

**EXACERBATIONS, HEALTH STATUS AND
SIBLING PAIR COMPARISONS IN SEVERE
ALPHA-1-ANTITRYPSIN DEFICIENCY**

by

MICHELLE NEEDHAM

A thesis submitted to
The University of Birmingham
for the degree of
DOCTOR OF MEDICINE

School of Medicine
The University of Birmingham
September 2007

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

ABSTRACT

Alpha-1-Antitrypsin Deficiency (AATD) is a risk factor for the development of early-onset emphysema and airflow obstruction. The current work defines exacerbations, lung function and health status in these patients including sibling pairs.

Exacerbations occur commonly in AATD and are associated with worse health status. Exacerbations were associated with a decline in the gas transfer of the lung over time, but show no relationship to changes in forced expiratory volume in one second (FEV₁). However, despite lung function decline, patients do not show a progressive loss in health status.

Index patients have worse lung function and health status and more emphysema than non-index siblings. These differences are not solely explained by smoking or ascertainment. Although FEV₁ values differ between sibling pairs, gas transfer does show significant correlation. Thus disease phenotype may also be influenced by other genetic modifiers.

These results provide a firm basis upon which to design, power and implement trials of interventions that may reduce exacerbations and improve health status in patients. Furthermore sibling pairs, particularly those with discordant disease or concordant parenchymal disease, are an ideal group to further investigate the contribution of other genes in the development of COPD or its phenotype in AATD patients.

DEDICATION

**This work is dedicated to the memory of my daughter Holly
and to my son Daniel**

ACKNOWLEDGEMENTS

I gratefully acknowledge the help and support of all of the team members of the ADAPT project: Becky, Anita and Carol for their organisation, appointments and administration; all of the members of the Lung Investigation Unit for pulmonary function testing; Diane, Helen and other nurse team members for their help with data collection; Darren and Ali for laboratory and computer support; the statistics department of the Queen Elizabeth Hospital for advice; Martin for help with the bubble plot; David and Andrew for discussion; Professor Stockley for his continuous support, direction and education; and finally the patients with alpha-1-antitrypsin deficiency who give up their time to help themselves and others through the project. The ADAPT project is funded by Bayer Pharmaceuticals.

TABLE OF CONTENTS

1	INTRODUCTION	1
1.1	Chronic Obstructive Pulmonary Disease	1
1.1.1	Definition of COPD	1
1.1.2	Diagnosis and Assessment of COPD	2
	<i>1.1.2.1 Symptoms</i>	2
	<i>1.1.2.2 Clinical examination</i>	2
	<i>1.1.2.3 Lung Function</i>	3
	<i>1.1.2.4 Radiology</i>	4
	<i>1.1.2.5 Inflammatory Response</i>	5
1.1.3	Pathology of COPD	5
	<i>1.1.3.1 Bronchial Pathology</i>	5
	<i>1.1.3.2 Small Airways Disease</i>	6
	<i>1.1.3.3 Alveolar Pathology</i>	6
1.1.4	Pathogenesis of COPD	7
	<i>1.1.4.1 Smoking</i>	7
	<i>1.1.4.2 Inflammation</i>	8
	<i>1.1.4.3 Protease-Antiprotease Imbalance</i>	8
1.1.5	Genetic Risk Factors	9
1.2	Alpha-1-Antitrypsin Deficiency	11
1.2.1	Alpha-1-Antitrypsin	11
1.2.2	Genetics of AATD	12
1.2.3	Incidence of AATD	13
1.2.4	Identification of Patients	13
1.2.5	Lung Disease	14
	<i>1.2.5.1 Molecular Basis of Lung Disease in AATD</i>	14
	<i>1.2.5.2 Emphysema</i>	16
	<i>1.2.5.3 Airways Disease and Bronchodilator Reversibility</i> ...	16
	<i>1.2.5.4 Chronic Bronchitis</i>	18
	<i>1.2.5.5 Bronchiectasis</i>	18
1.2.6	Factors Influencing Expression and Progression of Disease ...	19
	<i>1.2.6.1 Smoking</i>	19
	<i>1.2.6.2 Exacerbations</i>	20
	<i>1.2.6.3 Environmental Factors</i>	21
	<i>1.2.6.4 Bronchodilator Reversibility</i>	21
	<i>1.2.6.5 Age and Gender</i>	22
	<i>1.2.6.6 Base Lung Function</i>	22
	<i>1.2.6.7 Genetic Modifiers</i>	22
	<i>1.2.6.8 Alpha-1 Antitrypsin Replacement Therapy</i>	24
1.3	Exacerbations	25
1.3.1	Definition of Exacerbations	26
1.3.2	Exacerbations and Usual COPD	26
1.3.3	Aetiology of Exacerbations	27

1.3.3.1	<i>Bacteria</i>	28
1.3.3.2	<i>Viruses</i>	28
1.3.3.3	<i>Pollutants</i>	29
1.3.4	Factors Affecting the Frequency and Severity of Exacerbations	29
1.3.5	Effect of Exacerbations on Lung Function	31
1.3.6	Exacerbations and Inflammation	32
1.3.7	Exacerbations and AATD	33
1.4	Health Status	34
1.4.1	Health Status Measurements of Disease	35
1.4.2	Health Status and COPD	36
1.4.3	Health Status and COPD Disease Parameters	37
1.4.3.1	<i>Pulmonary Function</i>	37
1.4.3.2	<i>Computed Tomography</i>	38
1.4.3.3	<i>Exercise Capacity</i>	38
1.4.4	Changes in Health Status	39
1.4.5	Health Status and Exacerbations	40
1.4.6	Health Status and Therapeutic Interventions	40
1.4.6.1	<i>Pharmacological Interventions</i>	40
1.4.6.2	<i>Non-pharmacological Interventions</i>	41
1.5	Clinical Trials in Alpha-1-Antitrypsin Deficiency	42
2	AIMS	45
3	GENERAL METHODS	46
3.1	Patient Selection and Diagnosis	46
3.1.1	Referral and Consent	46
3.1.2	Diagnosis	46
3.1.3	Demographic Data Collection	47
3.2	Lung Function Testing	47
3.2.1	Spirometry and Reversibility Testing	47
3.2.2	Lung Volume Measurements	48
3.2.3	Gas Transfer Measurements	48
3.3	Health Status Measurements	49
3.3.1	St George's Respiratory Questionnaire	49
3.3.2	Short-Form 36	50
3.4	Computed Tomography	50
3.5	Statistics	51
3.6	Author Contribution	51

4	EXACERBATIONS AND HEALTH STATUS	53
4.1	Introduction	53
4.2	Methods	53
4.2.1	Subjects	53
4.2.2	Exacerbation Data	54
4.2.3	Lung Function Testing	54
4.2.4	Health Status	55
4.3	Results	55
4.3.1	Subjects Studied for 1 Year	55
4.3.1.1	<i>Baseline Characteristics</i>	55
4.3.1.2	<i>Exacerbations</i>	58
4.3.1.3	<i>Exacerbations and Baseline Characteristics</i>	60
4.3.1.4	<i>Lung Function</i>	61
4.3.1.5	<i>Health Status</i>	65
4.3.2	Subjects Studied for 3 Years	71
4.3.2.1	<i>Lung Function Progression</i>	71
4.3.2.2	<i>Health Status Change</i>	74
4.3.2.3	<i>Exacerbations over 3 Years</i>	76
4.3.2.4	<i>Exacerbations, Changes in Lung Function and Changes in Health Status</i>	77
4.3.2.5	<i>Linear Regression of Changes in Lung Function</i>	79
4.4	Discussion	79
5	SIBLINGS	87
5.1	Introduction	87
5.2	Methods	88
5.2.1	Subjects	88
5.2.2	Lung Function Testing	88
5.2.3	Health Status	89
5.2.4	CT Scanning	89
5.2.5	Smoking Correction Factor	89
5.3	Results	90
5.3.1	Patient Characteristics	90
5.3.2	Lung Function Data	91
5.3.2.1	<i>Index-Non-Index Pairs</i>	91
5.3.2.2	<i>Non-Index-Non-Index Pairs</i>	96
5.3.2.3	<i>Correction for Smoking</i>	97
5.3.3	Health Status Data	99

