Review of Literature
II. REVIEW OF LITERATURE

Chronic Obstructive Pulmonary Disease (COPD) is the most common chronic disease of the lungs characterized by a slowly progressive irreversible airflow obstruction (Stockley, 2000; Anto et al., 2001). Emphysema and chronic bronchitis are the most important conditions that compose COPD. Emphysema and chronic bronchitis occur together frequently as they share a common etiologic agent, tobacco smoke (Boschette et al., 2003).

In chronic bronchitis, irritation of the airways by smoke or other irritants results in excessive mucus secretion. Airway obstruction results from narrowing of airway by thick mucus and from bronchiolar inflammation and edema. Excess mucus secretion also results in plugs in the small peripheral airways. These plugs reduce the functional air exchange area and leads to the destruction of the alveoli. In contrast, emphysema is characterized by destruction of the distal airspaces including the bronchioles, alveolar ducts and alveolar sacs. This results in the loss of elastic recoil. The forced expiratory volume is maintained by the elastic recoiling. If decreased, the ductal airways will collapse during expiration and trap air (Goldsmith and Weber, 1996).

COPD begins with complex biochemical and cellular events in the small airways and surrounding alveoli. The lungs begin to increase in size with an increase in forced expiratory vital capacity (FVC). This leads to early physiological alterations that can be readily identified by simple spirometry.
By the time that both clinical and radiographic signs are present, COPD is in a moderate to advanced stage. Airflow obstruction in COPD patients is found to respond to various therapeutic efforts, but mostly once initiated this is largely irreversible (Petty, 2002).

COPD is becoming a greater health problem with the growing use of cigarettes around the world (Lomas and Silverman, 2001; Rennard and Farmer, 2002). The mortality data for COPD and allied conditions for men and women aged 35-74 years reported from industrialized countries is provided in Fig. 2 (Page No. 10).

COPD is responsible for >29 million disability adjusted life years and one million years of life lost per annum around the world (Silverman et al., 2002). COPD is currently the 12th leading cause of disability worldwide (Stang et al., 2000). It is expected to become the third leading cause of death after ischemic heart disease and cardiovascular diseases and the fifth leading cause of disability by 2020 (Lundback et al., 2003; Roche and Hu hon, 2004). COPD is also predicted to explode in developing countries such as India, Mexico, Cuba, Egypt, south Africa and China (Peto et al., 1999).

2.1 Genetic predisposition

Exposures to environmental factors such as tobacco smoke or occupational air pollutants play a significant role in the pathogenesis of COPD (Cohen, 1980; Higgins, 1991). WHO (1996) estimates that there are approximately one-third smokers in the world or approximately one third of the global population ≥15 years old are smokers.
Fig. 2. Age adjusted death rates for COPD by countries, by sex and ages, 35-75 years

A marked variability in the development of airflow obstruction in response to smoking has been reported (Burrows et al., 1987; Silverman, 2002). Interestingly many countries with high rates of smoking have a low prevalence of COPD. For example, despite the highest tobacco consumption, the prevalence of COPD in China is reported to be very low (Halbert et al., 2003). In Caucasians, only 10-20% of chronic heavy cigarette smokers develop symptomatic COPD. This suggests that other factors are likely to be important in determining which cigarette smokers are at the risk of developing airflow obstruction (Bascom, 1991; Sandford et al., 2002).

Population studies of families and twins have demonstrated familial aggregation of respiratory symptoms (Kueppers et al., 1977; Khoury et al., 1986; Tager et al., 1988; Larson and Barman, 1989; Redline et al., 1989). Larson and Barman (1965) showed aggregation of COPD in families favoring a genetic basis for COPD. Familial aggregation of reduced lung function was observed in relatives of COPD patients suggesting a genetic basis for the development of COPD (Kueppers et al., 1977; Redline et al., 1989). Case control studies also demonstrated an increased prevalence of COPD in relatives of COPD patients (Khoury et al., 1986; Tager et al., 1988).

Difference in the prevalence of COPD among different racial groups indicates that genetic factors may play an important role in the development of COPD (Roberts et al., 1977; Cox et al., 1980; Marcus et al., 1988; Buist et al., 1995; Blanco et al, 2001). COPD is more common in whites than blacks and other racial and ethnics groups (Cox et al., 1980; Zhu, 2001). The susceptibility to develop COPD was found to vary between different racial
and ethnic groups with Caucasians being more susceptible and Asians and Africans less susceptible (Barnes, 1999; Blanco et al, 2001). The prevalence of COPD in Japanese Americans was found to be very low when compared with Caucasian Americans (Roberts et al., 1977; Marcus et al., 1988). It is also uncommon in Chinese living in the USA (Buist et al., 1995). These reports indicate a genetic predisposition for the development of COPD.

The association of COPD to inherited severe deficiency of the serine protease inhibitor α1-AT has been known since 1963, and remains the only proven genetic risk factor for severe, early onset of COPD. Alpha-1-antitrypsin serves primarily as an inhibitor of neutrophil elastase (Snider et al., 1985). Laurell and Eriksson (1963) observed that members of families who have low concentrations of serum α1-AT have a high prevalence of pulmonary emphysema than the usual smoking population that acquired emphysema. Gross et al (1964; 1965) reported that when lung tissues of rats were treated the elastolytic enzyme papain, it degrades them to produce parenchymal destruction resembling centrilobular and panacinar emphysema.

