Chapter 1

Review of Literature
1.1 Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is a major and increasing global health problem. It is predicted by the World Health Organization to become the third most common cause of death and the fifth most common cause of disability in the world by 2020 (Lopez and Murray, 1998). Indeed, COPD is already the fourth most common cause of death that has increased over the last 30 years (Murray et al, 2001). Because of the enormous burden of disease and escalating health care costs, which now exceed those of asthma by more than 3-fold, there is now renewed interest in the underlying cellular and molecular mechanisms (Barnes PJ, 2000) and a search for new therapies (Barnes PJ 2001), resulting in a reevaluation of the disease (Barnes PJ, 2002, 2003). Prevalence of COPD in India varies from about 2 to 22 per cent in men and from 1.2 to 19 per cent in women, which have been shown in different reports (Reddy et al, 2004).

COPD, as published by the British Thoracic Society Guidelines (British Thoracic Society, 1997) and the American Thoracic Society standards (American Thoracic Society, 1995), is a slowly progressive condition characterized by airflow limitation, which is largely irreversible. A new definition of COPD has recently been adopted by the Global Initiative on Obstructive Lung Disease (GOLD): “A disease state characterized by airflow limitation that is not fully reversible. The airflow limitation is usually progressive and associated with an abnormal inflammatory response of the lungs to noxious particles and gases” (www.goldcopd.com/workshop/index.html). Cigarette smoking is the major etiological factor in this condition. More than 90% of patients with COPD are smokers, but not all smokers develop COPD (Snider GL, 1989). Only, 15–20% of cigarette smokers appear to be susceptible and develop the disease. COPD is unique among complex genetic diseases in that the environmental inducer of the disease is usually completely obvious, and that the level of exposure can usually be documented with some precision. Previous studies estimated that smoking contributes 15% to the variability of lung function (Burrows et al, 1977), whilst genetic factors account for a further 40% (Coultas et al, 1991). Family based studies support this: they have shown...
ancestral aggregation of spirometric measures in groups unselected for respiratory disease (Redline et al, 1989; Lewitter et al, 1984), and higher rates of airflow obstruction in first-degree relatives of patients with COPD (Kueppers et al, 1977). Moreover, the observation of differences in rate of decline of lung function between smokers (Kueppers et al, 1977) suggests an interaction between genetic and environmental influences.

A genotype-environment interaction is defined by a nonadditive contribution of gene and environment to the clinical phenotype (Hartl DG, 1997). Thus the two influences together confer a different level of risk than that expected by simply adding them. In a complex disease such as COPD there are likely to be many genes contributing to the overall phenotype, which may have additive or synergistic effects; these are known as epistatic interactions. The high mortality and morbidity associated with COPD, and its chronic and progressive nature, has prompted the use of molecular genetic studies in an attempt to identify susceptibility factors for the disease. The eventual aim of such studies is to develop effective therapy. In addition, the early identification of genetic susceptibility to COPD among cigarette smokers may be an essential element in prevention of disease.

1.1.1 Risk Factors
Risk factors for COPD include both host factors and environmental exposures, and the disease usually arises from an interaction between these two types of factors. The host factor that is best documented is a rare hereditary deficiency of alpha-1 antitrypsin (α1-AT). Cigarette smoking is by far the most common environmental cause of COPD, but there are several other risk factors, including air pollution (particularly indoor air pollution from burning fuels), occupational dusts exposure, male sex, advancing age, lower socioeconomic grouping and urban residence. Exposures to environmental tobacco smoke and exhausts of fuel combustion are also important especially amongst nonsmoker patients and women. Indicators of COPD are listed in Table 1.1
1.1.2 Symptoms

COPD includes chronic obstructive bronchitis with fibrosis and obstruction of small airways, and emphysema with enlargement of airspaces and destruction of lung parenchyma, loss of lung elasticity and closure of small airways (Figure 1.1). Most patients with COPD have three pathological mechanisms chronic obstructive bronchitis, emphysema, and mucus plugging as all are induced by smoking, but they may differ in the proportion of emphysema and obstructive bronchitis.

1.1.2.1 Chronic bronchitis

In this respiratory disorder, the air passages in the lungs are inflamed and the mucus-producing glands in the bronchi (the larger air passages of the lungs) are enlarged. The swelling makes it difficult to get air in and out of the lungs. These enlarged glands produce excessive amounts of mucus, which in turn triggers a cough. Tobacco smoke causes inflammatory cells (neutrophils and leukocytes) to arrive in the bronchi. These cells worsen airway obstruction by causing inflammation and thickening of the airways. In chronic bronchitis, the cough persists for at least three months of the year for two consecutive years.

1.1.2.2 Emphysema

Emphysema is the destruction or breakdown of the walls of the alveoli (tiny air sacs) located at the end of the bronchial tubes. The lungs are unable to contract fully and gradually lose elasticity. Irreversible holes develop in the lung tissue reducing the capacity for the lungs to exchange oxygen for carbon dioxide between the lungs and the blood. As a result, breathing may become labored and inefficient resulting in a persistent feeling of breathlessness. This involves dilatation and destruction of the respiratory bronchioles (McLean KA, 1958). This disruption of the alveolar walls and elastin leads to a decrease in the elastic recoil of the lungs, limiting the ability of the alveoli to passively shrink and to exhale. This accounts for the main limitation to exhalation seen in severe COPD.
Table 1.1: Indicators for COPD. These indicators are related to the presence of COPD in human and should ideally be present in animal models.

<table>
<thead>
<tr>
<th>Indicator</th>
<th>Human features</th>
<th>Experimental models</th>
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<tbody>
<tr>
<td>History of exposure to</td>
<td>Tobacco smoke</td>
<td>Exposure based experimental protocol</td>
</tr>
<tr>
<td>Risk factors</td>
<td>Occupational dusts and chemicals</td>
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<td></td>
<td>Indoor/Outdoor air pollution</td>
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<td>Airflow Obstruction</td>
<td>Decrease in FEV1</td>
<td>Lung function tests</td>
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<td>Cough</td>
<td>Chronic Intermittent or persistent</td>
<td>Cough assessment</td>
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<tr>
<td>Hypersecretion</td>
<td>Chronic Sputum production</td>
<td>Functional &amp; morphological assessment</td>
</tr>
<tr>
<td>Dyspnea</td>
<td>Progressive/Persistent/worse on exercise/worse in resp. Infection</td>
<td>Assessment of hypoxemia</td>
</tr>
<tr>
<td>Emphysema</td>
<td>Progressive impairment of lung Function</td>
<td>Morphological analysis of airspace enlargement</td>
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Figure 1.1 Mechanisms of airflow limitation in COPD. The airway in normal subjects is distended by alveolar attachments during expiration, allowing alveolar emptying and lung deflation. In COPD these attachments are disrupted because of emphysema, thus contributing to airway closure during expiration, trapping gas in the alveoli and resulting in hyperinflation. Peripheral airways are also obstructed and distorted by airway inflammation and fibrosis (chronic obstructive bronchiolitis) and by occlusion of the airway lumen by mucus secretions, which may be trapped in the airways because of poor mucociliary clearance.
1.2 Diagnosis and Severity of COPD, GOLD Criterion

The diagnosis of COPD should be considered in any patient with a chronic cough, sputum production, and risk factors, such as tobacco use, α1-AT deficiency, and occupational exposures to dust and chemicals (Pauwels et al., 2001). If a patient has a clinical picture consistent with COPD, then spirometry is used to confirm the diagnosis. All spirometric readings are conducted following treatment with a bronchodilator. To establish the COPD diagnosis, the FEV1 should be less than 80% of predicted value and the FEV1/FVC less than 70%. These abnormal findings indicate airflow limitation, which constitutes the obstructive component of COPD. Many patients have the clinical symptoms of COPD (cough, sputum production) before they develop decreased lung function. However, they are at risk of progressing to a more severe disease.