5.4	Discussion	99
6	DISCORDANCE IN LUNG FUNCTION IN PATIENTS WITH AATD	103
6.1	Introduction	103
6.2	Methods	103
	6.2.1 Lung Function Testing	104
	6.2.2 Health Status	104
5.3	Results	104
	6.3.1 Patient Characteristics	104
	6.3.2 Lung Function Data	106
	6.3.3 Health Status Data	108
	6.3.4 Predictors of Discordant Disease	108
6.4	Discussion	109
7	GENERAL DISCUSSION	112
8	REFERENCES	120
9	APPENDICES	137

LIST OF FIGURES

Figure		Page
1	The proportion (%) of exacerbations occurring in each month of the year.	59
2	The length of the exacerbations of symptoms.	60
3	The number of exacerbations over the first 12 months in patients grouped according to their GOLD stage.	64
4	The SGRQ total score at month 12 in patients grouped by exacerbation frequency.	68
5	The SF36 physical score at month 12 in patients grouped by exacerbation frequency.	69
6	The SGRQ Total score at month 12 in patients grouped according to their GOLD stage.	70
7	A frequency histogram of annual absolute change in FEV ₁ .	73
8	A frequency histogram of annual absolute change in K _{CO} .	74
9	SGRQ Symptom score at four annual time points in 76 patients.	76
10	The annual change in SGRQ symptom score in patients grouped by exacerbation frequency.	78
11	The numbers of index and non-index siblings as categorised by GOLD stage.	93
12	A scatter plot showing the FEV ₁ of the sibling pairs expressed as % predicted.	94
13	A scatter plot showing the K _{CO} of the sibling pairs expressed as % predicted.	95
14	A scatter plot showing the RV/TLC ratio of the sibling pairs.	96
15	A scatter plot showing the FEV ₁ of the non-index sibling pairs expressed as % predicted.	97
16	A scatter plot showing the residual FEV ₁ value of the sibling pairs.	98
17	A scatter plot showing the FEV ₁ and K _{CO} of the study group expressed as percent predicted.	107

LIST OF TABLES

Table		Page
1	Effect of pharmacological therapies on health status in COPD.	41
2	Baseline characteristics for the whole patient group, patients with and without exacerbations over the first year and for the subgroup followed for 3 years.	57
3	Lung function values at baseline for the whole patient group and patients with and without exacerbations over the first year.	62
4	Lung function values at year 1 for the whole patient group and patients with and without exacerbations over the first year.	63
5	Health status scores at baseline for the whole patient group and patients with and without exacerbations over the first year.	66
6	Health status scores at year 1 for the whole patient group and patients with and without exacerbations over the first year.	67
7	The lung function data at baseline and the end of year 3 are shown together with the change per year and the number of patients tested at the two time points.	72
8	The health status scores at baseline and the end of year 3 are shown together with the change per year and the number of patients tested at the two time points.	75
9	Patient characteristics of index and non-index siblings.	90
10	Lung function data of index and non-index siblings with paired statistical comparison of the 2 groups.	92
11	Health Status scores of index and non-index siblings with paired statistical comparison of the 2 groups.	99
12	Characteristics and FEV ₁ of discordant and concordant patients.	105
13	Health Status scores of discordant and concordant groups with statistical comparison.	108

LIST OF ABBREVIATIONS

AAT	Alpha-1-Antitrypsin
AATD	Alpha-1-Antitrypsin Deficiency
ADAPT	Antitrypsin Deficiency and Assessment Programme for Treatment
BMI	Body Mass Index
COPD	Chronic Obstructive Pulmonary Disease
CRQ	Chronic Respiratory Questionnaire
CT	Computed Tomography
FEV₁	Forced Expiratory Volume in 1 second
FVC	Forced Vital Capacity
GOLD	Global Initiative for Chronic Obstructive lung Disease
Ig E	Immunoglobulin E
IL	Interleukin
IQR	Interquartile Range
K_{CO}	Transfer factor for carbon monoxide per unit of effective alveolar volume
LTB₄	Leukotriene B ₄
MRC	Medical Research Council
NE	Neutrophil Elastase
NHLBI	National Heart Lung and Blood Institute
NS	Not Significant
PEFR	Peak Expiratory Flow Rate
PiZ	Z phenotype for alpha-1-antitrypsin
RV	Residual Volume
SE	Standard Error

SF36	Short Form 36
SGRQ	St George's Respiratory Questionnaire
SLPI	Secretary Leukoproteinase Inhibitor
TLC	Total Lung Capacity
TL_{CO}	Transfer factor for carbon monoxide
TNF-α	Tumour Necrosis Factor- α
UK	United Kingdom
USA	United States of America
VC	Vital Capacity

1 INTRODUCTION

1.1 Chronic Obstructive Pulmonary Disease

Chronic Obstructive Pulmonary Disease (COPD) is a disease of the lungs and is a major cause of morbidity worldwide. Prevalence studies have shown that between 4 and 10% of the population of Europe and North America have COPD (Halbert et al, 2003). COPD is also a major cause of mortality and led to 25, 765 deaths in 2003 in the United Kingdom alone (Office for National Statistics, 2004). Furthermore, the impact of this condition is increasing due to the aging population and the increased use of tobacco. Over the next two decades COPD is predicted to move from the twelfth to the fifth greatest worldwide medical burden (Lopez et al, 1998).

1.1.1 Definition of COPD

The terminology for this condition has varied in the past: chronic airflow limitation, chronic obstructive lung disease, chronic bronchitis and emphysema. Chronic Obstructive Pulmonary Disease is now the preferred description although chronic bronchitis and emphysema are still applicable to aspects of this condition. COPD is defined by “airflow limitation that is not fully reversible”. The current consensus definition also states that the airflow limitation in this disease is “usually both progressive and associated with an abnormal inflammatory response of the lungs to noxious particles or gases” (Pauwels et al, 2001).

1.1.2 Diagnosis and Assessment of COPD

1.1.2.1 Symptoms

Patients with COPD present to health services with a variety of symptoms including breathlessness, cough, sputum production and wheeze. In addition, the systemic effects of the disease may lead to fatigue, reduced exercise tolerance, weight loss and depression. These symptoms may vary between individuals and may change within an individual over time. Symptoms of chronic cough and sputum production may precede the development of airflow obstruction although not all individuals with these symptoms will develop COPD.

1.1.2.2 Clinical Examination

The physical signs that may be detected in patients with COPD are not specific to the condition and are often not sensitive for detecting or excluding the disease. Airflow obstruction may lead to prolonged forced expiration time and pursed lip breathing may be observed in patients in an attempt to reduce airway collapse in expiration. In advanced disease there may be evidence of cyanosis, weight loss and the use of accessory muscles of respiration. Overinflation may lead to increased thoracic kyphosis and a barrel-shaped chest, together with reduced distance between the cricoid cartilage and the suprasternal notch. Overinflation may also be demonstrated by reduced cardiac dullness to percussion and soft heart sounds. Auscultation may reveal reduced breath sounds with prolonged expiratory phase and wheeze may be present. Crackles are often detected particularly at the lung bases.

Furthermore, consequences of severe disease may be detected on examination including signs of pulmonary hypertension, hypercapnia, cachexia and depression.

1.1.2.3 Lung Function

Airflow limitation is detected by objective measurement using spirometry and this limitation largely remains when bronchodilator agents are given. The measurements made during spirometry are the forced expiratory volume in 1 second (FEV_1), the forced vital capacity (FVC) and the ratio of these 2 measures (FEV_1/FVC). Airflow obstruction is defined as an FEV_1/FVC ratio of less than 70% with or without a reduction in FEV_1 (Pauwels et al, 2001; Fabbri et al, 2003). COPD is not the sole cause of airflow obstruction and other conditions, such as asthma, bronchiectasis and bronchial carcinoma, need to be excluded. In early disease, conventional spirometry may be normal but measures of small airway function, such as flow rates at low lung volumes, may be abnormal. However, in routine clinical practice, conventional spirometry is usually abnormal by the time the patient presents to health care services with symptoms. Additional measurements of lung function may also show abnormalities in COPD patients. Patients may have an increase in the Total Lung Capacity (TLC), which is the volume of gas in the lungs and airways at full inspiration, due to overinflation. There may also be trapping of air within the lungs as demonstrated by an increase in Residual Volume (RV), which is the volume of gas remaining in the lungs at full expiration. Static lung volumes may be measured using steady state helium dilution (Meneely et al, 1949) or by whole body plethysmography (Dubois et al, 1956). The helium dilution method assumes that the lungs are well ventilated which may not be the case in patients with severe obstructive defects or bullous emphysema. Thus in some cases helium dilution may underestimate TLC.