Subsequently, it was demonstrated that the normal human neutrophil contained a potent serine elastase (Janoff and Schere, 1968). The serum from patients with α1-AT deficiency also showed less inhibitor capacity specifically for elastase (Turino et al., 1969). These observations led to the protease-antiprotease imbalance hypothesis for the development of lung destruction in pulmonary emphysema. The proteinase-antiproteinase hypothesis originated with the observation that subjects with inherited deficiency of plasma alpha-1-antitrypsin were particularly susceptible to the
development of emphysema. Thus research into human emphysema concentrated on the role of α1-AT and neutrophil elastase in the pathogenesis of the disease.

2.2 Alpha-1-antitrypsin: Structure, Function and Molecular Genetics

Alpha-1-antitrypsin is the archetypal member of the Serine Protease Inhibitors (Serpins) family that is widely distributed throughout the plant and animal kingdoms performing a diverse array of functions. The major function of α1-AT is to protect the elastic tissue from proteolytic attack (Carrell et al., 1982; Matsunaga et al., 1990; Potempa et al., 1994).

Alpha-1-antitrypsin exhibits broad substrate specificity, inhibiting a variety of serine proteases including neutrophil elastase, cathepsin G, kallikrein, pancreatic trypsin, rennin and urokinase (Laurell and Jeppsson, 1975; Beatty et al., 1980). However, kinetic studies have shown that the neutrophil elastase is the primary target for α1-AT especially in the lung (Heidlmann and Travis, 1986).

Alpha-1-antitrypsin is a suicidal protein, the inhibitor-proteases complex formation results in the inactivation of the protease and proteolytic cleavage of the inhibitor (Beatty et al., 1980). The rate of inactivation of different proteases by α1-AT varies considerably but is greatest with neutrophil elastase having an association constant of 6.5x10^7 M^-1 S^-1. Inhibition occurs by forming 1:1 equimolar complexes in which the proteinase binds to the α1-antitrypsin active site (Kurachi et al., 1981; ATS/ERS, 2003).
The liver is the predominant source of α1-AT. It is subsequently secreted into the blood stream where it accounts for 90% of the protease inhibitory capacity in serum (Fagerhol and Cox, 1981; Mornex et al., 1986; Perlmutter, 2002). Alpha-1-antitrypsin is also synthesized in the extra-hepatic tissues and cells including neutrophils, monocytes and macrophages, intestinal epithelial cells, breast carcinoma cells and the cornea (Moraga and Janciauskiene, 2000). The major site of action of α1-AT is alveoli of the lung, where it functions to protect elastic tissues from excessive hydrolysis by neutrophil elastase (Olsen et al., 1975; Gadek et al., 1980).

A normal serum concentration of α1-AT (1.3 g/L) is necessary to maintain the normal structure and function of the human lung (Jeppsson et al., 1978; Kurachi et al., 1981). Being an acute phase protein, α1-AT level is substantially raised during inflammation. As a result, during the host response to inflammation, serum α1-AT concentration may be misleading (Martin et al., 1987; Rosen, 1998).

The synthesis of α1-AT is controlled by a pair of genes at the Pi (Protease inhibitor) locus and is inherited as co-dominant alleles (Fagerhol and Cox, 1981; Gadek and Crystal, 1982). The gene-encoding human α1-AT resides in an approximately 320kb gene cluster of serine protease inhibitor genes. This region also includes the genes encoding α1-antichymotrypsin, protein C inhibitor, kallistatin, corticosteroid binding globulin and an antitrypsin related pseudo gene. These six genes are organized into two discrete sub clusters of three genes each, which have similar genomic organizations (Rollini et al., 2000).
The *aat* gene locus is highly polymorphic and is mapped to chromosome 14q31-32.3 (Carrell and Owen, 1979; Lai *et al*., 1983; Schroeder, 1985; Byth *et al*., 1994). Long *et al* (1984) obtained the complete cDNA sequence of the *aat* gene. The gene length was 12.2kb with a 1,434bp coding region. The *aat* gene structure is given in Fig. 3 (Page No. 16).

The gene is composed of seven exons separated by six introns (Morgan and Kalsheker, 1997). Exon I, the 5′prime portion of exon II and 3′ prime portion of exon V are non-coding regions. The first intron contains a 143 amino acid open reading frame, an Alu family sequence and pseudo transcription initiation region. The major transcription site starts from the middle of exon Ic. Cis acting promoter sequences are present in the 5′ to exon Ia and in the middle of exon Ic. Two different hepatocyte nuclear proteins bind in the region between Ib and Ic. C-jun protein binds within exon Ib region. The start codon (ATG) lies in the exon II followed by sequences coding for a 24 residue signal peptide. The sequences for the matured protein start in exon II and end in the exon V. Three identical carbohydrate attachment sites, two in the exon II (Asn46 and Asn83) and one in the exon III (Asn247) are present. The sequence for the active residue is in the exon V region. There is a promoter region specific for hepatocytes and an alternate promoter for monocytes and macrophages (Perlino *et al*., 1987; Crystal, 1990; Rollini and Fournier, 1997).