The GOLD expert panel classified COPD into 4 stages, ranging from 0 to 3 (Pauwels et al., 2001).

1.2.1 Stage 0
At Risk for COPD: Symptoms of chronic cough and sputum production may be present but patients have normal spirometry readings.

1.2.2 Stage 1
Mild COPD: Characterized by FEV1 ≥ 80%, FEV1/FVC < 70%. Patients may or may not have chronic cough and increased sputum production.

1.2.3 Stage 2
Moderate COPD: Characterized by a worsening of airflow (30% ≥ FEV1 > 80%). Patients with stage-2 disease often are symptomatic, seek medical attention, and have shortness of breath with exertion. Stage 2 has 2 subcategories: IIA and IIB. IIA patients have a FEV1 between 50% and 80%; stage IIB patient have a FEV1 between 30% and 50%. Patients with FEV1 below 50% are especially prone to acute exacerbations of disease.
1.2.4 Stage 3
Severe COPD: Characterized by an FEV1 below 30%. Patients are also included in stage 3 if they have respiratory failure or right heart failure. The quality of life is severely affected in these patients. Acute exacerbations in this patient population often require hospitalization and are frequently life threatening.

1.3 Theories
There are three main theories, i.e. protease-anti-protease, oxidant-antioxidant and inflammatory within the pathogenesis of COPD. The protease-anti-protease theory suggests that there is an imbalance between proteases that digest elastin, together with other components of the extra-cellular matrix, and anti-proteases that protect against this (Stockley et al, 1999; Shapiro et al, 1999). The oxidant-antioxidant theory states that disparity between levels of harmful oxidants and protective antioxidants leads to oxidative stress, which in turn influences the actions of anti-proteases, and expression of proinflammatory mediators (MacNee W, 2000). Both of these theories link to the third observation: the importance of inflammation in the pathogenesis of COPD (O'Donnell et al, 2006). These concepts are illustrated below.

1.3.1 Protease–Antiprotease Imbalance
It has long been proposed that various proteases break down connective tissue components, particularly elastin, in lung parenchyma to produce emphysema and that there is an imbalance between proteases and endogenous antiproteases that normally protect against protease-mediated effects (Figure 1.2).

1.3.1.1 Proteases
Evidence for elastin degradation in COPD is provided by the increased excretion of desmosine, derived from elastin cross-links, in smokers with rapid decline in lung function compared with those experiencing a normal decline (Gottlieb et al, 1996). Although early attention was focused on neutrophil elastase, many other proteases that have capacity to degrade elastin have now been implicated (Stockley RA, 2001).
1.3.1.1 Neutrophil Elastase

Neutrophil Elastase (NE) is a serine protease inhibited by \( \alpha1\)-AT in the lung parenchyma and almost certainly accounts for the emphysema in \( \alpha1\)-AT deficiency but its role in smoking-related emphysema is less certain. Furthermore, \( \alpha1\)-AT may be inactivated by cigarette smoke exposure raised the possibility that NE may also be important in smokers with normal plasma \( \alpha1\)-AT concentrations. There is an increase in the amount of NE/\( \alpha1\)-AT complexes in bronchoalveolar lavage fluid of COPD patients who are normal smokers (Yoshioka et al., 1995) and this is correlated with the rate of decline in FEV1 (Betsuyaku et al., 2000).

1.3.1.1.2 Other Serine Proteases

Neutrophils also store two other serine proteases, cathepsin G and proteinase 3, in their specific granules (Rao et al., 1991). These serine proteases have similar properties to NE and potent stimulants of mucus secretion and may have role in the mucus hypersecretion seen in chronic bronchitis (Sommerhoff et al., 1990; Witko-Sarsat et al., 1999). Proteinase 3 is potently inhibited by \( \alpha1\)-AT (Duranton and Bieth, 2003).

1.3.1.1.3 Cysteine Proteases

Lysosomal cysteine proteases (cathepsins) may also be involved in COPD (Chapman et al., 1997; Turk et al., 2001). Increased concentrations of cathepsin L have been detected in BAL fluid of patients with emphysema (Takeyabu et al., 1998) and alveolar macrophages from patients with COPD secrete more cysteine protease activity than macrophages from normal smokers or nonsmokers (Russell et al., 2002b).

1.3.1.1.4 Matrix Metalloproteinases

There is increasing evidence for a role of MMPs in COPD (Shapiro and Senior, 1999). In patients with emphysema, there is an increase in BAL concentrations and macrophage expression of MMP-1 (collagenase) and MMP-9 (gelatinase B) (Finlay et al., 1997; Betsuyaku et al., 1999; Culpitt et al., 1999). There is an increase in activity of MMP-9 in the lung parenchyma of patients with emphysema (Ohnishi et al., 1998) and this is correlated with FEV1 (Kang et al., 2003). Alveolar macrophages from normal smokers express more MMP-9 than those from normal subjects (Lim et al., 2000) and there is an ever greater increase in cells from patients with COPD (Russell et al., 2002a).
Figure 1.2 Protease–Antiprotease Imbalances in COPD. In COPD the balance appears to be tipped in favor of increased proteolysis, because of either an increase in proteases including neutrophil elastase, cathepsins and matrix metalloproteinases or a deficiency of antiproteases, which may include α1-antitrypsin, elafin, secretory leukoprotease inhibitor and tissue inhibitors of matrix metalloproteinases.
1.3.1.2 Antiprotease

Normally, proteases are counteracted by an excess of endogenous antiproteases. The major inhibitors of serine proteases are α1-AT in lung parenchyma and airway epithelium-derived secretory leukoprotease inhibitor (SLPI) in the airways. Other serine protease inhibitors include elafin and α1-antichymotrypsin. At least three tissue inhibitors of matrix metalloproteinases (called TIMP-1, TIMP-2, and TIMP-3) counteract matrix metalloproteinases. Serine protease inhibitors inactivate NE and other serine proteases such as proteinase-3 (Rooney et al., 2001). Cigarette smoking may induce inflammation and increased release of proteases that are counteracted by antiproteases in amounts sufficient to prevent parenchymal injury, but in smokers in whom COPD develops, the production of antiproteases may be inadequate to neutralize the effects of multiple proteases, perhaps because of genetic polymorphisms that impair the function or production of these proteins.