The ability of the lungs to transfer oxygen from air into the circulation may be reduced in COPD patients. This can be quantified by the measurement of the rate of gas transfer

across the alveolar membrane (TL_{CO}) and can be adjusted for alveolar volume to acquire a value of gas transfer per unit of lung (K_{CO}) (Blakemore et al, 1957). Furthermore, measurement of arterial blood gases may indicate hypoxaemia.

Disease staging of COPD patients is currently based on the severity of airflow limitation as detected by FEV_1 . However this staging has limitations and it does not account for differences in symptoms, functional ability or pathological changes within individuals. Thus patients within the same disease stage may vary greatly and comprehensive assessment of COPD patients should include measures of these other parameters.

1.1.2.4 Radiology

Plain chest radiography does not show any specific abnormalities in COPD patients and indeed may be normal. Abnormalities that may be seen include low and flattened diaphragms and an increase in the retrosternal airspace due to hyperinflation. There may also be a reduction in the size and number of pulmonary vessels and focal translucency may be seen in areas of bullous formation. Areas of increased lung markings may also be seen in regions of atelectatic or scarred lung.

Computed tomography (CT) is a more accurate method for radiological assessment of the lungs and is more sensitive than plain radiography in detecting emphysema (Sanders et al, 1988). Macroscopic emphysema is visualised on CT scans as areas of low attenuation with thinning of the corresponding vascular tree and has been shown to correlate with pathology scores (Kuwano et al, 1990). Furthermore, methods of objective quantification of emphysema on CT scans have been developed (Gevenois et al, 1995; Thurlbeck et al, 1994). Scans may be analysed to assess the number of pixels with a density lower than a predetermined threshold to obtain a measurement which may be used to compare patients or monitor groups of patients over time. However,

many factors may influence the results including scanner performance, the age of the patient and the ventilatory volume at the time of acquisition of the scan. Thus the usefulness of these measurements outside clinical trials may be limited. Validation studies of these methods of quantification of emphysema on CT scans continue.

1.1.2.5 Inflammatory Response

The “abnormal inflammatory response” that occurs in COPD which is described in the consensus definition has been well demonstrated in the research setting. However this is more difficult to measure in regular clinical practice and so individuals are often diagnosed with COPD based upon their history, clinical findings and pulmonary function tests.

1.1.3 Pathology of COPD

The underlying cause of the airflow limitation in COPD is complex. Pathological changes within large and small airways as well as destruction of the alveolar airspaces may all contribute to airflow limitation. The relative contribution of pathological changes within different parts of the lungs may vary between individuals with COPD and this may contribute to different phenotypic expression of disease.

The complex pathological changes in COPD can be simplified by considering the three affected compartments separately, namely the bronchi, peripheral airways and alveolar airspaces.

1.1.3.1 Bronchial Pathology

Healthy bronchi contain submucosal glands and goblet cells which decrease in number and size down to the peripheral bronchi and are absent in the bronchioles. Some patients

with COPD have chronic bronchitis which is defined clinically by an increase in sputum production. The bronchi of these patients show an increase in the number and size of submucosal glands particularly in the larger bronchi. The number and distribution of goblet cells also change in smokers but these changes are not consistent between individuals. Inflammation and scarring is also seen in bronchi of COPD patients and this contributes to airway thickening and luminal narrowing.

1.1.3.2 Small Airways Disease

Many pathological changes may be seen in small airways disease ranging from inflammatory infiltration to airway wall thickening and fibrosis. Mucus production and inflammatory cell infiltrate may lead to occlusion of the lumen which may further contribute to airflow obstruction.

1.1.3.3 Alveolar Pathology

Normal acinar structure consists of respiratory bronchioles, alveolar ducts, terminal alveolar sacs and alveoli. The alveolar walls are thin and contain types I and II pneumocytes, basement membranes, interstitium and endothelial cells. It is here that the gas transfer of oxygen and carbon dioxide occurs.

Emphysema may be a component of COPD and is defined structurally as “abnormal, permanent enlargement of the airspaces, distal to the terminal bronchioles, accompanied by destruction of their walls and without obvious fibrosis” (National Heart Lung and Blood Institute, 1985). Emphysema may be subdivided into 3 types based on anatomical distribution within the secondary lobule: centrilobular emphysema is clustered around the terminal bronchiole; panlobular emphysema is distributed throughout the acinar unit; and paraseptal emphysema occurs along the edge of the acinar unit. Centrilobular

and panlobular emphysema are more common and each of these subtypes is often associated with a characteristic distribution within the lungs. Centrilobular emphysema often affects the upper zones of lobes whilst panlobular emphysema is seen more in the lung bases. Alveolar attachments to small airways play an important role in maintaining airway patency and loss of these attachments in emphysematous lung allows surrounding airways to collapse, particularly in expiration, contributing to airway occlusion.

1.1.4 Pathogenesis of COPD

The risk of developing COPD is affected by a combination of genetic and environmental factors. Cigarette smoking is the most important environmental risk in this condition, particularly in the Western world. Other environmental risks include occupational mineral dust exposure (Oxman et al, 1993), biological dust exposure (Matheson et al, 2005), indoor pollution from fuel and outdoor air pollution.

In addition, it is likely that several genetic susceptibility factors influence the effect of smoke and other environmental agents on an individual. This gene/environment interaction may determine not only the development of COPD but also the rate of progression of the disease.

1.1.4.1 Smoking

Smoking may lead to symptoms of cough, sputum and wheeze and these symptoms may improve after smoking cessation (Tashkin et al, 1984). Moreover, there is also a clear dose-dependent relationship between the amount of cigarettes smoked and the risk of irreversible airflow obstruction (Burrows et al, 1977). However this relationship is not

absolute and only a minority of smokers develop COPD (Fletcher et al, 1977). In addition, a minority of patients with COPD have never smoked although they may have been exposed to other environmental agents such as coal.

Cigarette smoke contains many harmful compounds, including oxygen free radicals, polycyclic aromatic hydrocarbons, nitrosamines, nitrogen oxides and other oxidants. These compounds not only have direct cytotoxic effects but also lead to an inflammatory response within the lungs.

1.1.4.2 Inflammation

Sputum induction, bronchial lavage and bronchial biopsy studies have shown an increase in the number of T lymphocytes, macrophages, eosinophils and neutrophils in COPD patients. However the key causative inflammatory cell in the pathogenesis of COPD is probably the neutrophil. Neutrophils migrate to sites in response to upregulation of cell-surface adhesion molecules (such as e-selectin and ICAM-1) on endothelial and epithelial cells. The principal mediators of this inflammation are Leukotriene B₄ (LTB₄), Interleukin 8 (IL-8) and Tumour Necrosis Factor- α (TNF- α).

1.1.4.3 Protease-Antiprotease Imbalance

One theory on the development of emphysema in smokers is that an imbalance occurs between proteases released from neutrophils and antiproteases in the interstitium. Neutrophils are activated and migrate through endothelial cells and the interstitium into airspaces in response to cigarette smoke and chemoattractants. Proteases, including elastase, proteinase 3, cathepsin G and matrix metalloproteinases, are released from neutrophils during migration and these bind to elastin causing tissue destruction. Antiproteases, such as alpha-1-antitrypsin (AAT) and secretory leukoproteinase

inhibitor (SLPI), are found in the interstitium and may inactivate released elastase and limit the area of damage. However an insufficient quantity of antiprotease or an excess amount of protease will lead to an imbalance in this system and significant tissue destruction will ensue.

This hypothesis is supported by the observation that intrapulmonary instillation of elastolytic enzymes produces emphysema in animal models (Gross et al, 1965; Senior et al, 1977). Moreover, humans with reduced levels of antiprotease, such as that seen in alpha-1-antitrypsin deficiency, have an increased risk of emphysema particularly if their lungs are exposed to pollutants such as cigarette smoke.