The *aat* gene is expressed in liver hepatocytes and mononuclear phagocytes as a 1.75kb mRNA, translated and secreted into the ER. In the rough ER, N-linked carbohydrates are added to each of the 3-asparaginyl
Fig. 3. The structural organization of *aat* gene (Morgan and Kalshekar, 1997)
residues- 46, 83 and 247. The protein is translocated to the golgi bodies where
the high mannose carbohydrates are trimmed and the glycosylated $\alpha$1- AT is
secreted as a mature protein of 394 amino acids (Carrell et al., 1982). The
mature protein circulates in the plasma with a half-life of approximately
5 days and diffuses into all tissues where it functions to inhibit neutrophil
elastase (Long et al., 1984; Nukiwa et al., 1986; Perlmutter et al., 2000).

The 3D conformation of $\alpha$1-AT is determined by nine $\alpha$-helices and
three $\beta$ pleated sheets made of parallel and anti parallel strands. An exposed
mobile reactive loop presents a peptide sequence as a pseudo substrate for the
target proteinase. In $\alpha$1-AT, this loop is occupied by the $P_1$-$P_1$ residues
(methionine- serine) and act as “bait” for neutrophil elastase. The methionine
residue can be easily oxidized resulting in the functional inactivation of the
protein (Beatty et al., 1980; Hubbard et al., 1987; Moraga and Janciauskiene,
2000).

The crystallographic study of serpin protease complex indicates a
conformational change initiated by reaction of the active serine of the protease
with the active residue of $\alpha$1-AT (Fig.4, Page No. 18). The reactive loop
cleaves and moves to the opposite pole while displacing the tethered protease
along with it. After docking, the proteinase is inactivated by a mouse trap
action that swings it from the upper to the lower pole of the protein in
association with the insertion of the reactive loop as an extra strand in $\beta$ sheet
A (Huntington et al., 2000). This complex is then recognized by hepatic
receptors and cleared off from the circulation (Carrell and Lomas, 2001;
Lomas and Mahadeva, 2002).
Fig. 4. Mechanism of inhibition of Protease by α1-AT (Lomas and Mahadeva, 2002)
The reactive loop \( \beta \)-sheet A interaction of the serpin is crucial for their role as effective antiproteinases. It also renders them liable to undergo conformational transitions that cause diseases. Mutations in the mobile regions lead to the loss of function, with consequences that reflect in the physiologic role of proteinase inhibitor (Mahadeva et al., 1999; Lomas and Mahadeva, 2002).

2.3 Genotypes of Protease Inhibitor (Pi) System

During serum electrophoresis in an acid gradient, the \( \alpha_1 \)-AT protein moves towards the anode and splits into three major and five minor antitrypsin bands (Fagerhol and Laurell, 1970). Difference in the speed of migration of different \( \alpha_1 \)-AT variants on serum electrophoresis has been used to identify the Pi phenotype. These differences in migration relate to variations in protein charge resulting from nucleotide base alterations (Fagerhol and Cox, 1981). Based on the mobility the variants are named: F-Fast, M-medium, S-slow. The E, F, G and I variants are electrophoretically faster than the M protein, while the P, S, V, W, X and Z proteins are slower than the M protein (Brantly, 1996; Hutchison, 1998).

With respect to circulating \( \alpha_1 \)-AT levels, Pi alleles may be classified as "normal" (normal levels of functional \( \alpha_1 \)-AT protein), "deficient" (low serum \( \alpha_1 \)-AT protein level), "dysfunctional" (normal level of a non-functional \( \alpha_1 \)-AT) or "null" (no \( \alpha_1 \)-AT detectable) (Brantly et al., 1988; Crystal et al., 1989). From the viewpoint of \texttt{aat} gene evolution, \( \alpha_1 \)-AT variants can be categorized into two groups: the variants derived from the oldest human \texttt{aat}
gene- PiM1 Ala213 (example: PiZ, PiM Heerlen, and PiNull Granitefalls) and those derived from the newer *aat* gene PiM1 Val213 (example: PiM3, PiM2 and PiNull Bellingham) (Nukiwa *et al.*, 1986; Crystal *et al.*, 1989). The commonly found *aat* alleles are given in Table 1 (Page No. 21).

The normal M alleles represent by far the largest group of *aat* alleles. The normal *aat* alleles are characterized by their association with normal levels of α1-AT in serum and normal function of the α1-AT protein (Hall *et al.*, 1976; Gadek and Crystal, 1982; Travis and Salvesen, 1983; Nukiwa *et al.*, 1987a).

Inheritance of any homozygous or heterozygous combinations of the M family proteins is associated with "normal" levels of α1-AT. Among the Caucasians of northern European descent, M1 (Val213) allele is the most common allele (allelic frequency- 44-49%) followed by M1 ( Ala213)-20-23%, M2- 14-19% and M3- 10-11% (Dykes *et al.*, 1984; Nukiwa *et al.*, 1986; Blanco *et al.*, 2001).

The deficiency group is characterized by *aat* genes that code for α1-AT present in serum but in amounts insufficient to protect the lower respiratory tract from progressive destruction by neutrophil elastase. Deficiency alleles of *aat* gene represent the clinically relevant group and include mainly the PiZ and PiS alleles (Takahashi *et al.*, 1988; Crystal, 1990).