1.3.1.2.1 α1-Antitrypsin

Multiple genetic variants of α1-Antitrypsin (α1-AT) are now recognized that give rise to reduced circulating active α1-AT concentrations (Mahadeva and Lomas, 1998; Carrell and Lomas, 2002). The best described deficiency that results in early-onset emphysema is the ZZ type (PiZZ), in which a single amino acid substitution (Gly3423Lys) results in structural alterations in α1-AT, resulting in failure of its normal post-translational modification and secretion by hepatocytes, leading to very low plasma concentrations. Whether heterozygotes and other genetic variants that reduce circulating α1-AT concentrations to a lesser extent than the ZZ phenotype also predispose to emphysema is more debatable (Lomas and Mahadeva, 2002). ZZ α1-AT deficiency is a rare cause of emphysema accounting for less than 1% of patients but it was proposed long ago that cigarette smoking may oxidize α1-AT resulting in impaired antiprotease function and increased neutrophil elastase activity (Carp and Janoff, 1978). The mechanism appears to be due to oxidative stress and oxidation of methionine at positions 351 or 358 impairs anti-NE activity of α1-AT (Taggart et al., 2000).
1.3.1.2.2 Secretory Leukoprotease Inhibitor

Secretory Leukoprotease Inhibitor (SLPI) is the other major serine proteinase inhibitor in the airways (Vogelmeier et al., 1991). Like α1-AT, SLPI may be inactivated by oxidative stress, but also by cleavage through its active site by cathepsins L and S (Taggart et al., 2001). In patients with emphysema, proteolytic fragments of SLPI are found in BAL fluid, which contributes to the reduced anti-NE activity in these patients. SLPI down-regulates LPS-induced TNF-α and MMP secretion from monocytes (Jin et al., 1997; Zhang et al., 1997). The role of elafin and α1-antichymotryptase in COPD are less well defined (Ishii et al., 2000; Sallenave JM, 2000).

1.3.1.2.3 Tissue Inhibitor of Matrix Metalloproteinases

Four tissue inhibitors of matrix metalloproteinases (TIMP1–4) counteract MMPs (Cawston et al., 2001). TIMP-1 secretion from alveolar macrophages is increased in response to inflammatory stimuli, but the increase is blunted in cells derived from COPD patients, thus favoring increased elastolysis (Russell et al., 2002a, b). An increased frequency of loss-of-function mutations of TIMP-2 has been described in patients with COPD (Hirano et al., 2001).

1.3.2 Oxidant-Antioxidant Theory

Oxidative stress is an important feature of COPD and there is increasing evidence that it is involved in its pathophysiology. Oxidative stress occurs when reactive oxygen species (ROS) are produced in excess and result in harmful effects. There is increasing evidence that oxidative stress is an important feature in COPD (Repine et al., 1997; Henricks and Nijkamp, 2001; MacNee W, 2001). There is an increase in the concentration of H$_2$O$_2$ in the exhaled breath condensates of patients with COPD, particularly during exacerbations, (Dekhuijzen et al., 1996) and increased breath and urinary concentrations of 8-isoprostane, a marker of oxidative stress. (Pratico et al., 1998; Montuschi et al., 1999) Oxidative stress may exacerbate COPD through several mechanisms, including the activation of the transcription factor nuclear
factor kB (NF-kB), which switches on the genes for TNFα, interleukin-8, and other inflammatory proteins, (Barnes et al, 1997), and oxidative damage of antiproteases, such as α1-AT and secretory leukoprotease inhibitor, thus enhancing inflammation and proteolytic injury. Effects of oxidative stress and its molecular consequences are shown in Figure 1.3 and Figure 1.4.

1.3.2.1 Formation of Reactive Oxygen Species

Inflammatory and structural cells that are activated in the airways of patients with COPD produce ROS, including neutrophils, eosinophils, macrophages, and epithelial cells (MacNee W, 2001). Superoxide anions (O$_2^\cdot$) are generated by NADPH oxidase and this is converted to H$_2$O$_2$ by superoxide dismutases. H$_2$O$_2$ is then dismutated to water by catalase. O$_2^\cdot$ and H$_2$O$_2$ may interact in the presence of free iron to form the highly reactive hydroxyl radical (OH$^\cdot$). O$_2^\cdot$ may also combine with NO to form peroxynitrite, which also generates OH$^\cdot$ (Beckman and Koppenol, 1996; Figure 1.4). Oxidative stress leads to the oxidation of arachidonic acid and the formation of a new series of prostanoid mediators called isoprostanes, which may exert significant functional effects (Morrow, 2000), including bronchoconstriction and plasma exudation (Kawikova et al., 1996; Okazawa et al, 1997; Janssen, 2001). The lung is also exposed to exogenous oxidants, which presumably summate with endogenous ROS production to further enhance oxidative stress in the lungs.

1.3.2.2 Evidence for Increased Oxidative Stress

There is considerable evidence for increased oxidative stress in COPD (Repine et al, 1997; MacNee W, 2001). As discussed above, oxidant stress is derived from cigarette smoke and from inflammatory cells, such as activated macrophages and neutrophils. Epidemiological evidence indicates that reduced dietary intake of antioxidants may be a determinant of COPD, and population surveys have linked a low dietary intake of the antioxidant ascorbic acid with declining lung function (Britton et al, 1995; Schunemann et al, 2001).
Figure 1.3 Oxidative stress and its effects. Oxidants contained within cigarette smoke irritate epithelial cells (1) releasing activating cytokines that prompt the recruitment of neutrophils and the release of cell derived oxidants (2) and proteases (3). Antioxidants inhibit oxidant mediated damage to the lung (4), but when an imbalance arises (perhaps because of gene polymorphisms) oxidative stress results (5). The consequences of oxidative stress include activation of macrophages (6), leading to the production of more proteases, mucus hypersecretion, epithelial cell apoptosis, inflammation and inhibition of the action of antiproteases.
Figure 1.4 Molecular consequences of oxidative stress. The relatively weak superoxide anion from both cellular and environmental sources can be transformed into more damaging and potent reactive oxygen and reactive nitrogen species, such as hyperchlorous acid, the hydroxyl radical, and peroxynitrite, through a series of enzymic and non-enzymic steps. Xenobiotic radicals from the environment can be long lived and undergo redox cycling, such as the semi-quinones and result in further superoxide anion formation as well as more powerful radical formation in the presence of free metal ions through Haber–Weiss and fenton chemistry. Endogenous antioxidant defenses glutathione transferase, glutathione peroxidase, SOD, and catalase neutralize and remove these ROS and RNS. ROS, reactive oxygen species; RNS, reactive nitrogen species; O$_2^\cdot$$, superoxide anion; H$_2$O$_2$, hydrogen peroxide; OH$^\cdot$, hydroxide radical; HOCl, hyperchlorous acid; NO, nitric oxide; ONOO$^\cdot$, peroxynitrite; GS-X, glutathione-xenobiotic conjugate; GSSG, oxidized glutathione dimmers; GSH, reduced glutathione; GRx, glutathione reductase; GPx, glutathione peroxidase; LPO, lipid peroxidation; G6PDH, glucose-6-phosphate dehydrogenase.
1.3.2.2.1 Pulmonary Oxidative Stress
There is abundant evidence for increased oxidative stress in the lungs of patients with COPD. A specific marker of lipid peroxidation, 4-hydroxy-2-nonenal, which forms adducts with basic amino acid residues in proteins, has been detected in lungs of patients with COPD (Rahman et al., 2002). This signature of oxidative stress is localized to airway and alveolar epithelial cells, endothelial cells, and neutrophils.