1.1.5 Genetic Risk Factors

Airflow obstruction is not seen in every individual who smokes and there appears to be a sub-population that is susceptible to accelerated decline in lung function. The hypothesis of genetic susceptibility factors has been supported by the observation that early-onset COPD may be seen in more than one subject within a family. Severe early-onset COPD subjects may have a high carrier rate for COPD susceptibility genes as may be seen in early onset cases of other complex diseases such as breast cancer. One study of early onset COPD is the Boston Early-Onset COPD Study which investigated 44 severe COPD patients and 204 of their first degree relatives with comparison to 83 control subjects (Silverman et al, 1998). The relatives of these early-onset COPD probands showed significantly lower FEV₁ values, despite similar age and pack year smoking history, with a relative risk of three for reduced FEV₁ and also for chronic bronchitis. However, this increased risk seems to be limited to current or ex-smokers, as there was no increased risk for reduced FEV₁ seen in relatives who were lifelong non-

smokers. This suggests that these genetic risk factors for COPD are expressed in response to cigarette smoking. Interestingly, this risk may be influenced by gender as female smoking first-degree relatives in one study were significantly more likely to demonstrate profound reductions in FEV₁ than male smoking first-degree relatives (Silverman et al, 2000).

Identification of genes that may influence the risk of developing COPD has proved difficult. Association studies have shown inconsistent results which might be explained by genetic heterogeneity of the study populations. Conversely, case-control association studies may find associations purely due to stratification of the populations. Linkage studies which identify areas of the genome which are likely to contain susceptibility genes may prove more helpful. One such linkage study has demonstrated evidence for an early-onset COPD-susceptibility locus on chromosomes 2 and 12 and further suggestive evidence for linkage of spirometry-related phenotypes to several other genomic regions (Silverman et al, 2002b; Silverman et al, 2002a). Palmer et al again found significant linkage to airflow obstruction susceptibility loci on chromosomes 2q as well as 8p (Palmer et al, 2003). Linkage of spirometric measurements to a locus on chromosome 2 has also been replicated in the general population (Malhotra et al, 2003). Taken together these studies suggest that the genetic risk factors for COPD are likely to be multiple and many remain ill-defined. However, one important genetic risk factor for the development of COPD that has long been well established is severe alpha-1-antitrypsin deficiency.

1.2 Alpha-1-Antitrypsin Deficiency

Alpha-1-antitrypsin deficiency (AATD) is a risk factor for the development of respiratory symptoms, early-onset emphysema and airflow obstruction in adult life and was first described by Eriksson and Laurell in 1963 (Eriksson, 1963; Laurell et al, 1963). AATD may also lead to the development of acute or chronic liver disease in childhood or adulthood and has been linked to other systemic diseases such as panniculitis and fibromyalgia.

1.2.1 Alpha-1-Antitrypsin

Alpha-1-Antitrypsin (AAT) is a 394 amino acid, 52 kDa protein which is encoded on chromosome 14q31-32.1 (Billingsley et al, 1993) and is a member of the serpin (*serine proteinase inhibitor*) family. The main biological role for AAT is to inactivate neutrophil elastase (NE) (Beatty et al, 1980) through irreversible binding via the reactive site. AAT is mainly synthesised in hepatocytes (Koj et al, 1978), although mononuclear phagocytes, intestinal and bronchial epithelial cells may synthesise small amounts (Cichy et al, 1997; Mornex et al, 1986; Perlmutter et al, 1989). AAT is an acute phase protein and the levels in the serum and airways may increase in an individual during times of systemic inflammation or stress. However the average serum concentrations are determined within an individual by the genetic alleles carried. Some genes lead to reduced levels of AAT and these subjects are said to have alpha-1-antitrypsin deficiency. The serum concentration of AAT in severely deficient patients is usually less than 11 μM although the actual concentration may change slightly over time as part of an acute phase response.

1.2.2 Genetics of AATD

The genetic locus for alpha-1-antitrypsin is highly polymorphic and over 100 alleles and their consequent protein variants have been described. The most common variants in white populations are the M alleles which are associated with normal serum AAT levels. The S allele is associated with slightly reduced AAT levels and the Z allele, which has a single base pair substitution (Jeppsson, 1976), leads to markedly reduced serum AAT. Null alleles are carried by a small percentage of individuals and lead to no detectable protein due to deletion of coding regions or defective gene translation. Testing for AAT is frequently performed using serum isoelectric focusing which detects bands of proteins separated by their differing charge. A protein strip which shows only a Z band may represent protein translated from 2 Z alleles or one Z and one null allele, and so, as isoelectric focusing cannot distinguish between these two possibilities, these individuals are referred to as phenotype PiZ. If required, actual genotyping can be elucidated using allele specific hybridisation.

The altered protein structure of Z AAT protein, caused by the point mutation at the base of the reactive loop of the molecule, leads to polymerisation of the Z AAT. These polymers accumulate in the endoplasmic reticulum of hepatocytes. Furthermore this polymerisation impairs secretion of AAT from the liver resulting in reduced serum levels of around 10% of the amount produced from a normal M allele.

AATD is inherited in an autosomal co-dominant manner and offspring of 2 heterozygote parents have a 1 in 4 chance of being homozygotes and consequently having severe deficiency.

1.2.3 Incidence of AATD

The incidence of AATD varies between studied populations and the highest frequency of the Z gene is seen in Scandinavia (Hutchison, 1998) which may represent a common mutational origin for the vast majority of Z alleles. Low Z allele frequencies are seen in populations of Asian and African descent. Many studies on incidence are limited by ascertainment bias as asymptomatic individuals may not present to health services and therefore remain undetected. Population screening is not widely performed although one large comprehensive study has been performed in Sweden. In this study 200,000 babies born over a 2 year period were screened and 122 children were identified as PiZ phenotype, giving a prevalence of 1 in 1640 (Sveger, 1976).

Studies on the prevalence of PiZ individuals amongst patients with a diagnosis of COPD show variable results between 1 to 10% (Fagerhol et al, 1969; Lieberman, 1969; Lieberman et al, 1986). These figures are affected not only by the incidence within the population but also by variations in screening practice.

1.2.4 Identification of Patients

Patients may be diagnosed with alpha-1-antitrypsin deficiency after presentation with symptoms or through family screening of an index case. The proportion of patients identified by screening is influenced by local and national practice and thus studies of groups of patients may show some differences. In the UK registry of AATD, 76% of PiZ patients have been identified through investigation of respiratory symptoms or disease and 19% through family screening. Similar proportions are reported in the National Heart Lung and Blood Institute (NHLBI) registry (McElvaney et al, 1997). A small number of subjects are identified through abnormal radiological, pulmonary or

blood tests or through the development of liver disease. In the UK registry, only 3% of patients have been identified as a result of liver disease, although it is possible that greater numbers of such patients are identified and treated elsewhere.

There can often be a delay in the diagnosis of AATD and one study has shown a duration of seven years between mean age of onset of symptoms and the mean age of subsequent diagnosis (Stoller et al, 1994). Patients often present in the third or fourth decade with symptoms of breathlessness and other common symptoms include cough, phlegm, wheeze (with and without infections) and fatigue (McElvaney et al, 1997; Eden et al, 2003).

A few studies have followed deficient individuals after detection through neonatal screening and these are more representative of the population of individuals with this condition. However, the largest cohort has only been studied for 25 years so far (Piitulainen et al, 2002), providing little data on the natural history of the condition through adult life.

1.2.5 Lung Disease

1.2.5.1 Molecular Basis of Lung Disease in AATD

Alpha-1-antitrypsin diffuses from the serum through the pulmonary endothelium into the lung interstitium where it protects against tissue destruction by proteinases. Neutrophils migrate from the circulation into the airways and release elastase from their azurophil granules. The concentration of elastase is around 5mmol which is three times the concentration of normal alpha-1-antitrypsin (Liou et al, 1995). Thus, even with normal AAT levels there has to be a region of uninhibited enzyme activity close to the

site of enzyme release. In conditions of reduced AAT levels, such as that seen in deficient patients, both the radius and duration of catalytic activity is increased which leads to a larger area of connective tissue destruction by migrating neutrophils. Furthermore uninhibited elastase may stimulate macrophages to produce LTB₄ (Hubbard et al, 1991). This chemoattractant leads to increased migration of neutrophils and thus amplifies the inflammatory process. This mechanism is supported by the observation that AAT deficient individuals have significantly greater neutrophil counts in bronchoalveolar lavage samples (Morrison et al, 1987) and in tissue samples. Moreover sputum from patients with AATD has higher concentrations of LTB₄ and IL8 and greater chemotactic activity than controls (Woolhouse et al, 2002) which leads to greater neutrophil traffic.

