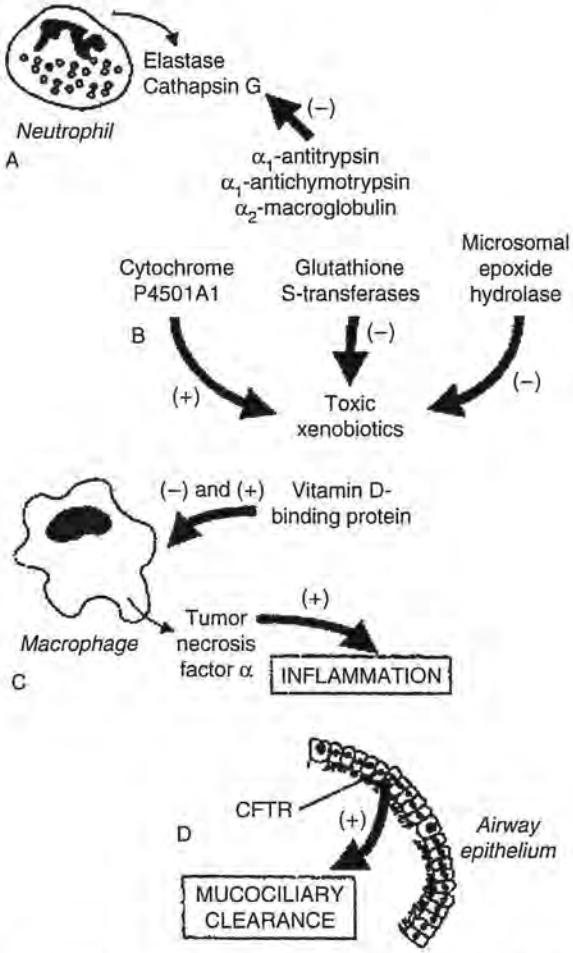

Figure 3 Genes implicated in the pathogenesis of COPD divided into four categories according to their function: antiproteolysis (A), xenobiotic metabolism (B), inflammation (C), and mucociliary clearance (D) alteration associated with cystic fibrosis transmembrane regulator (CSTR).

Intermediate α_1- Antitrypsin Deficiency

The most common α_1-AT gene variants are alleles M, S, and Z. The M allele is the normal (or wild-type) form, whereas the S42 alleles are associated with α_1-AT deficiency. MSRMZ heterozygotes have reductions in α_1-AT levels to roughly 80% and 60% of normal, respectively. The results of many

case-control studies have shown an increased prevalence of M2 heterozygotes in COPD patients vs. controls. Similarly, Madison et al. (70) demonstrated that men with intermediate α_1-AT deficiency (M2 genotypes) and a family history of COPD were at risk for an increased rate of decline of FEV_1 over a 6-year period. In another 10-year prospective study of 516 subjects, there were significant differences in the rate of change of several measures of lung function in M2 subjects compared to MM subjects (71). Therefore, one can conclude that M2 genotype is a risk factor for COPD. However, the increase in risk is modest.

α_1-AT polymorphism, which is not associated with α_1-AT deficiency, may still contribute to the development of COPD. A polymorphism in the 3′ region of α_1-AT gene has been associated with COPD in some populations (72,73). This could attenuate the acute-phase response, leading to reduced levels of α_1-AT during inflammation. This may then fail to protect against enhanced proteolytic tissue destruction during infection. In contrast to the in vitro data, the 3′ polymorphism was not associated with a reduced α_1-AT acute-phase response in patients who had cystic fibrosis (74). The role of 3′ polymorphism in the pathogenesis of COPD, therefore, remains unclear.

Xenobiotic Metabolizing Enzymes

Cigarette smoke contains many toxic and highly reactive compounds that can cause tissue injury and inflammation. Alteration in several enzyme systems, designed to detoxify reactive substances, may contribute to increased risk for developing COPD in some smokers.