1.3.2.2.2 Exhaled Markers of Oxidative Stress
There are several markers of oxidative stress that may be detected in the breath, and several studies have demonstrated increased production of oxidants in exhaled breath condensates (Kharitonov and Barnes, 2001; Montuschi and Barnes, 2002; Paredi et al., 2002). Ethane, a volatile hydrocarbon formed through lipid peroxidation, is increased in the breath of COPD patients (Paredi et al., 2000). There is an increased concentration of H$_2$O$_2$ in exhaled breath condensate of patients with COPD, particularly during exacerbations (Dekhuijzen et al., 1996; Nowak et al., 1999). There is also an increase in the concentration of 8-iso prostaglandin F$_{2\alpha}$ (8-isoprostane) in exhaled breath condensate, which is found even in patients who are ex-smokers (Montuschi et al., 2000) and is increased further during acute exacerbations (Biernacki et al., 2003). 8-Isoprostane is also increased in the breath of normal smokers, but to a lesser extent than in COPD, suggesting that there is an exaggeration of oxidative stress in COPD. Malondialdehyde and thiobarbituric acid reactive substances, which are markers of lipid peroxidation, are also increased in exhaled breath condensate of patients with COPD (Nowak et al., 1999; Corradi et al., 2003).

1.3.2.2.3 Systemic Markers of Oxidative Stress
There is also evidence for increased systemic markers of oxidative stress in patients with COPD as measured by biochemical markers of lipid peroxidation (Rahman et al., 1996). Increased plasma concentrations of malondialdehyde have been reported in COPD patients (Calikoglu et al., 2002). 8-Isoprostane is increased
in the urine of patients with COPD and further increased during exacerbations (Pratico et al., 1998). The interaction of $O_2^{-}$ and NO forms peroxynitrite, which forms stable 3-nitrotyrosine adducts.

### 1.3.2.3 Effects on Airway Function

ROS have several effects on the airways, which would have the effect of increasing the inflammatory and destructive response in COPD. These effects may be mediated by direct actions of ROS on target cells in the airways but may also be mediated indirectly via activation of signal transduction pathways and transcription factors and via the formation of oxidized mediators, such as isoprostanes and hydroxyl-nonenal.

#### 1.3.2.3.1 Effects on Transcription Factors

ROS activate NF-κB, which switches on multiple inflammatory genes resulting in amplification of the inflammatory response (Barnes and Karin, 1997). The molecular pathways by which oxidative stress activates NF-κB have not been fully elucidated but there are several redox-sensitive steps in the activation pathway (Janssen-Heininger et al., 2000). Many of the stimuli that activate NF-κB appear to do so via the formation of ROS, particularly $H_2O_2$. ROS activate NF-κB in an epithelial cell line (Adcock et al., 1994) and increase the release of proinflammatory cytokines from cultured human airway epithelial cells (Rusznak et al., 1996). Oxidative stress results in activation of histone acetyltransferase activity, which opens up the chromatin structure and is associated with increased transcription of multiple inflammatory genes (Rahman, 2003; Tomita et al., 2003). As with NF-κB, there are several redox-sensitive steps in the activation pathway (Xanthoudakis and Curran, 1996).

#### 1.3.2.3.2 Effects on Signal Transduction Pathways

Oxidants also activate mitogen-activated protein (MAP) kinase pathways. $H_2O_2$ is a potent activator of extracellular regulated kinases (ERK) and p38 MAP kinase pathways that regulate the expression of many inflammatory genes and survival in certain cells and spreading of macrophages (Ogura and Kitamura, 1998). Indeed,
many aspects of macrophage function are regulated by oxidants through the activation of multiple kinase pathways (Forman and Torres, 2002).

### 1.3.2.3.3 Effects on Target Cells

H$_2$O$_2$ directly constricts airway smooth muscle in vitro (Rhoden and Barnes, 1989), and OH$^-$ potently induce plasma exudation in airways (Lei et al., 1996). 8-Isoprostane, the predominant isoprostane formed by the nonenzymatic oxidation of arachidonic acid in humans, is a potent constrictor of animal and human airways in vitro, an effect that is largely mediated via thromboxane receptors (Kawikova et al., 1996). The effect of oxidative stress may be mediated via the activation of epidermal growth factor receptors (EGFR) on submucosal glands (Takeyama et al., 2000). Oxidative stress may induce proliferation of airway epithelial cells, and this effect also appears to be mediated via activation of EGFR (Tamaoki et al., 2004).

### 1.3.2.3.4 Effects on Inflammatory Response

The increased oxidative stress in the airways of COPD patients may play an important pathophysiological role in the disease by amplifying the inflammatory response in COPD. This may reflect the activation of NF-$\kappa$B and activator protein-1, which then induce a neutrophilic inflammation via increased expression of IL-8 and other CXC chemokines, TNF-$\alpha$ and MMP-9. Oxidative stress may therefore serve to amplify the ongoing chronic inflammatory response in COPD and may be an important mechanism leading to increased inflammation during acute exacerbations.

### 1.3.2.3.5 Effect on Proteases

Oxidative stress may also impair the function of antiproteases like $\alpha$1-AT and SLPI and thereby accelerates the breakdown of elastin in lung parenchyma (Taggart et al., 2000).

### 1.3.2.3.6 Effects on Apoptosis

Oxidative stress may also induce apoptosis in endothelial and epithelial cells (Haddad, 2004). Apoptosis of type 1 pneumocytes may be contributory to the
development of emphysema, and this might be induced by cytotoxic T lymphocytes or by inhibition of vascular-endothelial growth factor receptors (Kasahara et al., 2000; Majo et al., 2001). ROS may induce apoptosis by activating the NF-κB pathway, by direct DNA damage via activation of poly-ADP-ribose, and via the generation of 4-hydroxy-nonenal. Apoptosis signal-regulating kinase-1 is held in an inactive conformation by thioredoxin, and when oxidized by ROS, this triggers apoptotic pathways (Gotoh and Cooper, 1998).

1.3.2.3.7 Systemic Effects
The systemic oxidative stress in COPD may contribute to the systemic effects seen in severe disease. For example, impaired redox balance in skeletal muscle cells may be contributory to the muscle weakness, fatigability, and wasting seen in some patients (Langen et al., 2003).