Not only is there less AAT in AATD but the Z protein may also be a less effective inhibitor of neutrophil elastase than the normal M protein (Lomas et al, 1993; Ogushi et al, 1987). Furthermore this “functional deficiency” may potentially be worsened by reduction in AAT activity through oxidation in patients who smoke (Gadek et al, 1979; Janoff et al, 1979).

More recently it has been suggested that polymerisation of Z protein within the lungs of deficient patients may also contribute to the pathogenesis of this condition (Elliott et al, 1998). Loop-sheet polymers of AAT are inactive and consequently their genesis within the lung may further reduce the level of functional protease inhibitor that is available to protect the lungs from damage. Furthermore polymeric alpha-1-antitrypsin has chemotactic activity for neutrophils in vitro (Parmar et al, 2002) and may amplify the inflammatory response and subsequent damage.

Thus the mechanisms behind the development of COPD in alpha-1-antitrypsin deficiency appear to be complex and the relative contribution of smoking, infections, cytokines and AAT polymerisation remains undefined.

1.2.5.2 Emphysema

The close association of AATD and the development of emphysema was first described in 1963 (Eriksson, 1963). The disease in these patients is usually of an earlier onset compared to patients with usual Chronic Obstructive Pulmonary Disease (COPD) and often appears to be out of proportion to their smoking history. The typical pattern shows panlobular emphysema with lower zone predominance although the emphysema may affect all zones. Plain chest x-rays may show evidence of hyperinflation with reduced lung markings and bulla formation may also be associated with emphysematous lung (Guest et al, 1992). Lung function tests on symptomatic patients often show evidence of increased lung volumes and air trapping as well as impaired gas transfer. However, this picture is not universal and some patients with severe airways obstruction and prominent emphysema on CT scan may maintain normal gas transfer (Wilson et al, 2000).

Furthermore the pattern and distribution of emphysema may influence the lung function impairment in these patients. Parr et al have shown that basal emphysema is associated with greater impairment of FEV₁ but less impairment of gas exchange and alveolar-arterial oxygen gradient than the apical emphysema in this disease (Parr et al, 2004).

1.2.5.3 Airways Disease and Bronchodilator Reversibility

Airflow obstruction, as demonstrated by a reduced FEV₁ and reduced FEV₁/FVC ratio, may be seen in deficient subjects. The amount of airflow limitation measured may fall

anywhere in the range between none to severe obstruction and it is often out of proportion to the smoking history of the patient.

Airflow limitation in these patients is not always fixed and the symptoms and signs in AATD can be similar to features of asthma. Individuals can often be given a diagnosis of asthma in childhood or early adulthood and 15% of patients in a Swedish cohort identified by neonatal screening had been diagnosed as having asthma by the age of 22 (Piitulainen et al, 2002). In the same cohort, 29% of the patient group reported recurrent wheezing. In the larger NHLBI registry, 21% of the total group and 12.5% of those with normal FEV₁ had asthma as defined by reversible airflow obstruction, recurrent wheezing, and a reported diagnosis of asthma or allergy with or without elevated IgE levels (Eden et al, 2003). Studies on airway hyperresponsiveness in these patients are limited and group together patients of different phenotypes but they suggest that airway hyperresponsiveness is not more prevalent in these patients compared to control subjects (Malerba et al, 2003).

There is a wide variation in bronchodilator response in these patients. In the NHLBI registry, 28% of patients during the first visit showed reversibility of $\geq 12\%$ and $>200\text{ml}$ in FEV₁ and this was seen in 49% over all visits in the study (Eden et al, 2003). In these patients, the median increase in FEV₁ was 330ml with a range of 202ml to 1,492ml, which explains why many patients who present at a young age are given an initial diagnosis of asthma. Indeed, bronchodilator responses are also seen in patients without a reduced FEV₁ below 80% predicted and it may be that this is more common in the early stages of the disease.

Although airflow obstruction and parenchymal destruction can often be seen together in the same patient, this is not always the case. Some patients have marked emphysema

with very little airways disease and the reasons for this relative preservation of airway function are unclear. By contrast, some patients have severe airflow obstruction with little parenchymal disease and show preserved gas transfer. These patients with discordant lung function measurements have been poorly studied and it is not known whether they represent a discrete phenotypic expression of this disease or have simply been assessed at an earlier stage of the disease process.

1.2.5.4 Chronic Bronchitis

Chronic bronchitis is defined clinically by the presence of cough productive of sputum for at least 3 months in 2 successive years if other causes of chronic cough have been excluded (Medical Research Council, 1965). Patient registries of alpha-1-antitrypsin deficient subjects show that as many as 43% of patients have chronic sputum expectoration, as defined by Medical Research Council (MRC) criteria, even in non-smokers. The patients with chronic bronchitis tend to have more severe airflow obstruction and more extensive emphysema than those without despite similarities in age and smoking history (Dowson et al, 2002).

1.2.5.5 Bronchiectasis

Bronchiectasis is a condition of the lungs characterised by persistent abnormal dilatation of the bronchi. The presence of bronchiectasis in patients with AATD is well recognised and in series using CT scanning for detection, the incidence varies from 41% to 43% (Guest et al, 1992; King et al, 1996; Shin et al, 1993). However, in the largest study the incidence of bronchiectasis was 26% (Dowson et al, 2002) which is similar to that seen in usual COPD (O'Brien et al, 2000), suggesting that the incidence is not increased. In support of this, Cuvelier et al compared a group of patients with bronchiectasis, mainly

diagnosed by CT scan, and a group of control subjects and found similar AAT phenotypes and gene frequencies in the 2 groups (Cuvelier et al, 2000). A greater frequency of PiZ alleles was seen in those patients with bronchiectasis who also had emphysema but the number of patients with the PiZ phenotype was too small to provide firm conclusions. Although the bronchiectasis seen may be severe and associated with chronic sputum production, sputum expectoration itself is not a sensitive nor specific feature for detecting the presence of bronchiectasis and CT scanning remains the most reliable tool (Dowson et al, 2002).

1.2.6 Factors Influencing Expression and Progression of Disease

The degree of lung function impairment can vary greatly amongst patients with the same phenotype for alpha-1-antitrypsin (Tobin et al, 1983). Furthermore, case reports have shown that even siblings with the same phenotype show different disease expression (Apprill et al, 1990; Stableforth, 1978). Some environmental factors have been shown to affect the development and progression of disease in these patients but it is likely that other host factors, including other genetic modifiers, are also important.

1.2.6.1 Smoking

The most important risk factor for the development of emphysema and airflow obstruction in AATD is active smoking. Piitulainen et al have demonstrated an accelerated decline in FEV₁ over twelve months in current smokers (70ml/year, CI 58-82) compared to ex-smokers (41ml/year, CI 36-48) and never-smokers (47ml/year, CI 41-53) (Piitulainen et al, 1999). Even higher rates of decline in smokers were seen in other studies (Janus et al, 1985; Seersholm et al, 1995). Furthermore, there appears to be

a dose-response relationship between the cigarette consumption and change in FEV₁ over time. Active smoking can affect lung function as early as the age of 18, with a significantly lower FEV₁ and FEV₁/VC in those who are smoking compared to non-smokers (Piitulainen et al, 1998a). However, active smoking does not explain all of the variability and patients who have never smoked still show variation in their clinical course (Black et al, 1978).

Passive smoking with an exposure of greater than 10 years has been associated with chronic bronchitis in non-smoking individuals (Piitulainen et al, 1998b) but there has been no evidence to support an association between passive smoking in adulthood and lung function decline. However, parental smoking has been associated with some changes in lung function in adolescents with this condition (Piitulainen et al, 1998a) and it may be that passive smoking in childhood reduces the potential maximum lung capacity in early adulthood.