Microsomal epoxide hydrolase (MEH) enzyme polymorphism may cause two common amino acid substitutions. It was observed that in patients who had COPD, there was a significant increase in homozygosity for the slow-activity MEH allele (77).

Glutathione-*S*-transferases (GSTS) also play an important role in detoxifying various aromatic hydrocarbons found in cigarette smoke. GST-mA is expressed in the bronchiolar epithelium, type 1 and type 2 pneumocytes, and alveolar macrophages (78). Homozygous deficiency for GST-M1 was associated with emphysema in patients who had lung cancer (79) and in heavy smokers with severe chronic bronchitis (80).

Cytochrome P4501A1 (CYP1A1) often metabolizes xenobiotic into bioactive agents. A mutation in exon 7 of *CYP1A1* causes an amino acid substitution, which increases *CYP1A1* activity in vivo (81). This high-activity allele was associated with increased susceptibility to develop centriacinar emphysema in patients with lung cancer (82).

In addition to the genes [α_1-antitrypsin (*AAT*), α_1-antichymotrypsin (*AACT*), α_2-macroglobulin (*A2M*), cytochrome P4501A1 (*CYP1A1*),

glutathione-*S*-transferase (*GST*), microsomal epoxide hydrolase (*EPHX1*), vitamin D-binding protein (*VDBP*), tumor necrosis factor-α (*TNF-α*), and cystic fibrosis transmembrane regulator (*CFTR*)] listed in Figure 3, polymorphisms of several other genes are also implicated in the pathogenesis of COPD. These include matrix metalloproteinase (*MMP*), hemoxygenase-1 (*HMOX1*), interleukin-1 (*IL-1*), interleukin-1 receptor antagonist (*IL1RN*), and β_2-adrenergic receptor (*ADRB2*). However, they are rare, and evidence of susceptibility to COPD is weak (75,76). The majority of the case-control studies, implicating these genes as biologically plausible candidates for a direct association to COPD, have limitations.

I. Inflammatory Mediators

Inflammation is crucial in the pathogenesis of COPD. Genetic polymorphisms in augmenting inflammation or impairing anti-inflammatory pathways contribute to variability in development of COPD in smokers. Gc globulin, a vitamin D-binding protein, enhances neutrophil chemotactic activity and is an inflammatory mediator (83). It also can act as a macrophage-activating factor (MAF) (84). Three protein isoforms result due to substitution in exon 11 of the gene. These isoforms may be associated with COPD because they affect the ability of the protein to act as a MAF.

TNF-α and TNF-β are proinflammatory cytokines, important in the pathogenesis of COPD. These cytokines cause neutrophil recruitment and their subsequent activation. *TNF* genes exhibit several polymorphisms. The polymorphisms of *TNF* genes are associated with the level of TNF-α and TNF-β production in vitro in certain inflammatory diseases (85). In other studies, *TNF* polymorphism was found not relevant in regulation (86). However, the TNF-α-308 A allele was associated with COPD in patients who had chronic bronchitis and impaired lung function, and its prevalence was greatly increased in patients compared to controls (87).

J. Mucociliary Clearance

The rate of clearance of particulate matter from the lung is highly variable in individuals with COPD (88). The study in monozygotic and dizygotic twins revealed a higher rate of clearance in monozygotic twins (89). This suggests a role for genetic factors.

Mutations of the CFTR gene were identified as the cause of cystic fibrosis. The most frequent CF-causing variant is DF508, and the heterozygosity for this mutation is increased in patients who have disseminated bronchiectasis (90). However, the prevalence of DF508 was not increased in patients who have chronic bronchitis (91). Other *CFTR* mutations were increased in patients who have disseminated bronchiectasis and normal

sweat chloride levels (92,93). One of these mutations is a variable-length thymine repeat in intron 8 of the *CFTR* gene (IV58). Study of IV58-5T has resulted in variable and conflicting results.