1.3.2.4 Antioxidants
The normal production of oxidants is counteracted by several endogenous antioxidant mechanisms in the human respiratory tract (Cantin et al., 1990). Antioxidants may be enzymatic or nonenzymatic. The major enzymatic antioxidants in the airways are catalase, superoxide dismutase (SOD), glutathione peroxidase, glutathione-S-transferase, xanthine oxidases, and thioredoxin (Figure 1.4). The nonenzymatic category of antioxidant defenses includes low molecular weight compounds such as glutathione, ascorbate, urate, α-tocopherol, bilirubin, and lipoic acid. Concentrations of these antioxidants vary, depending on both subcellular and anatomic location. For example, glutathione is 100-fold more concentrated in the airway epithelial lining fluid compared with plasma (van der Vliet et al., 1999b). Oxidant stress activates the inducible enzyme heme oxygenase-1, converting heme and hemin to biliverdin with the formation of carbon monoxide (Choi and Alam, 1996). Biliverdin is converted via bilirubin reductase to bilirubin, which is a potential antioxidant. Heme oxygenase-1 is widely expressed in human airways (Lim et al., 2000a), and carbon monoxide production is increased in COPD (Montuschi et al., 2001). In the lung, intracellular antioxidants are expressed at relatively
low levels and are not induced by oxidative stress, whereas the major antioxidants are extracellular (Comhair and Erzurum, 2002). Extracellular antioxidants, particularly glutathione peroxidase, are markedly up-regulated in response to cigarette smoke and oxidative stress. The glutathione system is the major antioxidant mechanism in the airways. There is a high concentration of reduced glutathione in lung epithelial lining fluid (Cantin et al., 1990), and concentrations are higher in cigarette smokers. Extracellular glutathione peroxidase is an important antioxidant in the lungs and may be secreted by epithelial cells and macrophages, particularly in response to cigarette smoke or oxidative stress (Avissar et al., 1996). Extracellular glutathione peroxidase inactivates H$_2$O$_2$ and O$_2^•−$ but also reactive nitrogen species (Comhair and Erzurum, 2002). Extracellular antioxidants also include the dietary antioxidants vitamin C (ascorbic acid) and vitamin E (α-tocopherol), uric acid, lactoferrin, and extracellular SOD3. SOD3 is highly expressed in human lung, but its role in COPD is not yet clear (Bowler and Crapo, 2002).

1.3.2.4.1 GSH and its Redox System

The GSH redox system is crucial in maintaining intracellular GSH homeostasis, which is critical to normal cellular physiological processes and represents one of the most important antioxidant defense systems in the lung (Cantin and Begin, 1991). This system uses GSH as a substrate in the detoxification of peroxides such as H$_2$O$_2$ and lipid peroxides, a reaction that involves glutathione peroxidase (GPx). This reaction generates oxidized GSH (GSSG), which is subsequently reduced by glutathione reductase in a reaction that requires the hexose monophosphate shunt pathway utilizing NADPH (Figure 1.5). Physiologically, the glutathione reductase reaction is driven strongly in favor of GSH, with the GSH-to-GSSG ratio normally >90%. Maintenance of the high GSH-to-GSSG ratio minimizes intracellular accumulation of disulfides. However, oxidant stress or other stress alters this ratio. The protective functions of GSH involve enzymatic as well as nonenzymatic processes. GSH is a strong nucleophile and often inactivates electrophilic reactive compounds either by nonenzymatic direct conjugation or by an enzyme-catalyzed reaction involving glutathione-S-transferase (GST).
Figure 1.5 GSH redox cycle. GSH converts hydrogen and lipid peroxides to nontoxic hydroxy fatty acids and/or water. Glutathione disulfide (GSSG) is subsequently reduced to GSH in presence of NADPH and glutathione reductase, which are linked with hexose monophosphate (HMP) shunt. G-6-PD, glucose-6-phosphate dehydrogenase.
1.3.2.4.2 Effects of Antioxidants

In view of the persuasive evidence presented above that oxidative stress is important in the pathophysiology of COPD, antioxidants are a logical approach to therapy (MacNee W, 2000; Barnes PJ, 2001). Several antioxidants have also been administered to patients with COPD to explore their effects on lung function. N-Acetyl cysteine (NAC) was developed as a mucolytic agent but also acts as an antioxidant by increasing the formation of glutathione. Although small-scale trials failed to demonstrate any clear clinical benefit, more recent meta-analyses have shown a small but significant clinical benefit in COPD, particularly in reducing exacerbations (Grandjean et al., 2000; Poole and Black, 2001). This benefit is not shared by other mucolytics and is therefore likely to be due to the antioxidant effect of NAC. These results should encourage the development of more effective antioxidants in the future. Currently available antioxidants are rather weak, but more potent drugs, including spin-trap antioxidants (nitrones) and stable glutathione analogs, are currently in clinical development (Cuzzocrea et al., 2001).

1.3.2.5 Damaging Effects of Oxidative Stress

ROS have several damaging effects including decrease in antiproteases, activation of NF-κβ, increased isoprostane production and several direct effects which have been shown in Figure 1.6. Beside that ROS also cause DNA, protein and lipid damage.

1.3.2.5.1 DNA damage

Genetic material in the living organism is potentially vulnerable to ROS both directly and indirectly. ROS react with either the sugar or the base component of the DNA molecules, which leads to the fragmentation of sugar or loss of base causing strand brake. Hydrogen peroxide is a potent inducer of DNA single stranded breaks (SSB) and in presence of L-histidine also induces double stranded breaks (DBS). Multiple chemical modification occur in all four bases including the deamination of guanine and adenine suggesting the reaction with hydroxy radical or deaminating species, which leads to the mutation in many cells (Repine J E et al., 1997 and Dutta K et al., 2000).
Figure 1.6 Oxidative Stress and its damaging effects in COPD. Reactive oxygen species from cigarette smoke or from inflammatory cells have several damaging effects, including decreased antiprotease defenses; activation of nuclear factor-κB, resulting in increased secretion of the cytokines interleukin-8 and tumor necrosis factor-α; increased production of isoprostanes; and direct effects on airway functions. $O_2^-$ denotes superoxide anion, $H_2O_2$ hydrogen peroxide, $OH^-$ hydroxyl radical, and $ONOO^-$ peroxynitrate.
1.3.2.5.2 Protein Damage
ROS cause the protein oxidation, which leads to the peptide bond cleavage, protein-protein cleavage, and side chain modification with formation of peroxide and carbonyl (Dutta K et al, 2000).

1.3.2.5.3 Lipid Peroxidation
Free radical triggers lipid peroxidation chain reaction by abstracting a hydrogen atom from a side chain methylated carbon of a poly-unsaturated fatty acid by singlet oxygen and hydroxyl radical (radical formation or initiation). Resulting carbon centered radical then react with the O₂ in aerobic cells to give a peroxy radical that subsequently propagates chain reaction, which transform polyunsaturated fatty acid into lipid hydroperoxide, which can impair membrane functions, inactivate membrane bound receptor and enzymes disturb membrane fluidity and increases membrane permeability lipid hydroperoxide can interact with antioxidant or decomposes after reacting with metal ion leaving hydrocarbon gas and unsaturated aldehyde (malondialdehyde) as byproduct (oxygenation or peroxidation). These events may be followed by a detoxification process, in which the reaction chain is stopped where two radical combine to give a nontoxic and non-radical product, such steps may called as termination (Dutta K et al, 2000).

1.3.3 Inflammatory Mediators
COPD is a complex inflammatory disease that involves several types of inflammatory cells mediators, inflammatory effects, and responses to treatment. (Barnes et al, 2003). Analysis of the cell profile in alveoli and small airways shows an increase in all of the cell types implicated in COPD, including macrophages, T lymphocytes, B lymphocytes, and neutrophils (Retamales et al, 2001). Inflammatory Mechanisms in COPD is given in Figure 1.7.