1.2.6.2 Exacerbations

Lower respiratory tract infections may also affect the clinical course of the disease (Silverman et al, 1989) and prior infections are associated with symptoms of cough and wheeze (Mayer et al, 2000). The effect of exacerbations may be more apparent in patients with mild to moderate disease and an increasing number of exacerbations has been shown to correlate with the decline in gas transfer (Dowson et al, 2001a). It is therefore likely that interventions that reduce the frequency of exacerbations may also reduce this decline. Indeed augmentation therapy, which may moderate lung function decline in some patients (Group, 1998), may also be associated with a reduction in the frequency and severity of exacerbations (Lieberman, 2000). It has been shown that infusions of AAT reduce airway leukotriene B₄ concentrations (Stockley et al, 2002)

and this neutrophil chemotactic factor is thought to be central to the exacerbation episodes. Although this provides a mechanism for this association, further prospective clinical trials are still required to support this assumption and to explore whether all or only a subset of patients would benefit from this therapy.

1.2.6.3 Environmental Factors

Domiciliary kerosene heater usage and an agricultural occupation for a duration of at least 10 years have been shown to be associated with increased symptoms and decreased lung function in non-smoking PiZ patients in the Swedish registry (Piitulainen et al, 1998b). Self reported occupational exposure to gas, fumes or dust was found to be an independent risk factor for lung function impairment in older patients who had never smoked (Piitulainen et al, 1997). Mineral dust exposure, as detected by self-reported questionnaires, has also been shown to be independently associated with chronic cough and with airflow limitation after adjusting for age and smoking in a group of American patients with more severe disease (Mayer et al, 2000). Nevertheless, it is still not clear if this is due to high total inhalational exposure to many agents in these patients or due to a specific effect of mineral dust. Taken together, these studies suggest that environmental exposures may be associated with the development of respiratory symptoms in these patients and may also be a contributory factor to lung function decline. Further studies are needed to isolate the effects of individual agents and also to identify groups of individuals that may be more susceptible to these effects.

1.2.6.4 Bronchodilator Reversibility

Bronchodilator reversibility has been shown to be associated with more rapid FEV₁ decline and is an independent predictor of decline after accounting for age, gender and

smoking status (Eden et al, 2003; The Alpha-1-Antitrypsin Deficiency Registry Study Group, 1998; Dowson et al, 2001a) . Other features of asthma, such as attacks of wheeze and raised IgE, have not been associated with greater lung function decline in these studies.

1.2.6.5 Age and Gender

Advancing age, male gender and previous symptoms of wheezing were found to be independent predictors for lung function impairment in 225 Pi Z patients who had never smoked (Piitulainen et al, 1997) but the relationship between age and the rate of decline is less well understood due to other confounding factors. Eden et al found that those patients aged between 30 and 44 years had the most rapid decline and age remained an independent predictor of decline after adjustment for other confounders (Eden et al, 2003). It is unclear whether this reflects the natural history of the disease or represents a cohort effect.

1.2.6.6 Base Lung Function

In a study on PiZ patients from the UK registry, there was a correlation between the initial FEV₁ and its subsequent decline with the more severely affected patients showing the least change (Dowson et al, 2001a). Although this accelerated decline in less severely affected patients may illustrate a change in the disease course over time, it may be that survivorship bias “selects” some severe patients who are declining more slowly.

1.2.6.7 Genetic Modifiers

There has been little evidence to support genetic modifiers in families with more than one homozygote member although studies of heterozygote relatives do suggest familial aggregation. The “St Louis AAT Study” has investigated a series of 52 AAT deficient

subjects and 118 of their relatives using pulmonary function and a respiratory epidemiology questionnaire (Silverman et al, 1989). The investigators found that variability in pulmonary function was partially related to age and pack years of smoking. However the study also showed that Pi MZ relatives of PiZ patients with airflow obstruction showed lower FEV₁ values than Pi MZ relatives of unaffected PiZ subjects (Silverman et al, 1990b). Furthermore parents of diseased PiZ individuals were more likely to report a history of emphysema than parents of unaffected PiZ individuals in the same study, which is consistent with COPD aggregation in families. Lung function measurements were only obtained in a subset of these families but the figures acquired showed a suggestive trend to worse lung function in parents of PiZ individuals with airways disease compared to those of individuals with preserved function. Taken together, these studies suggest that familial factors other than Pi type and smoking behaviour may influence the development of COPD in relatives of homozygotes. If other factors do play a role in this regard, they are also likely to modify the development of lung disease in PiZ individuals.

In a separate study, segregation analysis of the residual phenotypes in 44 nuclear families was performed (Silverman et al, 1990a). Statistical evidence was found for an additional major gene, other than the Pi locus, influencing FEV₁ in the study population. The evidence of this gene diminished to below significance after adjusting for the effects of pack-years and the interaction between Pi type and pack years. This would be consistent with a gene that exerted influence by altering susceptibility to smoke.

Another approach to identify modifier genes is to select genes on the basis of known pathogenesis of COPD. Endothelial nitric oxide synthase has been thus studied and two

polymorphisms have been associated with more severe airflow obstruction in AATD which may suggest a modulatory function (Novoradovsky et al, 1999).

Genome wide linkage studies, similar to those performed in usual COPD, in families of PiZ individuals may identify other putative genes in the future. Such studies may be improved by careful selection of a study population containing well characterised families with disparate lung function.

1.2.6.8 Alpha-1-Antitrypsin Replacement Therapy

Alpha-1-antitrypsin replacement therapy may be a logical choice for this condition and is a recognised treatment in some countries although is not currently used in the United Kingdom. Studies have adequately demonstrated that weekly or monthly infusions of AAT are safe and can increase plasma and lung fluid levels of AAT (Hubbard et al, 1988; Wewers et al, 1987). However it is less clear whether this treatment holds any clinical benefit as, to date, any trials have been underpowered or not adequately controlled.

One retrospective internet survey of patients suggested that patients receiving augmentation therapy had less exacerbations than those not receiving such therapy (Lieberman, 2000) but this observation needs to be tested in a prospective controlled trial. Seersholm et al have compared FEV₁ decline in patients from Germany, where augmentation therapy is available, to patients from Denmark where it is not available (Seersholm et al, 1997). The German patients receiving therapy showed less decline in lung function although this effect was limited to patients with moderate airflow obstruction. In addition, a study of a single group of 96 patients before and after augmentation therapy showed a significant reduction in decline in FEV₁ in a subgroup of patients with previous rapid decline, although this subpopulation contained only 7

individuals (Wencker et al, 2001). To date there has only been a single published randomised controlled trial of augmentation therapy with results suggesting that therapy may reduce emphysema progression as assessed by lung densitometry on CT scans (Dirksen et al, 1999). However this study included only 56 patients and the reduction seen did not achieve conventional levels of statistical significance.

With the absence of supportive data for efficacy, alpha-1-antitrypsin replacement is not currently licensed for use in the United Kingdom.

1.3 Exacerbations

Exacerbations of Chronic Obstructive Pulmonary Disease (COPD) are an important feature of the disease and have a substantial effect on the morbidity and mortality from the disease. Exacerbations are heterogeneous in nature and aetiological agents include viruses, bacteria and air pollution. However the precise cause for a particular exacerbation in an individual is frequently not identified. The biology and impact of exacerbations has recently been reviewed (Celli et al, 2007; Hurst et al, 2007).

Studies of exacerbations may be affected by the type of COPD population chosen due to the heterogeneous nature of the disease and other confounding factors. Furthermore the definition of an exacerbation used by investigators is an important consideration as there has not been a consistently used definition in clinical practice or in the research setting.

1.3.1 Definition of Exacerbations

One definition of exacerbations that has been often used is based on criteria defined by the major symptoms of increased dyspnoea, sputum production and sputum purulence (Anthonisen et al, 1987). In addition, exacerbations defined by these criteria may be subdivided into three types: type 1 exacerbations are those with all major symptoms present; type 2 exacerbations occur when two of these symptoms are present; and type 3 exacerbations show only one symptom. Minor exacerbation symptoms may also be present and these include fever, cough, wheeze, sore throat and nasal discharge.