K. Modifier Genes

Genetic factors influence smoking behavior. In studies of adolescent twin pairs, likelihood of smoking attributable to genetic factors ranged from 31% to 84% (94,95). Similarly, studies of older twins demonstrate different genetic effects on smoking initiation and persistence.

Cytochrome P4502A6 (CYP2A6) in the liver is a major enzyme involved in the metabolism of nicotine. It has been proposed that polymorphisms in the nicotine-metabolizing enzymes may be a major factor in inter-individual differences in smoking behavior. An amino acid substitution in the *CYP2A6* gene results in an enzyme with reduced activity. Individuals who have the deficiency allele are less likely to be smokers, and those who are smokers consume fewer cigarettes. However, these observations were not found in a case-control study of patients with lung cancer and self-reported cigarette consumption (96). Assuming that cigarette consumption will be reflected by nicotine dependence, several studies attempted to evaluate *CYP2A6* polymorphisms and smoking behavior (97). It is apparent from the review of literature that current available evidence provides no conclusive support that genetic polymorphisms in genes are the determinant factor involved in an individual's smoking behavior (97).

Nicotine increases brain dopamine levels and hence individuals when exposed to nicotine would like to smoke more (98). The D_2 dopamine receptor (*DRD2*) gene contains a polymorphism in the 3′ flaking region (Tqe1A) of the 5′ flanking region (Taq 1B). The prevalence of Taq 1A1 allele was increased in ever-smokers vs. never-smokers in two separate populations (98).

The dopamine transporter (*SLC6A3*) regulates the clearance of dopamine from the synapse. Polymorphisms that affect the rate of clearance would affect the synaptic dopamine concentration and may, therefore, affect an individual's response to nicotine. Individuals with *SLC6A3* allele 9 may result in less efficient dopamine clearance and, therefore, higher levels of synaptic dopamine (99). They may have less need to stimulate dopamine release with nicotine. In another population study, there was a lower prevalence of *SLC6A2* allele 9 in current smokers than in former smokers (42% vs. 52%), suggesting an effect on smoking cessation (100).

Ventilatory responses to hypoxia and hypercapnia are highly variable among individuals (101). Patients with a large ventilatory response to hypoxia and hypercapnia may develop into pink puffer type and those with

minimal responses into blue bloater type. Although no specific gene has been implicated in the variable response to hypoxia and hypercapnia, the results of twin and family studies have indicated the possibility of a genetic component to this variation. Studies demonstrating ventilatory response to CO_2 show the intrapair variance in MZ and DZ twins (102). Recently, Kawakami et al. (103) found that the ventilatory responses to both hypoxia and hypercapnia had significantly lower intrapair variance in MZ than DZ twins. This was the only study to show a genetic component to the response to CO_2, the reason being that CO_2 may have influenced and masked any genetic component to the hypercapnic response. Kawakami et al. had measured the response to CO_2 at normal arterial oxygen tension (103).

In a different approach, ventilatory responses in relatives of patients with COPD with either normal or decreased ventilation were studied. Mountain et al. (104) did not find significant differences in lung function between COPD patients with $Paco_2$ less than 38 mmHg and more than 42 mmHg. In another study, offspring of hypercapnic/hypoxic patients with COPD had decreased responses to hypoxia but not hypercapnia (105). All these studies suggest that individual's response to hypoxia and possibly hypercapnia is partly determined by genetic factors.

IV. Pathogenesis

COPD has distinct anatomical characteristics with a persistent presence of activated T lymphocytes (CD8+), macrophages, and neutrophils in the bronchial wall and lumen. During airway exacerbations an increase in eosinophils also occurs. The pathological hallmark of emphysema is destruction of alveolar walls and inflammation of the peripheral airways. Emphysema is defined morphologically as the distension of airspaces distal to the terminal bronchiole by destruction of alveolar walls. It is classified according to the specific pattern of involvement of the air-exchanging units of the lung. Although several morphological patterns of emphysema are found, two important ones in relation to COPD are centriacinar and panacinar types. In cigarette smokers, centriacinar pattern is characteristic, and panacinar is the predominant type of emphysema in α_1-AT deficiency. Quite often both morphological patterns are present in a lung of a COPD patient.