The PiZ variant differs from the PiM variant by a single nucleotide substitution of G by A at codon 342 exon V resulting in the amino acid substitution of Glu (GAG) to Lys (AAG). Based on the assumption of random
<table>
<thead>
<tr>
<th>S. No</th>
<th>Subclass</th>
<th>Allele</th>
<th>Base allele</th>
<th>Base change</th>
<th>Variant site</th>
<th>Codon</th>
<th>Exon Involved</th>
<th>Abnormal intracellular process</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal</td>
<td>M1 Ala213</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2.</td>
<td>-do-</td>
<td>M1 Val213</td>
<td>M1</td>
<td>C→T</td>
<td>GCG→GTG Ala→Val</td>
<td>213</td>
<td>III</td>
<td>-</td>
</tr>
<tr>
<td>3.</td>
<td>-do-</td>
<td>M2</td>
<td>M3</td>
<td>G→A</td>
<td>CGT→CAT Arg→His</td>
<td>101</td>
<td>II</td>
<td>-</td>
</tr>
<tr>
<td>4.</td>
<td>-do-</td>
<td>M3</td>
<td>M1 Val213</td>
<td>A→C</td>
<td>GAA→GAC Glu→Asp</td>
<td>376</td>
<td>V</td>
<td>-</td>
</tr>
<tr>
<td>6.</td>
<td>-do-</td>
<td>Z</td>
<td>M1 (Val213)</td>
<td>G→A</td>
<td>GAG→AAG Glu→Lys</td>
<td>342</td>
<td>V</td>
<td>Intracellular aggregation</td>
</tr>
<tr>
<td>7.</td>
<td>-do-</td>
<td>Mprocida</td>
<td>M1 (Val213)</td>
<td>T→C</td>
<td>CTG→CCG Leu→Pro</td>
<td>41</td>
<td>II</td>
<td>Intracellular degradation</td>
</tr>
<tr>
<td>8.</td>
<td>-do-</td>
<td>Mmalton</td>
<td>M2</td>
<td>Phe51/Deletion</td>
<td>TTC TTC</td>
<td>52 or 51</td>
<td>II</td>
<td>Intracellular aggregation</td>
</tr>
<tr>
<td>10.</td>
<td>-do-</td>
<td>Granitefalls</td>
<td>M1 (Val213)</td>
<td>C</td>
<td>TAC→GTG</td>
<td>160</td>
<td>II</td>
<td>-do-</td>
</tr>
<tr>
<td>11.</td>
<td>-do-</td>
<td>Hongkong</td>
<td>M2</td>
<td>TC</td>
<td>Leu318 5’Shift Delete →Stop (TAA)</td>
<td>334</td>
<td>IV</td>
<td>Intracellular aggregation of α1-A1 protein</td>
</tr>
<tr>
<td>12.</td>
<td>-do-</td>
<td>Isola-di Procida</td>
<td>-</td>
<td>17kb del</td>
<td>-</td>
<td>-</td>
<td>II-V</td>
<td>No stable αat mRNA</td>
</tr>
</tbody>
</table>
recombination, origin of PiZ was proposed to be approximately 2000 years old. With frequency higher in the northern Europe it has been accepted that the PiZ gene had first arose in the northern Europe and subsequently spread to other European countries. It has an allelic frequency of 1-2% in Caucasians of northern European descent (Jeppsson, 1976; Crystal, 1989).

Individuals carrying the PiS gene exhibited reduced α1-AT plasma levels (60% for PiSS, and 80% for PiMS (Gitlin and Gitlin, 1975; Owen and Carrell, 1976). Molecular characterization of the PiS allele revealed an A to T transversion resulting in a Glu to Val substitution at residue 264, exon III (Owen and Carrell, 1976; Long et al., 1984).

It is hypothesized that the origin of PiS allele occurred around 10,000 to 15,000 years ago making the PiS mutation much older than the PiZ allele. The PiS allele has an allelic frequency of 2-4% in Caucasians of northern European descent and varies from 10% in the southern Europe to 5% in the north, a distribution gradient with opposite direction to Z allele frequency. PiS frequency is highest in the Iberian Peninsula (Crystal, 1990; Blanco et al., 2001).

Null phenotype is defined as the total absence of immunologically cross-reactive α1-AT in serum (Martin, 1975). The two parental α1-AT genes are not expressed, such that they produce no or insufficient α1-AT to be detected in the serum (Sifers et al., 1989; Frasier et al., 1989).

It is estimated that among the Caucasians, null α1-AT alleles have a haplotypic frequency of approximately 0.001 (Talamo et al., 1973). When
inherited with certain deficient haplotypes such as Z or with other null haplotypes, the affected individuals are at high risk for the development of emphysema (Blundall et al., 1974). The molecular mechanisms responsible for absence of serum α1-AT include splicing abnormalities, deletion of aat coding exons and premature stop codons. Some of the null variants are Null Granitefalls, Null Bellingham, Null Hong kong (Crystal, 1990).

Dysfunctional variants are present in normal levels but do not function normally (Owen et al., 1983). In the P1-P10 loop of Pi Pittsburg, methionine is replaced by arginine residue. This results in the inhibition of the highly effective coagulation proteases-thrombin instead of neutrophil elastase. The consequence is life threatening hemorrhagic disease (Lewis et al., 1978).