1.3.3.1 Neutrophils
Increased numbers of activated neutrophils are found in sputum and BAL fluid of patients with COPD (Lacoste et al., 1993; Keatings et al., 1996), yet they are increased relatively little in the airways or lung parenchyma (Finkelstein et al, 1995). Neutrophils secrete oxidants and serine proteases including NE, cathepsin G and proteinase-3 as well MMP-8 and MMP-9, which may contribute to alveolar destruction and induce tissue damage. These serine proteases are also potent mucus stimulants. The role of neutrophils in COPD is not yet clear. There is a correlation between the number of circulating neutrophils and fall in FEV₁ (Sparrow et al, 1984) and the rate of decline in lung function.
1.3.3.2 Macrophages

Macrophages appear to play a pivotal role in the pathophysiology of COPD and can account for most of the known features of the disease (Shapiro, 1999; Barnes, 2004). There is a marked increase (5- to 10-fold) in the numbers of macrophages in airways, lung parenchyma, BAL fluid, and sputum in patients with COPD. Furthermore, macrophages are localized to sites of alveolar wall destruction in patients with emphysema (Finkelstein et al., 1995; Meshi et al., 2002). Macrophages may be activated by cigarette smoke extract to release inflammatory mediators, providing a cellular mechanism that links smoking with inflammation in COPD. Alveolar macrophages also secrete elastolytic enzymes, including MMP-2, MMP-9, MMP-12, cathepsins K, L, and S, and neutrophil elastase taken up from neutrophils (Punturieri et al., 2000; Russell et al., 2002b). The predominant elastolytic enzyme secreted by alveolar macrophages in COPD patients is MMP-9.

Macrophages are phagocytic for bacteria and play an important role in host defense. The phagocytic potential of macrophages from COPD patients has not been explored but it is possible that impaired phagocytosis may result in the increased bacterial load in the respiratory tract of patients with COPD. Macrophages recognize apoptotic cells via expression of phosphatidylserine, which interacts with specific receptors on the macrophage surface (Fadok et al., 2000). Ingestion of apoptotic granulocytes by macrophages induces the secretion of transforming growth factor (TGF)-β1 (Huynh et al., 2002). NE cleaves the phosphatidylserine receptor and may thus impair the ability of macrophages to take up apoptotic neutrophils, resulting in increased numbers of apoptotic neutrophils in the airways (Vandivier et al., 2002).

1.3.3.2 T Lymphocytes

There is an increase in the total numbers of T lymphocytes in lung parenchyma and peripheral and central airways of patients with COPD, with the greater increase in CD8+ than in CD4+ cells (Finkelstein et al., 1995; O’Shaughnessy et al., 1997; Saetta et al., 1999; Majo et al., 2001; Retamales et al., 2001). There is a correlation among the number of T cells, the amount of alveolar destruction and the severity of airflow obstruction. The role of T cells in the pathophysiology of COPD is not yet certain. CD8+ cells have the capacity to cause cytolysis and apoptosis of alveolar epithelial cells through release of perforins, granzyme-B, and TNF-α (Hashimoto et al., 2000). There is an association between CD8+ cells and apoptosis of alveolar cells in emphysema (Majo et al., 2001).
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Figure 1.7 Inflammatory Mechanisms in COPD. Cigarette smoke and other irritants activate macrophages and airway epithelial cells in the respiratory tract, which release neutrophil chemotactic factors, including interleukin-8 and leukotriene B4. Neutrophils and macrophages then release proteases that break down connective tissue in the lung parenchyma, resulting in emphysema, and also stimulate mucus hypersecretion. Proteases are normally counteracted by protease inhibitors, including α1-antitrypsin, secretory leukoprotease inhibitor and tissue inhibitors of matrix metalloproteinases. Cytotoxic T cells (CD8⁺ lymphocytes) may also be involved in the inflammatory cascade. MCP-1 denotes monocyte chemotactic protein 1, which is released by and affects macrophages.
1.4 Nitric Oxide

Nitric Oxide (NO) is generated in COPD from the enzyme inducible NO synthase (iNOS) and endothelial NO synthase (eNOS), which is expressed in macrophages and lung parenchyma, particularly in patients with severe disease (Ichinose et al., 2000; Maestrelli et al., 2003; Ahsan et al., 2004). NO is markedly increased during exacerbations (Maziak et al., 1998; Agusti et al., 1999) in patients with COPD. The reason why exhaled NO may be elevated in COPD may be because exhaled NO levels are depressed by cigarette smoking and oxidative stress, since NO combines avidly with superoxide anions to form peroxynitrite. This is supported by the fact that nitrate concentrations, formed by metabolism of peroxynitrite, are increased in breath condensate and sputum of cigarette smokers and patients with COPD (Corradi et al., 2001; Kanazawa et al., 2003). There is also an increase in the tyrosine nitration of proteins in sputum of COPD patients compared with normal controls and this is correlated with disease severity (Sugiura et al., 2004). Peroxynitrite activates MAP kinase pathways in airway epithelial cells and may induce apoptosis of epithelial cells, particularly through the activation of the ERK pathway (Nabeyrat et al., 2003). Peroxynitrite is a potent pulmonary vasoconstrictor in an isolated perfused rat lung preparation but it does not affect hypoxic vasoconstrictor responses (Nossaman et al., 2004).

1.5 Peptide Mediators

1.5.1 Endothelins

Endothelin-1 (ET-1) is induced in sputum and bronchoalveolar lavage fluid of patients with COPD (Chalmers et al., 1999; Bacakoglu et al., 2003), particularly during exacerbations (Roland et al., 2001). Plasma ET-1 concentrations are also elevated in COPD patients, particularly in patients who develop nocturnal hypoxemia during the night (Trakada et al., 2001; Spiropoulos et al., 2003). The release of ET-1 may contribute to pulmonary vasoconstriction and pulmonary hypertension in COPD patients. ET-1 is a potent vasoconstrictor and also causes vascular smooth muscle hyperplasia. There is increased expression of ET-1 in pulmonary endothelial cells of patients with COPD, who have secondary pulmonary hypertension (Giaid et al., 1993) suggesting that ET-1 may contribute to the vascular remodeling associated with hypoxic pulmonary hypertension.
1.5.2 Bradykinin
There is very little information about kinins in COPD. It is likely that kinins are generated in the airways of COPD patients as plasma exudation occurs. Furthermore, proinflammatory cytokines that are found in COPD airways increase the expression of bradykinin B1- and B2-receptors in pulmonary cells (Tsukagoshi et al., 1995; Trevisani et al., 1999). Bradykinin is a potent bronchoconstrictor of human airways, particularly small airways (Hulsmann et al., 1994), stimulates mucus secretion (Nagaki et al., 1996).

1.5.3 Tachykinins
Increased substance P concentrations have been reported in induced sputum of patients with chronic bronchitis (Tomaki et al., 1995), this is presumably derived from sensory nerve endings, although nonneuronal sources of tachykinins are now recognized. Tachykinins are potent stimulants of submucosal gland and goblet cell secretion. The effects of cigarette smoke on mucus secretion is also blocked by tachykinin antagonists in experimental animals, indicating that tachykinin release from sensory nerves mediates these effects (Tokuyama et al., 1990).