The morphological hallmark of chronic bronchitis is hypersecretion of mucus in the large airways associated with hypertrophy of submucosal glands, progressing to small airways with inflammation of mucous glands and airway walls. Unlike emphysema, chronic bronchitis is clinically well

defined and diagnosed as persistent cough with sputum production for at least 3 months for 2 years or more. In chronic bronchitis, important morphological abnormalities found in the small airways are hyperplasia of goblet cells, peribronchial fibrosis, edema, increased smooth muscle, intraluminal mucous plugs, and inflammatory cells. These changes in the small airways of the lung contribute significantly to the early manifestations of chronic bronchitis. In both disease entities, similar pathological features are often common, possibly due to a common etiological origin for both these diseases from cigarette smoke or other toxic environmental particles or gases.

V. Mechanisms of Disease Development

Emphysema causes central airway and parenchymal destruction that is thought to be responsible for a majority of the chronic airway limitation in COPD. Increased accumulation of neutrophils and destruction of α_1-AT in the lungs of cigarette smokers was described as a cause of lung destruction and the development of emphysema in cigarette smokes (106). Increased presence of alveolar macrophages, activated T lymphocytes, and eosinophils is also characteristic at the sites of inflammation, and these cells play distinctive roles in the genesis of emphysema. Experimental and clinical evidence suggests that destruction of alveolar walls occurs as a result of protease imbalance caused by α_1-AT deficiency. α_1-AT is an inhibitor of protease (elastase) secreted by the infiltrating neutrophils during the inflammation encountered in COPD. Cigarette smoke, which has been reported to contain about 10^{17} oxidant molecules per puff (107), is implicated in the destruction of α_1-AT protease inhibitor (α_1-PI), thereby creating protease/antiprotease imbalance. In addition, inflammatory infiltrates, particularly stimulated neutrophils, are also capable of inhibiting α_1-AT by the generation of oxygen free radicals. In the peripheral airways of young cigarette smokers, pathological changes consistent with inflammatory infiltrates were demonstrated, providing support for the role of cigarette smoke in the pathogenesis of COPD (108).

In addition to α_1-AT and protease imbalance in the pathogenesis of COPD, other enzymes, cytokines, secretory factors, and reactive oxygen/nitrogen species are postulated to be involved in disease development and exacerbation of COPD. There is accumulating evidence suggesting that oxidative stress, cytokines, cysteine, serine, metalloproteinases, and heme oxygenases may have important roles in COPD (109–111). Recently it was reported that alveolar macrophages from COPD patients degraded more elastin than cells from the control group of nonsmokers or healthy smokers

without COPD (112). MMPs are cell surface–associated proteinases important in tissue remodeling. It was demonstrated that MMPs have important roles in the pathogenesis of emphysema. In a study of 110 smokers and 94 nonsmokers, it was shown that smoking-induced emphysema development was strongly associated with the polymorphism of MMP-9 (113). It was also reported that the extracellular metalloproteinase Mmp12 preferentially degrades elastin and may play a significant role in the development of pulmonary emphysema (114). Recent animal studies have demonstrated a new in vivo pathway for the loss of an epithelial integrin, which is known to cause a local deficiency in active transforming growth factor-β (TGF-β), leading to an increased expression of Mmp12 by alveolar macrophages and to the development of emphysema (115).