2.4 Alpha-1-Antitrypsin Deficiency in Emphysema and COPD

The normal function of the respiratory system is to exchange oxygen (O2) and carbon dioxide (CO2) so that O2 is delivered to and CO2 is removed from the blood. CO2 is the major stimulus for the respiratory centre, which is located in the medulla of the brain. When the partial pressure of CO2 (PaCO2) is increased, ventilation is stimulated resulting in increased removal of CO2. This process is supported by the components present in the respiratory tract-a conducting system, respiratory exchange unit and a vascular supply. The conducting system (upper respiratory system) is composed of the nose, pharynx, trachea and bronchi. The respiratory exchange units (lower respiratory tract) include the bronchioles, alveolar ducts, alveolar sacs and alveoli (Netter, 1980; Goldsmith and Weber, 1996; Pierce, 1997).
The normal acinus is supplied by a terminal bronchiole. The terminal bronchiole undergoes three orders of branching, first into respiratory bronchioles with alveolar walls then into alveolar ducts and finally into alveolar sacs. The alveoli (tiny air sacs) remove carbon dioxide from the blood, releasing it into the lung to be breathed out, and also absorb oxygen, transferring it into the blood. This exchange is essential to survival and is the key function of the lungs. Normal airway integrity is maintained through ...relationship of pressure in and around the airways and the elasticity of the airways (Netter, 1980; Goldsmith and Weber, 1996; Pierce, 1997).

During inflammation, activation of airway neutrophils release neutrophil elastase (Lucey et al., 1988). Neutrophil elastase, a 29kDa extra cellular protease is produced by white blood cells to help fight bacteria and clean up dead lung tissue (Travis, 1988). Extra cellular release of neutrophil elastase occurs after cell death, phagocytes or neutrophil activation by a variety of stimulants. Despite its useful role in fighting infection, neutrophil elastase also has the potential to be harmful, damaging healthy lung tissue and when in excess, the protective effects are overcome by the destructive effects (Beith, 1985; Senior et al., 1989).

The elastases also stimulate macrophages to release the chemoattractant leukotriene B4 (LTB4). This further recruits neutrophils to the site of inflammation. In the lung, elastases and proteases from polymorphonuclear leucocytes (PMN) are capable of digesting parenchymal tissues including basement membranes, capillary endothelium and alveolar
walls. The consequences result in the connective tissue destruction especially the elastin breakdown (Beatty et al., 1980; Campbell, 2000).

Alveoli have fragile, thin walls, which are easily damaged. The bronchioles distribute the air throughout the lung to the individual alveoli. The respiratory tissues are normally protected from neutrophil elastase specific antiprotease, particularly by α1-AT (Wewers et al., 1987; Crystal et al., 1989). The homozygous (PiZZ) and compound heterozygous (PiZS, PiMZ and PiMS) carriers of aat alleles and null alleles lead to severe deficiency of serum α1-AT (Gadek and Crystal, 1982; Faber et al., 1990). This results in an imbalance between proteinases and inhibitors within the lung (WHO, 1997; Sandford et al., 1999; Hogg and Senior, 2002).

In alpha-1-antitrypsin deficiency, the alveoli and bronchial tubes are destroyed due to excessive proteolytic action of the uninhibited neutrophil elastase (Fig. 5, Page No. 26) (Gadek and Crystal, 1982; Buist et al., 1995). The oxygen-carbon dioxide transfer becomes much less efficient and the stale air is trapped in the isolated sacs. More air is required to provide the same amount of oxygen to the blood via the parts of the lung that are still functioning (Netter, 1980; Goldsmith and Weber, 1996; Pierce, 1997). This need for more air eventually leads to lung over-inflation. As the lung over expands, it gradually enlarges, completely filling the chest cavity and causing a sense of shortness of breath (Sandford et al., 1999; Hogg and Senior, 2002).

In addition to obstructive pulmonary disease, liver disorders are also found. Liver disease in association with alpha-1-antitrypsin deficiency was
Fig. 5. Diagrammatic representation of lung destruction in alpha-1-antitrypsin deficiency (Stockley, 2000)  
LTB4 – Leukotriene B4
first recognized by Sharp et al (1969) and has a less common prevalence than lung diseases. It is associated with PiZ homozygotes, PiM Malton, Pi Siiyama and the compound heterozygotes PiSZ, PiZ-aat variants (Curiel et al., 1989; Lomas et al., 1992; Seyama et al., 1995). Two major distinct clinical entities have been identified. They are neonatal liver disease and adult-onset liver disease (Berg and Eriksson, 1972; Perlmutter, 1991).

Homozygous PiZZ alpha-1-antitrypsin deficiency, which has an incident of 1 in 1,600 to 1 in 2,000 live births, is the most common genetic cause of liver disease in children (Povey, 1990; Perlmutter, 1993). The Swedish neonatal screening program reported that about 70% of PiZZ neonates have abnormal liver function tests and about 10% develop clinically significant cholestasis. Approximately 2.5% of individuals with α1-AT deficiency die of cirrhosis by age of 18 years (Massi, 1996; Rosen, 1998).

The suggestive mechanism in the development of liver abnormalities in these variants is that the base change from the normal sequence reduces the rate at which the α1-AT peptide folds to form the tertiary structure. This slow folding allows the peptide monomers to come together by a loop sheet insertion mechanism to form polymer, which is retained within the ER (Cox et al., 1986; Curiel et al., 1989; Huber and Carrell, 1989; Primhak and Tanner, 2001; Carrell and Lomas, 2001).