1.6 Genetic predisposition to COPD
Since only a minority of smokers (approximately 15 to 20%) develop symptoms and COPD, a genetic predisposition has been hypothesized. The identification of susceptibility genes for complex diseases like COPD, which are likely influenced by multiple genetic factors, multiple environmental factors, as well as gene-by-gene and gene-by-environment interactions, remains challenging. The most commonly applied approach is to select a candidate gene from known or suspected COPD pathophysiology and to test genetic variants within that gene for association to COPD—typically in cases versus controls. Genome wide linkage analysis could also be performed to identify the general locations of COPD susceptibility genes, followed by association analysis with assessment of the following: (1) positional candidate genes from COPD pathophysiology (2) positional candidate genes selected from gene expression studies or (3) dense SNP panels across regions of linkage. Rare variant analysis involves systematically searching for all variants in a gene of interest, often followed by functional studies. Genome wide association analysis has great potential in COPD susceptibility gene identification.
Next to α1-AT deficiency, several candidate genes have been suggested to be linked to COPD induction. Since it was difficult to replicate some of these findings among different populations, future studies are needed. Also, whole genome screening in patients and unaffected siblings displays a promising genetic approach to identify genes associated with COPD. Other studies have examined xenobiotic metabolizing enzymes such as microsomal epoxide hydrolase (mEPHX) and glutathione-s-transferases (GSTM1, GSTT1 and GSTP1) (Sandford et al 2002). Many diverse sources of data have shown that there is a substantial variation in the DNA sequence between two individuals at many points throughout the genome. Human genome polymorphisms are expected to play a key role in defining the etiologic basis of phenotypic differences between individuals in aspects such as drug responses, common disease predisposition and response to the environment. With the completion of Human Genome Project, producing a consensus reference human DNA sequence, the next important step is to study the variation in the DNA sequence both among individuals and different human populations (Cheung et al, 2000). At the individual level, difference in DNA sequence may determine differences in physiological processes and disease susceptibility. At the population level, the different frequencies of the variants may be because of ethnic variations, positive or negative selection pressures and geographical acclimatization or adaptation. DNA sequence variation, patterns of the variation among populations can be used to trace population histories and determine how modern human beings spread around the world (Tishkoff et al, 1998; Cavalli-Sforza et al, 2003).

1.7 Single Nucleotide Polymorphisms

Single nucleotide polymorphisms, variants in DNA sequence better known as SNPs and pronounced as snips, provide a shortcut to comparing genes and genomes within and among species (Lewis CM, 2002). SNPs are single base pair positions in genomic DNA at which different nucleotide sequence alternatives (alleles) exist in normal individuals in populations, wherein the least frequent allele has an abundance of 1% or greater. In principle, SNPs should be bi-, tri-, or tetra-allelic polymorphisms. However, in humans, tri-allelic and tetra-allelic SNPs are rare or almost to the point of non-existence, therefore, SNPs are sometimes simply referred to as bi-allelic markers.
1.7.1 Functional Significance of SNPs

The functional significance of SNPs is mainly in population genetics, evolutionary biology, association studies, diagnostics, risk profiling and pharmacogenomics. SNPs have important implications in human genetic studies. First, a subset of SNPs occur in the coding regions of genes (cSNPs) or in the regulatory regions (Brookes AJ, 1999). It is possible that one of the polymorphic forms may give rise to the expression of a defective or a variant protein, and may potentially lead to a genetic disease. These variations may also reflect evolutionary processes that molded the modern genome (Lewis CM, 2002). Natural selection has left an imprint on SNP distribution. Most are in the regions of the genome that does not encode proteins, including vast DNA that separate genes and the introns within genes. Linkage disequilibrium (LD) mapping ultimately assesses and exploits co-segregation of marker and trait-influencing alleles within families or populations and as such assumes that markers used have an indirect association with the trait. As an alternative to assessing indirect association, one could test alleles at polymorphic sites for direct and physiologically relevant associations with a trait or disease. The identification of a polymorphic site whose alleles are causally related to a trait or disease is the ultimate goal of many genetic analysis studies.

1.7.2 Genetic Variations and Disorders

The investigation of heritable susceptibility to disease is an effort to associate disease phenotype with underlying genotype. Such genotype-phenotype associations have been demonstrated for a large number of monogenetic disorders (Kulle et al, 2005; Lee et al, 2005). The variations in genes can be used as markers in genome wide association mapping studies to identify the related disorder susceptibility loci (Riley et al, 2000). SNP mapping approach is widely being used for the identification of various disorders. The scientific resources to systematically pursue the identification of gene variation that causes heritable susceptibility to polygenic disorders, using genome wide SNP association studies are maturing. These include the availability of a large number of SNP markers and definition of the exact nature of LD in the human population.
1.8 Genes of Detoxification and Oxidative Stress as the Candidates of COPD

The present study would stress on the genetic variations of detoxifying and oxidative stress genes in relation to cigarette-smoke associated toxicity and susceptibility in COPD. These enzymes include Cytochrome p450 (CYPs), mEPHX, GSTs, and NADPH oxidase p22phox. CYPs are phase I enzymes involved in the oxidative metabolism of many substances, including steroids, fatty acids, prostaglandins, chemical carcinogens, and other environmental contaminants. The polymorphic CYP1A1 is located on chromosome 15q22-q24 (Kawajiri et al., 1986). CYP1A1 is key to the metabolic activation of polycyclic aromatic hydrocarbons (PAH), such as those found in cigarette smoke, which are considered carcinogenic (Gonzalez 1990). mEPHX is an enzyme involved in the first-pass metabolism of smoking-induced highly reactive epoxide intermediates and is expressed at varying levels in most tissue and cell types (Oesch et al., 1973, 1977). The human mEPHX gene is localised to the long arm of chromosome 1 (Skoda et al., 1988) and two common aberrant alleles can be detected, which confer slow and fast enzyme activity (Hassett C, 1994b). GSTs are a superfamily of enzymes involved in the conjugation of a wide range of electrophilic substances with glutathione, thereby facilitating detoxification and further metabolism and excretion. GSTs are separated into the following classes: alpha, mu (GSTM), pi (GSTP), theta (GSTT), sigma and kappa. The GST M1, T1 and P1 genes are located on chromosomes 1p13, 22q11.2 and 11q13, respectively. Among the isoenzymes of GST, the homozygous GSTM1-null genotype has been reported to show some association with the pathogenesis of emphysema (Harrison et al., 1997).

1.8.1 Cytochrome P450

Cytochrome P450 (CYPs) enzymes, which represent a large multigene family with different substrate specificities, are important in phase I detoxification reactions. One of the important xenobiotic substrates for CYP enzymes is PAH and aromatic amines, which in its native form is relatively harmless in small doses but upon bioactivation by CYP enzymes, can become very toxic substances for the lungs. The CYP1A1 gene product, aromatic hydrocarbon hydroxylase, catalyzes the first oxidative step in the metabolism of PAH to carcinogens.
CYP1A2 primarily activates aromatic amines (Hammons et al., 1997; Turesky et al., 1998). Both the genes encoding each of these enzymes are located on chromosome 15 and have several allelic variants. CYP1A1 consists of seven exons, six introns and spans 5810 base pairs (Kawajiri et al., 1986). Several SNPs in CYP1A1 gene (15q22.2-q22.4) have been identified (Cascorbi et al., 1996), two of which have been associated with cancer risk: the first (Spurr et al., 1987) is a T/C polymorphism in the 3’UTR region at 3801 nucleotide. This mutation determines three different genotypes, called *1A*1A, which are homozygotes for the wild-type allele, *1A*2A, and *2A*2A, which are, respectively, the heterozygotes and the homozygotes for the mutant allele. The second is an A/G polymorphism, at nucleotide 2455, which results in an isoleucine/valine substitution in the heme-binding region in exon 7, codon 462 (I462V). The *1A*1A genotype corresponds to the wild type, and *1A*2C and *2C*2C to the heterozygous and homozygous genotypes for the mutant allele, respectively (Hayashi et al., 1991). Its function has still not been completely defined and may depend on its link to the MspI polymorphism or to other polymorphisms, for example, in the regulatory region important for CYP1A1 inducibility that can affect CYP1A1 transcription levels followed by threefold elevation in aryl hydrocarbon hydroxylase enzyme activity. (Croft et al., 1994). CYP1A2 promoter polymorphism, −3860G/A (CYP1A2*1C), was reported to decrease enzyme activity in Japanese smokers (Nakajima M, 1999).