Oxidative stress and susceptibility to COPD is well recognized. Polymorphism of genes (*TNF-α*, *MEH*, etc.), oxidant–antioxidant imbalance, and activation of transcription factors (such as NF-κB) are thought to be involved in the molecular genesis of COPD (109–111). The potential for smoking or environmental smoke exposure to cause a decrease in antioxidants was demonstrated in a study of smokers, passive smokers, and nonsmokers (116). Smokers and passive smokers had significantly lower plasma beta carotene and higher α-tocopherol concentrations than nonsmokers. Smokers also had significantly lower plasma ascorbic acid than nonsmokers and passive smokers. (116). These antioxidant levels in smokers, passive smokers, and nonsmokers were independent of differences in dietary antioxidant intake and other factors, such as sex, age, race, body mass index, alcohol intake, triacylglycerol concentration, and fruit and vegetable intake.

In addition to the protease imbalance and the role of oxidant-derived activation of molecular mechanisms, recent studies point to the role of T lymphocyte as a potentially important factor in the inflammatory process leading to COPD (117). Cosio et al. reported on the increased presence of T lymphocytes (CD8+) in smokers with COPD. They propose that CD8+ T cells in concert with other cells orchestrate pulmonary responses that lead to the progressive development of COPD possibly through an antigenic stimulus, originating in the lung (117).

Chronic bronchitis is induced by repetitive sustained airway inflammation leading to mucus gland enlargement and goblet cell metaplasia. In chronic bronchitis inflammatory cells infiltrate the epithelium into airway walls. Mucus gland hypertrophy and goblet cell hyperplasia is considered to be stimulated by the irritants in cigarette smoke. It is believed that the toxic components of cigarette smoke injure epithelial cells and promote inflammation through arachidonic acid metabolites, which have been suggested as potent signals to recruit neutrophils (117). In chronic

bronchitis, there is persistent narrowing of peripheral airways causing increased resistance to air flow.

Although genetic predisposition to develop COPD with α_1-AT deficiency is well documented, recent studies demonstrates that less than 1% of patients with COPD have α_1-AT deficiency. Because of the likelihood of multifactorial origin of COPD, both genetic and environmental factors must be evaluated. In this respect, *TNF*-α gene promoter (TNF-α-308 and receptor 1 and 2 genes), known to be associated with TNF-α secretion, was widely investigated in several studies (87,118–121). In a study of Taiwanese population, *TNF*-α polymorphism was reported to be associated with increased TNF-α production and a 10-fold elevation in the risk of developing COPD (87). The results reported from several of these studies show varying degrees of association of polymorphism of TNF-α-308 alleles to COPD and predisposition to disease. In a study of *TNF*-α gene polymorphisms at positions −376 G/A, −308 G/A, −238 G/A, and ±489 G/A in 169 Dutch COPD patients compared with 358 controls, it was shown that patients without emphysema exhibited a significant difference in the TNF-α +489 G/A genotype compared with controls (121). However, such an association with increased risk for COPD has not been demonstrated in other population studies (122–127). In contrast to the human studies with inconsistencies, Churg et al. (128) recently reported, in an experimental study of mice with knocked-out p55/p75 TNF-α receptors, cigarette smoke–induced inflammation and connective tissue breakdown, which are important precursors in the development of emphysema. Subsequently, it was also demonstrated that macrophage MMP-12 mediated acute cigarette smoke–induced inflammation by releasing TNF-α from macrophages (129). Therefore, it is reasonable to assume that inconsistencies in human studies are caused by genetic heterogenicity of populations or poor phenotype definition in reported studies. It is likely that certain homozygosity may predispose to COPD and better phenotype-controlled studies are important to evaluate this genetic risk factor.