Alpha-1-antitrypsin deficiency shows a recessive mode of inheritance in familial conditions. Individuals with α1-AT deficiency have two deficient alleles for the protein. Brothers and sisters of deficient individuals have a 25%
chance of inheriting the condition. Children of deficient individuals can be expected to be heterozygotes for the deficiency. The risk is high only if the partner of the deficient individual is a carrier (Cox, 1989).

Alpha-1-AT deficiency related emphysema patients have the lowest survival rate (Burrows et al., 1987). Estimates of longevity in patients with α1-AT deficiency predicted a life span shortened by 10 to 15 years, compared with the normal population. They also have the highest rate of decline in pulmonary function (Brantly et al., 1988; Boschetto et al., 2003).

Symptomatic obstructive lung disease in α1-AT deficiency usually presents at a mean age between 32 and 41 years in individuals with a history of smoking (Eriksson, 1965; Brantly et al., 1988). Alpha-1-antitrypsin deficiency in smokers is associated with an accelerated development of emphysema and premature mortality (Gadek et al., 1980). Cigarette smoke is thought to cause emphysema by creating a functional protease imbalance in peripheral lung areas (Hunninghake and Crystal, 1983). In α1-AT deficient smokers, more neutrophils were found within airspaces than in emphysematous lungs of individuals with normal α1-AT plasma levels (Janoff, 1983; MacNee et al., 1989). Hutchison et al (1987) found a decline of the overall forced expiratory volume in 1 second (FEV₁) in smokers. This was supported by other studies indicating a significant decline in FEV₁ in smokers when compared with non-smokers (Janus et al., 1985; Pittulainen and Sveger, 2002).
Cigarette smoke also stimulates neutrophils and macrophages to increase the production of proteinase, stimulating them to produce oxidants such as HOCl₂. H₂O₂, a component of cigarette smoke is involved in the inactivation of α₁-AT (Beith, 1985; Luisetti and Travis, 1996). The wild type α₁-AT is also susceptible to oxidative impairment due to conversion of the reactive site methionine (Met358) to its sulfoxide derivative by oxidants contained in cigarette smoke or released from phagocytes resulting in loss of inhibitory activity (Johnson and Travis, 1991; Taggart et al., 2000).

2.5 Intermediate deficiency

The heterozygous PiMS and PiMZ genotypes are present at a frequency of approximately 10% and 3% respectively in the Caucasian populations. SZ compound heterozygotes are rare but have levels even lower than approximately 40% of normal level of α₁-AT (Alvarez Granda et al., 1997).

Lieberman (1969) suggested that heterozygosity for the deficient alleles might predispose them to lung disease. A significant loss of elastic recoil and lung function abnormalities were observed in studies comparing PiMZ smokers and PiMZ non-smokers (Larson, 1978; Tattersal et al., 1979, Eriksson et al., 1986). In a random population study consisting of 143 PiMZ heterozygous subjects, Bruce et al (1984) demonstrated that PiMZ heterozygotes do not have a clearly increased risk of lung damage.

Case control studies in Caucasians show an increased prevalence of PiMZ heterozygosity in COPD patients (Lieberman et al., 1986; Janus, 1988).
Bell et al (1990) observed a high prevalence of heterozygous PiMZ alleles in adults with chronic liver diseases when compared to normal healthy donors. An elevation of liver enzymes was observed in PiMZ and PiMS infants indicating hepatic dysfunction (Pitschieler, 1991).

In a 10-year follow-up study of 28 PiMZ subjects, a reduced maximal expiratory flow and mechanical properties of the lungs were observed in PiMZ individuals (Tarjan et al., 1994). The decrease in elasticity, which is the primary pathophysiological damage in alpha 1-antitrypsin deficiency, demonstrated that between COPD patients the PiMZ heterozygotes have a more rapid decline in lung function. Alvarez Granda et al (1997) observed an increased risk for smokers who are SZ heterozygous for the Pi locus. A cohort study on Danish population of 1551 PiMZ individuals with a follow up for an average of 7.5 year reported that subjects heterozygous for α1-AT deficiency were at increased risk of hospital admission for COPD only if they were 1st degree relatives of the PiZ genotype index cases (Seersholm et al., 2000).

In the Copenhagen study on Danish population, Dhal et al (2001) observed a greater rate of decrease of FEV₁ in PiMZ heterozygotes and suggested that in a given population at large, PiMZ heterozygosity may account for a fraction of COPD cases. Lomas and Silverman (2001) suggested that either all individuals with PiMZ are at slightly increased risk for the development of COPD or that a subset of the PiMZ subjects are at substantially increased risk of pulmonary damage if they smoke. Hence screening for the heterozygous deficient alleles in populations is essential and
will help to reduce the incidence of this disease by implementation of appropriate preventive programme.

However whether subjects heterozygous for α1-AT deficiency are "at risk" for development of COPD is currently under debate with case control studies indicating an increased frequency of PiMZ heterozygotes in COPD patients and population studies showing less frequency.

2.6 Population Genetics of α1-AT Deficiency

In a Swedish population, about 5% of the people were reported to be carriers of the deficiency variant PiMZ (Eriksson, 1965). The prevalence of α1-AT deficiency in neonates estimated for a period of 2 years in the Spanish population showed that out of 200,000 children, 127 had PiZZ genotype giving a frequency of 1 in 1,600 neonates (Sveger, 1976).