More recently a precise consensus definition has been reached which describes an exacerbation as “*a sustained worsening of the patient's condition, from the stable state and beyond normal day-to-day variations, that is acute in onset and necessitates a change in regular medication in a patient with underlying COPD*” (Rodriguez-Roisin, 2000). It is likely that future studies on exacerbations will incorporate this consensus definition or other definitions. The ongoing INSPIRE study is using two parallel definitions: an event based exacerbation that requires use of healthcare resources and a separate symptom based definition (Seemungal et al, 2007).

1.3.2 Exacerbations and Usual COPD

Exacerbations are a common feature of usual COPD and place a large burden on National Health Service resources and expenditure (McGuire et al, 2001). Moreover, these episodes adversely affect patients and those with more frequent exacerbations have been shown to have worse quality of life (Seemungal et al, 1998) and are more likely to become housebound (Donaldson et al, 2005).

The precise incidence of exacerbations in usual COPD is difficult to quantify due to differences in definitions between patients, clinicians and researchers. Patients may not present to health care services with their exacerbations as they may be accustomed to changes in their symptoms and feel that medical intervention is not required. In the East London COPD Study of moderate-severe usual COPD patients, 49% of the exacerbations went unreported to the research team despite being noted on daily diary cards (Seemungal et al, 1998). The median exacerbation rate in this study, including episodes that were not directly reported, was 3 per patient per year.

Some patients may consult their primary care physician at the time of exacerbations (Stockley et al, 2000) and more severe episodes may require hospitalisation. The usual treatment for exacerbations is empirical and includes increased β_2 -agonists, anticholinergic agents, oral steroids and antibiotics. Although the evidence to support each of these measures is inconsistent, patient recognition of an exacerbation and early intervention is associated with a more rapid recovery and a reduction in the need for hospitalisation (Wilkinson et al, 2004).

1.3.3 Aetiology of Exacerbations

The precise trigger for an individual exacerbation is often not identified. Even in the research setting this may be difficult as up to a third of stable COPD patients are colonised with bacteria at any one time which makes it difficult to be precise about the exact role of each pathogen during an exacerbation (Monso et al, 1995; Monso et al, 1999).

1.3.3.1 Bacteria

Common bacterial agents that may be isolated from sputum samples during exacerbations of COPD include *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Moraxella catarrhalis*, *H parainfluenzae* and *Pseudomonas sp.* The proportion of patients with positive bacterial cultures increases during exacerbations and the bacterial load is usually much greater than that seen in the stable state which supports a bacterial aetiology, at least in a subset of exacerbations. In addition, increased bacterial load is associated with greater levels of inflammatory markers (Hill et al, 2000) and the type of bacterial species may also affect the degree of inflammation. Bacterial eradication has been shown to be associated with resolution of this increased inflammation (White et al, 2003). However, those patients in whom bacteria continue to be cultured only have partial resolution of inflammation which may reflect continued stimulation by the reduced bacterial load.

1.3.3.2 Viruses

Viral agents may cause direct damage to the airways of COPD patients and may promote an inflammatory response. Furthermore viral infection may increase the risk of acquiring a secondary bacterial infection which may perpetuate or worsen an exacerbation. Viral agents that may be associated with exacerbations include Rhinovirus, Influenza, Parainfluenza, Respiratory Syncytial Virus and Adenovirus. Many studies have investigated the presence of viruses in COPD patients during exacerbations and suggest that up to a third of events are associated with viral infections (Greenberg et al, 2000; Seemungal et al, 2000b). However, one must consider that local epidemics, time of year, immunisation policy and disease severity may all confound the results of such studies.

1.3.3.3 Pollutants

It is difficult to prove directly that air pollution is the cause of acute exacerbations and the mechanisms for any causal link are largely unknown. However, epidemiological studies have shown links between increases in pollutants (such as sulphur dioxide (SO₂), nitrogen dioxide (NO₂), ozone and particulate matter) and increased respiratory symptoms, medical intervention and mortality in COPD patients (Anderson et al, 1997; Garcia-Aymerich et al, 2000; Sunyer et al, 2000). It is estimated that up to 9% of hospital admissions due to COPD exacerbations may be caused by pollutants (Sunyer et al, 1993).

1.3.4 Factors affecting the Frequency and Severity of Exacerbations

Disease severity may affect the incidence of exacerbations and patients with more severe disease may have more frequent exacerbations. Furthermore the exacerbations may have a greater impact on patients with more severe disease and there may be more frequent medical care use, hospitalisation or even an increased likelihood of death in these individuals.

Additional host factors may influence the susceptibility to exacerbations as some individuals have frequent episodes whilst others have none. Predictors for frequent exacerbations include symptoms of wheeze, cough and sputum production (Seemungal et al, 1998). One large prospective study of 5887 smokers with COPD showed that those patients with chronic bronchitis had more frequent infections than those without (Kanner et al, 2001). Patients with chronic bronchitis may have decreased mucociliary clearance (Mossberg et al, 1986) and reduced lung defences may facilitate bacterial

adherence, replication and subsequent invasion of the mucosa leading to an increase in exacerbations.

This susceptibility to exacerbations persists as one predictor of frequent exacerbations is a previous history of frequent exacerbations (Ball et al, 1995; Seemungal et al, 1998). A mechanism for this increased susceptibility may be reduced defences including reduced proteinase inhibitor levels. Secretory leukoproteinase inhibitor (SLPI) is an important component of airways defence and has antiviral and antibacterial properties as well as proteinase inhibitor action. Levels of SLPI in sputum are significantly reduced in COPD patients with frequent exacerbations, even in the stable clinical state (Gompertz et al, 2001). Whether reduced SLPI activity predisposes to increased frequency of exacerbations, either directly due to reduced antipathogen function or indirectly via uninhibited epithelial damage due to greater elastase activity, remains unknown. Reductions in other host defence mechanisms such as lysozyme may also play a role (Taylor et al, 1995).

These host factors may be important in explaining some of the genetic susceptibility to COPD. Recently, familial aggregation of exacerbations has been noted (Foreman et al, 2007) although these results may be influenced by other confounding factors such as passive tobacco smoke exposure.

Environmental factors may also affect exacerbation frequency and a fall in indoor or outdoor temperature appears to be a risk factor (Donaldson et al, 1999). Whether this is related to increased viral infections or to other mechanisms is unclear.

1.3.5 Effect of Exacerbations on Lung Function

One prospective study has closely assessed the effect of exacerbations on lung function (Seemungal et al, 2000a). In this study of 101 patients with moderate to severe COPD, 504 exacerbations were recorded over a two and a half year period. Patients recorded daily morning peak expiratory flow rate (PEFR) and changes in respiratory symptoms on diary cards and a subgroup of 34 patients also recorded daily spirometry using a handheld device. Significant falls were seen in PEFR, FEV₁ and FVC during exacerbations. Symptom changes during the episodes did not reflect those of lung function, but an increase in symptoms did predict some exacerbations, with dyspnoea or colds characterising the more severe episodes. The median time to recovery of peak flow was 6 days, although lung function recovery was incomplete in 7.1% of exacerbations at 91 days after the onset. This suggests that some episodes may contribute to deterioration in these parameters over time although further studies are required to support this hypothesis.

Enhanced decline in lung function is well recognised in AATD (Group, 1998; Ewald et al, 1990) and it has been suggested in a small study that exacerbation frequency may have an influence on this decline (Dowson et al, 2001a).

It is therefore possible that interventions that reduce the frequency of exacerbations may also reduce decline in lung function. Inhaled corticosteroids have been shown to reduce the rates of exacerbation and the decline in health status in usual COPD, although they have not been shown to alter the decline in FEV₁ (Burge et al, 2000; Jones et al, 2003). In addition, host factors such as the degree of airway obstruction may also be important as they have been shown to influence the treatment outcome of exacerbations (Dewan et

al, 2000) and thus may also influence the effect of exacerbations on other measures such as lung function.

1.3.6 Exacerbations and Inflammation

COPD is a condition in which, by the nature of its definition, there is an abnormal inflammatory response. During periods of exacerbation this background inflammatory process is increased although the precise mechanism remains unclear. The role and importance of inflammatory mediators during exacerbations appears to be complex and may vary between different clinical phenotypes within this disease.