VI. Prevention Strategies with Antioxidants

Oxidative stress has been recognized as a central feature of cigarette smoke–induced COPD (109–111,130–133). There is considerable evidence documenting that cigarette smoking results in oxidative stress and inflammation in the lungs. The oxidative stress in cigarette smokers is caused by the reactive oxidants present in the cigarette smoke as radicals, H_2O_2, peroxynitrate, and peroxynitrite in the gas and tar phase (134). In addition to these cigarette smoke–derived oxidants, influx of inflammatory cells,

stimulated by cigarette smoke, generates enhanced quantities of oxygen radicals. Morrison et al. (135) have demonstrated the potential of leukocytes from smokers to produce increased amounts of oxidants compared to those from nonsmokers. Therefore, an oxidant–antioxidant imbalance, resulting from cigarette smoking, is conceivable and well documented in several studies (130,135,136).

Cigarette smoke exposure of fresh human plasma in vitro was shown to cause lipid and protein oxidation with a subsequent consumption of endogenous plasma antioxidants (137). In cigarette smokers who develop COPD, elevated levels of markers of oxidative stress and decreased antioxidant capacity in blood, bronchoalveolar fluids, breath, air spaces, and urine have been documented (109–111,130–133,135,136).

Several studies document convincing evidence for increased oxidant generation and oxidative stress in COPD, particularly during exacerbations (138). Repine et al. in a "state of the art" review provided a myriad of evidence that suggests that oxidative stress is a major contributing factor for COPD (109). In studies of smokers with COPD this increased oxidant burden is much greater as documented by elevated markers of oxidative damage in blood, pulmonary lavage fluid, urine, and tissues. Among the various oxidative products, 4-hydroxy-2-nonenal, a lipid peroxidation product, is implicated in signaling events in lung inflammation are expression of proinflammatory mediators and protective antioxidant genes in COPD (139). Pacht et al. (140) demonstrated a deficiency of vitamin E and ascorbic acid in smoker's lavage fluid compared to nonsmokers. On the other hand, up-regulation of antioxidant enzymes, such as superoxide dismutase and catalase, in alveolar macrophages of young smokers has been reported and may represent an effort to protect the lungs from oxidant injury (141). Rahman et al. (136) demonstrated that in patients with acute exacerbations of COPD there is an enhanced production of superoxide, which returned to normal levels when the patient was clinically stable. In a study of smokers with or without emphysema, down-regulation of heme oxygenase-1 gene promoter (HO-1) was shown to reduce HO-1 inducibility by reactive species in cigarette smoke (142). HO-1 is a major antioxidant; its inhibition would promote the development of emphysema in smokers. In addition, polymorphisms of antioxidant genes, associated with glutathione-*S*-transferase, GSTM1, GSTT1, GSTP1, and HO-1 in smokers, were shown to have an association with rapid decline in lung function (143). These studies further provide compelling evidence on the role of oxidative stress in the pathogenesis of COPD. Furthermore, several animal experimental studies provide conclusive evidence for the role of ROS mechanisms in cigarette smoke–induced COPD. Since accumulating evidence indicates a pivotal role of ROS in COPD, the potential that enhancing the

antioxidant capacity in the lung may modulate or prevent the progression or development of COPD and improve lung function is worthy of investigation. In a recent report of 30 patients with COPD matched for age and sex with 20 nonsmoker controls, oral supplementation with vitamin E was reported to be beneficial in decreasing lipid peroxidation products in the blood but had an insignificant effect on pulmonary function measured by spirometry (144). In a cross-sectional general population study of randomly selected residents of western New York between the ages of 35 and 79 who were free of respiratory disease, a strong correlation was demonstrated between serum levels of antioxidants and pulmonary function (145). These authors postulate that antioxidants positively influence pulmonary function.