Other studies on Caucasian populations gave a frequency of 1 in 2,857 (Silverman et al., 1989), 1 in 5,097 (Wall et al., 1990) and 1 in 3, 694 (Colp et al., 1993). Crystal (1990) reported that α1-AT deficiency occurs at a frequency of 1 in 2000-7000 Caucasians but only rarely in African or Asian populations. The geographical distribution of PiZ allele worldwide is given in Fig. 6 (Page No.33).

In a recent analysis on the worldwide racial and ethnic distribution of α1-AT deficiency, new data were presented demonstrating that α1-AT deficiency is also found in populations of African blacks, Arabs and Jews in the middle east, central, far east and southeast Asians, whites in Australia,
Europe, New Zealand and north America (de Serres, 2002; de Serres et al., 2005). The frequency distribution of \textit{aat} carriers and deficiency allele combinations in different geographic regions of the world is given in the Table 2 (Page No. 34).

2.7 Diagnosis

Identification of the \textit{aat} phenotype or genotype provides important information relevant to the relative risk for emphysema and/or liver disease and plays an important role in the laboratory diagnosis of \(\alpha_1\)-AT deficiency. The accurate diagnosis of \(\alpha_1\)-AT deficiency is critical for proper evaluation, treatment and the genetic counseling of the individuals and their family members. The main advantage of early diagnosis is that patients may be persuaded to stop smoking. Hence diagnosis will enable the individuals to institute lifestyle changes that may slow down the progress of deterioration.

Alpha-1-antitrypsin deficiency is diagnosed from measurements of plasma or serum concentration of antitrypsin. This is done by radial immunodiffusion on commercially available agarose plates that contain specific antibody. Other widely used techniques include immuno electrophoresis, turbidometric assays and enzyme-linked immunoassays (Pierce, 1997).

The laboratory diagnosis of \(\alpha_1\)-AT deficiency is most frequently made by using isoelectric focusing of \(\alpha_1\)-AT in serum (Pi typing) but is very tedious and prone to errors (Jeppsson and Franzen, 1982). Recent molecular methods such as restriction fragment length polymorphism (RFLP), allele specific
Fig. 6. Geographical distribution of \textit{aat} PiZ allele worldwide (Blanco \textit{et al.}, 2001)
Table 2  Summary of the estimates of the numbers of carriers and deficiency allele combinations in different geographic regions of the world (de Serres, 2002)

<table>
<thead>
<tr>
<th>Geographic Region</th>
<th>Cohorts</th>
<th>Mean Gene Frequencies</th>
<th>Total Population</th>
<th>Normal PiMM</th>
<th>Population at Risk</th>
<th>Carriers</th>
<th>Deficiency Allele Combinations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
<td>Size</td>
<td>PiM</td>
<td>PiS</td>
<td>PiZ</td>
<td>No.</td>
<td>Size</td>
</tr>
<tr>
<td>Africa</td>
<td>23</td>
<td>3,887</td>
<td>0.9444</td>
<td>0.0310</td>
<td>0.0048</td>
<td>263,933,984</td>
<td>218,489,235</td>
</tr>
<tr>
<td>Australia and New Zealand</td>
<td>15</td>
<td>8,243</td>
<td>0.9410</td>
<td>0.0395</td>
<td>0.0151</td>
<td>22,830,939</td>
<td>20,116,672</td>
</tr>
<tr>
<td>Central Asia</td>
<td>48</td>
<td>6,151</td>
<td>0.9872</td>
<td>0.0043</td>
<td>0.0040</td>
<td>1,258,908,811</td>
<td>1,226,938,018</td>
</tr>
<tr>
<td>Central Europe</td>
<td>58</td>
<td>31,122</td>
<td>0.9708</td>
<td>0.0192</td>
<td>0.0074</td>
<td>320,961,495</td>
<td>302,491,194</td>
</tr>
<tr>
<td>Far East Asia</td>
<td>24</td>
<td>8,685</td>
<td>0.9937</td>
<td>0.0007</td>
<td>0.0004</td>
<td>1,435,853,427</td>
<td>1,414,534,485</td>
</tr>
<tr>
<td>Middle East and North Africa</td>
<td>18</td>
<td>3,859</td>
<td>0.9738</td>
<td>0.0133</td>
<td>0.0056</td>
<td>42,446,942</td>
<td>38,832,049</td>
</tr>
<tr>
<td>North America</td>
<td>43</td>
<td>33,147</td>
<td>0.9529</td>
<td>0.0328</td>
<td>0.0092</td>
<td>313,855,364</td>
<td>262,662,463</td>
</tr>
<tr>
<td>Northern Europe</td>
<td>38</td>
<td>21,005</td>
<td>0.9577</td>
<td>0.0176</td>
<td>0.0153</td>
<td>31,653,753</td>
<td>29,054,741</td>
</tr>
<tr>
<td>Southeast Asia</td>
<td>20</td>
<td>4,547</td>
<td>0.9732</td>
<td>0.0159</td>
<td>0.0036</td>
<td>473,595,032</td>
<td>458,747,460</td>
</tr>
<tr>
<td>Southern Europe</td>
<td>77</td>
<td>33,769</td>
<td>0.9272</td>
<td>0.0564</td>
<td>0.0125</td>
<td>177,610,448</td>
<td>150,836,300</td>
</tr>
<tr>
<td>Western Europe</td>
<td>9</td>
<td>6,941</td>
<td>0.9370</td>
<td>0.0451</td>
<td>0.0128</td>
<td>63,308,721</td>
<td>55,613,995</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>373</td>
<td>161,356</td>
<td>0.9727</td>
<td>0.0295</td>
<td>0.0107</td>
<td>4,404,958,925</td>
<td>4,178,316,620</td>
</tr>
</tbody>
</table>
prevalence of α1-AT deficiency have demonstrated that α1-AT deficiency will, in the near future, become one of the most common serious hereditary disorders in the world (Abound et al., 2001; Lusetti et al., 2002). Thus studies to identify α1-AT deficient individuals in all populations should be undertaken as unidentified individuals lose opportunities for important lifestyle changes and preventive measures.