### 1.8.2 Microsomal Epoxide Hydrolase

Microsomal Epoxide Hydrolase (mEPHX) is expressed in a variety of different cell types, including hepatocytes bronchial epithelial cells and metabolises highly reactive epoxide intermediates in cigarette smoke (Bartsch et al., 1992; Tingle et al., 1993). There are 2 known SNPs in this gene that affect enzyme activity by a single amino acid substitution. An exon-3 thymine (T) to cytosine (C) mutation changes tyrosine residue 113 to histidine (Tyr113His), and enzyme activity is reduced by ≥50% (slow allele). The second mutation, an adenine (A) to guanine (G) transition in exon 4 of the gene, changes histidine residue 139 to arginine (His139Arg), and produces an enzyme with an activity increased by ≥25% (fast allele). The distance between exon 3 and exon 4 is 6,696 base pairs (Hassett C, 1994a). In both cases the
His variant is associated with lower levels of enzyme activity (Hassett et al, 1994b; Hosagrahara et al, 2004). Both polymorphisms only account for a modest change in activity level (Hosagrahara et al, 2004), hence it may be that there is also variation in the gene's regulatory regions (Raaka et al, 1998). Patients carrying both His variants were at the highest risk of developing COPD and emphysema in a Scottish population (Smith et al, 1997). This result was replicated in those with more advanced COPD in Japan (Yoshikawa et al, 2000) despite the differing frequency of genotypes between the two racial groups. The His139 variant alone was associated with a spirometric diagnosis of COPD in the Boston early-onset COPD cohort (Hersh et al, 2005). The contribution of this gene to the heterogeneity of COPD has been examined in more detail in the National Emphysema Treatment Trial (NETT) Genetics Ancillary Study (Hersh et al, 2006). The authors studied a number of polymorphisms and looked for correlation between genotype and functional capacity phenotypes in two separate patient groups, hypothesizing that there is a genetic basis to the observed phenotypes. The exon 3 SNP (Tyr113His) was associated with poor exercise capacity, whilst the exon 4 SNP (His139Arg) was connected to relatively greater gas transfer. This study was powered to detect a moderate effect of each genotype on overall phenotype, so taken with the previous positive studies it seems likely that these polymorphisms contribute to the COPD phenotype. Their link to specific subgroups of COPD patients will need further study.

1.8.3 Glutathione-S-Transferases

The Glutathione-S-Transferases (GSTs) genes code for a family of enzymes that detoxify some of the harmful contents (aromatic hydrocarbons) of tobacco smoke (Mannervik B 1985). GST conjugate electrophilic substrates with glutathione and this facilitates further metabolism and excretion. Polymorphisms in the genes are known to have functional consequences, and have been examined in COPD (He et al, 2002, 2004; Ishii et al, 1999). The two variants with the most evidence supporting a role in the disease are GSTP1 and GSTM1. GSTP1 is expressed in the liver and the lung at a higher level (Cantlay et al, 1994). GSTP1 contains two known SNP, though only one is known to have an effect on the catalytic activity of the enzyme. This is an A→G change at nucleotide +313 in exon 5, resulting in a single amino acid substitution of 105Ile→105Val (Ali-Osman et al, 1997)
shown to increase the metabolism of carcinogenic aromatic epoxides (Sundberg et al, 1998). Another, C→T change in exon 8 resulting in 114Ala→114Val (Val) mutation. Individuals with the Val105 (mutant) allele have a higher risk of developing lung cancer than those with the Ile105 (wild-type) allele (Ryberg et al, 1997). Studies of the relationship of this variant to lung disease have varied in their results. It would be expected that the 105Ile variant would be associated with higher levels of lung damage, since it is less active against oxidants – this was confirmed by an association with airflow obstruction in a Japanese population (Ishii et al, 1999) and replicated in a Caucasian population in the Lung Health Study (LHS), where this polymorphism together with a family history of COPD was linked to rapid decline of FEV1 (He et al, 2002). Conversely the same group showed that the 105Val variant was associated with low baseline lung function and rapid decline in the higher baseline group (He et al, 2004), whilst Gilliland (2002) demonstrated reduced annual growth rates for FEV1 and FVC in children homozygous for the 105Val variant. The latter results are difficult to explain on the basis of this gene's action alone, but might be understandable if there are gene-smoking or gene-gene interactions affecting the expression of the gene product. No gene-smoking affects were seen in the LHS (He et al, 2004), but there may be an additive effect of polymorphisms in GSTP1 and other GST genes (He et al, 2002), suggesting that a consequence might not be seen unless a change in several gene products were present.

1.8.4 NADPH oxidase p22Phox

The NAD(P)H oxidase system is the most important source of superoxide production in in phagocytes, endothelial and vascular smooth muscle cells. Its activity requires 5 proteins one of them is p22phox, which is encoded by the cytochrome b245 (CYBA) gene, located on chromosome 16q24. The p22phox subunit is membrane bound, it is expressed in vascular cells and antisense studies have shown that the p22phox subunit is a critical component of the NAD(P)H oxidase system (Ushio-Fukai et al, 1996). Several polymorphisms of the CYBA have now been reported and two have been studied for association with coronary artery disease (CAD). The C242T polymorphism is located in the potential haem-binding site and codes for the substitution of histidine by tyrosine, the A640G polymorphism is located in the 3’-untranslated region of the gene. Inoue and co-
workers (1998) found the T/T genotype of the C242T polymorphism to be associated with a lower risk of developing CAD in a Japanese cohort. Other studies, however, found no association or the opposite that is the C/T and T/T genotype to be associated with CAD and disease progression (Cai et al., 1999; Cahilly et al., 2000). In contrast, Gardemann and co-workers (1999) found no association with the C242T polymorphism, but association between the A640G polymorphism (A/A genotype) and CAD. Another −930A/G variant is a functional promoter polymorphism.

1.9 Objectives

The goal of this study is to find association of the genetic makeup of human beings with susceptibility to respiratory disorders like COPD induced by environmental pollutants, taking into account the level of various risk factors. It is in context with the polymorphisms in the genes related with detoxification & oxidative stress that the following objectives have been set.

1. Screening of candidate genes like mEPHX, GSTP1, CYP1A1, CYP1A2 and CYBA for polymorphisms study in relation to COPD.

2. Estimation of related biochemical markers such as MDA, GSH levels, CAT and GPx activity.

3. Correlation analysis between gene polymorphisms & biochemical parameters.

4. Association studies of these polymorphisms with COPD & related respiratory disorders.