Development of novel antioxidant therapies with sustained bioavailability and potency may be important in the prevention of injury and progression of the disease. In this regard, attempts have been made to supplement smokers and/or COPD patients with antioxidants and vitamins to quench free radicals and ameliorate or prevent the progression of toxic molecular damage caused by the reactive oxygen species (ROS). The effect of antioxidants, such as vitamin C and vitamin E, in combination with a lipid peroxidation biomarker F_2 isoprostane levels in plasma was investigated in a randomized double-blind placebo-controlled trial in 126 smokers. It was shown that a 2 month daily supplementation with 500 mg of vitamin C decreased plasma F_2 isoprostane levels by 28.8 pmol/L when compared with the placebo control group (146). GSH or its precursors have also been tried to supplement lung GSH because it is known that under conditions of oxidative stress GSH may provide protection. Nebulized glutathione was also attempted therapeutically, but this has been shown to induce bronchial hyperreactivity and bronchoconstriction in patients with mild asthma (147). Cysteine administration was reported to be harmful due to its oxidation producing a neurotoxic compound. The cysteine-donating compound *N*-acetylcysteine (NAC) is a cellular precursor of GSH and becomes deacetylated in the gut to cysteine after oral administration. It reduces disulfide bonds and has the potential to interact directly with oxidants. The use of NAC to enhance GSH in patients with COPD has met with varying success (148,149). NAC given orally (600 mg three times a day) is rapidly deacetylated to cysteine with a temporary corresponding increase in plasma and bronchoalveolar lavage fluid (150). However, it was demonstrated in other studies that high oral doses of NAC do not produce a sustained increase in glutathione levels to render antioxidant protection. Irrespective of this drawback, studies reported from Europe have shown that long-term treatment with controlled-release NAC tablets in patients with chronic bronchitis provided a significant reduction in sick-leave days and a reduction in the number of exacerbations (150). However, in a previous

study reported by the British Thoracic Society Research Committee, using 200 mg of NAC three times a day this antioxidant provided only moderate improvement in exacerbations in patients with chronic bronchitis and severe airway obstruction (151). These contradictory results may be due to differences in doses of NAC used, use of controlled-release tablets, and types of chronic bronchitis, i.e., severe vs. mild or moderate airways obstruction.

N-acystelyn (NAL) is a lysine salt of *N*-acetylinecystiene. It has neutral pH and is an oxidant thiol compound with mucolytic and antioxidant properties. It can be aerosolized into the lung without causing significant side effects (152). It was recently shown that NAL may modulate IL-8 release in the lungs and may have therapeutic potential in controlling inflammation (153). Studies comparing the effects of NAL and NAC have found that both drugs enhanced intracellular GSH in alveolar epithelial cells and inhibited H_2O_2 and $O_2^{\bullet -}$ anion release from neutrophils harvested from peripheral blood of smokers and patients with COPD (154). GSH ethyl ester has been tried to increase GSH in vitro (155). Similarly, thiazolidine is a potentially useful compound for cysteine delivery and can be shown to protect against oxidative injury (155). However, there are no studies in humans that validate these compounds for clinical trials. Gene transfer technique to increase cellular GSH by increasing γ-GCS activity has great promise in the management of oxidant-mediated injury in lungs. This is currently considered as an expensive treatment for COPD. However, knowledge of γ-GCS regulation may allow development of other compounds that may act to enhance GSH.

VII. Conclusions

To summarize, one can say with certainty that COPD develops in genetically susceptible individuals after prolonged exposure to cigarette smoke. Presently, most of the genes contributing to this are unknown. α_1-AT deficiency is a risk factor for COPD, but other genetic associations with this disease must still be considered as tentative. The key to establishing that a gene modifies the risk for a disease is replication of the association in different populations. This is a difficult task. Besides α_1-*AT*, only the *GST-M1*, *VDBP*, and *CFTR* genes have been implicated as risk factors in more than one population. Identification of other candidate genes would further enhance the understanding of COPD pathogenesis at the molecular level. There is also good evidence that the propensity to smoke cigarettes and the likelihood of quitting smoking are influenced by genetic factors. This can be useful in efforts directed at cessation of smoking. The responses to hypoxia and hypercapnia also seem to be influenced by genetic factors.

Identification of the genes involved could yield important insights into the pathogenesis of COPD and provide new targets for therapeutic interventions for this debilitating disease.

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