2.8 Other Diseases in Alpha-1-Antitrypsin Deficiency

Panniculitis is the inflammation of the fat layer immediately under the skin characterized by inflammatory and necrotizing lesions of the skin. The reported frequency by WHO was 1 in 1000 (WHO, 1997; Bazex et al., 2002).

A high incidence of α1-AT deficiency has been reported in patients with antineutrophil cytoplasmic autoantibody (ANCA) systemic vasculitis in association with antibodies against proteinase 3 (PR3) (Callea et al., 1997; Gagho et al., 2000). Schneuwly et al. (1996) suggested that heterozygous and homozygous α1-AT deficiency is the genetic risk factor for the development of intracranial aneurysms. However Audran et al. (2001) could not observe any association of α1-AT deficiency in the development of ANCA positive vasculitides and suggested that it may have a secondary effect.

2.9 Current Therapy

Intravenous administration of a pasteurized pooled human plasma α1-AT product (Prolastin, Bayer Corporation, Clayton, North Carolina) is used to increase α1-AT levels in deficient individuals (Wewers et al., 1987;
Sandhaus, 1993; Stoller et al., 2003; Nita et al., 2005). In individuals with moderate airflow obstruction, augmentation therapy has found to confer better benefits than in those with severe airflow obstruction (Blank and Brantly, 1994, ATS-FRS, 2003; Stoller, 2003).

Transplantation has been found successful in severe cases of liver or lung diseases. In advanced liver diseases, liver transplantation is the therapeutic option for some patients. Hood et al. (1980) observed restoration of alpha-1-antitrypsin levels to the normal range after orthotopic liver transplantation in seven white patients who had end-stage liver disease due to alpha-1-antitrypsin deficiency.

In a randomized controlled trial in patients with severe emphysema, lung reduction surgery was found to improve FEV₁, walking distance and quality of life (Geddes et al., 2000, Fischer et al., 2002; Kayler et al., 2002). Lutte et al. (2004) observed a significant improvement in dyspnea and lung function in patients with advanced emphysema undergoing lung volume reduction surgery.

New therapeutic approaches involve restoring endogenous capacity for α1-AI synthesis and secretion through gene therapy. Garver et al. (1987) used a retroviral vector to insert human aat cDNA into the mouse fibroblasts and observed human α1-AI in the sera and epithelial surface of the lungs. Duan et al. (2004) used SV40 derived vectors to deliver aat Z ribozyme to transgenic mice carrying the mutant human aat allele. Treated transgenic mice showed marked decreases of human aat mRNA and the protein in the liver and serum.
Pulmonary rehabilitation is defined as a multidimensional service directed to persons with pulmonary diseases and their families usually by an interdisciplinary team of specialists with the goal of achieving and maintaining the individual’s maximum levels of independence and functioning in the community (Celli, 1995; Mahler, 1998; ATS/ERS, 2003).

Other measures include nonspecific or supportive measures for the clinical manifestations of liver or lung diseases. They include bronchodilator medications such as beta agonist salmeterol and formoterol. This is the mainstay of current drug therapy for COPD and causes a small increase in FEV₁ in COPD patients. These drugs may improve symptoms by reducing hyper inflammation and thus dyspnoea. They also improve the spirometric measurements. Additional treatment includes antibiotics, oxygen therapy and systemic glucocorticosteroid (Goldsmith and Weber, 1996).

2.10 Other Candidate Genes Involved in COPD

Next to **α**1-antichymotrypsin (α₁-ACTI), microsomal epoxide hydrolase, vitamin D binding protein, glutathione S transferse, IL-11, cytochrome P450 1A1, immunoglobulin A (IgA), tumor necrosis factor (TNF) α and haemoxxygenase have been implicated with the development of COPD. The genes implicated in the pathogenesis of COPD have been found to be involved in anti-proteolysis, metabolism of toxic substances in cigarettes smokers, the inflammatory response to cigarette smoke and the efficiency of mucociliary clearance in the
lung (Beatty et al., 1984; Poller et al., 1992; Huang et al., 1997; Yim et al., 2000; Yamada et al., 2000; Ishii et al., 2000; Hoidal, 2001).

World wide, COPD is the only leading cause of death that still has a rising mortality. It has been estimated that by year 2020, COPD will be fifth among the conditions that will be the most burden to society. It is therefore, essential that strategies be implemented on a global scale to assess the prevalence of COPD, and to study the causes and outcomes of the disease and how best the burden of COPD might be mitigated. Understanding the pathogenesis of and developing novel tools for the early diagnosis for COPD represent enormous challenges.