One study has shown a relationship between interleukin (IL)-8 and IL-6 levels in induced sputum samples during the stable clinical state and exacerbation frequency (Bhowmik et al, 2000). However the patients included in the study were an unselected group with no stratification for co-existing bronchiectasis, smoking status, severity of airflow obstruction and bacterial colonisation. In contrast, Gompertz et al found no significant difference in IL-8 levels when all of these parameters were accounted for (Gompertz et al, 2001). Such differences in characteristics of the study population, as well as differences in the definition of exacerbations and methodology, are seen in many studies on inflammation during COPD exacerbations. This can make comparisons and interpretation difficult.

Histological studies on exacerbations are rare due to the difficulty in acquiring samples from symptomatic patients, particularly those with severe disease. However, two studies of bronchial biopsies during exacerbations have shown evidence of increased inflammation with recruitment of eosinophils and an increase in the numbers of CD4+ lymphocytes (Saetta et al, 1994; Zhu et al, 2001).

Exacerbations also lead to an increase in the numbers of neutrophils in the airways which is associated with the presence of or increase in sputum purulence (Stockley et al, 2001). The colour of the sputum relates to the amount of myeloperoxidase present and has been shown to relate to the concentrations of the underlying markers of bronchial inflammation.

The inflammation that occurs during an exacerbation is not limited to the lung parenchyma. Systemic inflammation is also seen as evidenced by a rise in C reactive protein (CRP) and serum IL-6 levels (Bhowmik et al, 2000; Stockley et al, 2000). Furthermore the time course of recovery of systemic inflammation after exacerbation is different between those who have frequent and infrequent exacerbations which suggests that CRP may be used as a predictor for future episodes (Perera et al, 2007).

1.3.7 Exacerbations and AATD

Exacerbations in patients with usual COPD are well documented but less is known about the occurrence, time course and effect of exacerbations in patients with alpha-1-antitrypsin deficiency (AATD).

However, the episodes are known to be associated with a greater degree of inflammation than in non-deficient patients (Hill et al, 1999a). At the start of an exacerbation, patients with AAT deficiency have lower sputum AAT, lower secretory leukoprotease inhibitor (SLPI) and higher elastase activity compared to COPD patients without deficiency, reflecting their deficient anti-proteinase defence. After treatment with antibiotics, there are significant changes in many sputum proteins including a rise in SLPI levels, and a reduction in myeloperoxidase (MPO) and elastase activity. Furthermore the sputum chemoattractants interleukin-8 (IL-8) and leukotriene B4

(LTB4) fall and protein leak (sputum/serum albumin ratio) reduces. These changes are rapid and provide good support to the advice that exacerbations in AATD should be treated promptly to minimise ongoing inflammation and consequent tissue damage.

Exacerbations have a direct impact on patients with AATD and may also have an influence on the progression of disease over time. Thus it is important to investigate the effect of any therapies for this disease on the frequency and severity of exacerbations as well as other markers of disease severity. In order to include exacerbations as an end point in future trials, it is necessary to assess the occurrence and natural history of these episodes in this population.

1.4 Health Status

COPD is associated with a variety of symptoms which may vary between individuals and may change within an individual over time. The symptoms of COPD are not limited to the respiratory system and may include severe systemic effects. The symptoms of COPD, and their consequences for the individual, may have a tremendous impact on all aspects of a patients' life including work, sports, recreation, home life, and activities of daily living. All of these effects may affect the patient's quality of life.

Maintaining a good quality of life is important for patients and many treatments for the condition aim to improve the patient's symptoms and to reduce the adverse effect that these symptoms have on their well-being. The functional and psychosocial effects of COPD can have as great an impact on the individual as the physiological effects of the disease. Recognition of this has led to the development of a more scientific, standardised and objective way of quantifying how a person's health status (or health-related quality of life) is affected by their illness.

1.4.1 Health Status Measurements of Disease

Health status measurements are taken by questionnaires which ask about the impact of the disease on symptoms, daily activities, physical ability, emotions and psychological well-being. Many questionnaires are available which vary in their length and complexity and different questionnaires emphasise different aspects of the impact of the disease. In order to be useful, health status tools should be validated in groups of patients to confirm repeatability and also to confirm the detection of differences between individuals. An ideal health status measurement should also be sensitive to changes within individuals over time.

Disease-specific questionnaires have been developed with respiratory illness or COPD in mind and can be used for patients when assessing this particular disease. Generic questionnaires assess general health and may have more usefulness when comparing the impact of different illnesses on health.

Health status measurements have become an important outcome measure for clinical trials as they are used to detect improvements in symptoms, physical functioning or other aspects of the patients' quality of life. Disease-specific tools may be more useful in this context and may be more sensitive to the longitudinal change that would be assessed in interventional trials. However many trials use both a disease-specific measure and a generic measure to provide additional information and to aid comparison with other studies. Furthermore, health status measurements can be related to other measures of disease severity such as pulmonary function tests but the correlation between these measures and physiological tests is not strong and this confirms that they are assessing different aspects of the disease. However for a treatment or intervention to

be effective for patients, clinicians and researchers should look for improvements in symptoms and/or quality of life as well as improvements in other disease markers and these tools can be used to quantify any such changes.

An important consideration when using these questionnaires for trial purposes is how to translate statistically important changes in health status into clinically significant changes for patients and their clinicians. Some instruments, such as the Chronic Respiratory Questionnaire (CRQ) (Guyatt et al, 1987a) and St George's Respiratory Questionnaire (SGRQ) (Jones et al, 1992), have a validated threshold figure which equates to a clinically significant change and is useful when analysing the effect of a treatment on a group of patients over time. The usefulness of health status measurements on individual patients over time is less clear. However, clinically important thresholds can be translated back into the type of symptomatic or functional improvement required in an individual to change the score by that amount and these symptomatic or activity changes can then be elucidated through careful history taking.

1.4.2 Health Status and COPD

Physiological, functional, demographic and psychological factors may all affect health related quality of life in COPD. Some of these factors are also interrelated and they all may vary in their influence on health status between individuals and also within an individual over time. Many health status instruments are designed to assess more than one aspect of COPD and so give an assessment of more than one of these factors. However, in general, it has been shown that health status measures correlate better with breathlessness scores than with physiological parameters such as FEV₁. This reinforces that health status measures assess other aspects of COPD within individuals and can

complement the data obtained by physiological tests. Depression, anxiety and emotional dysfunction also have an important influence in COPD patients and will affect health status and its measurement.

COPD patients show reduced health status compared to healthy subjects without COPD. Mahler et al used the generic Medical Outcomes Study questionnaire to assess the general health status of 110 stable but symptomatic male COPD patients who had no other significant comorbidity (Mahler et al, 1992). The same health status tool was used by Stewart et al to assess the health status of 11,186 patients from the general population who had visited a primary care physician or specialist (Stewart A.L et al, 1988). Comparison of the two studies show that the patients with stable COPD showed significantly lower scores, indicating worse health status, than the general patient population particularly for physical and role functioning.

COPD patients have also been shown to have worse health status than patients with other diseases. In one study health status was measured in 50 COPD patients using the generic Short-Form 36 questionnaire and compared to the health status of 50 patients with unresectable non-small cell lung cancer (Gore et al, 2000). Patients with COPD showed worse health status than those with lung cancer and this was statistically significant for all of the SF36 components, apart from role emotional and role physical.

1.4.3 Health Status and COPD Disease Parameters

1.4.3.1 Pulmonary Function

The most widely used physiological measure of COPD severity is the forced expiratory volume in one second (FEV₁). Some studies have shown a correlation between this

measure and health status whilst others have shown no relationship. The demonstrated weak relationship between these measures implies that some individuals with severely impaired spirometry may maintain good health, whilst others with mild airflow obstruction may have a poor quality of life.

1.4.3.2 Computed Tomography

One small study of 22 patients with COPD and severe alpha-1-antitrypsin deficiency (PiZ phenotype) has investigated the relationship between the change in health status and the change in emphysema (Stolk et al, 2003). Health status was assessed in these individuals at baseline and at follow up at 30 months using the St George's Respiratory Questionnaire which scores higher values for worse health status. Patients also had an assessment of the quantity of emphysema at these two time points using lung density measurements on spiral computerised tomography (CT) scans of the chest. The study showed a significant correlation between increasing emphysema, as quantified by lung density, and worsening health status, as measured by increasing SGRQ scores (Spearman correlation coefficient 0.60, $p=0.003$). Neither the changes in health status nor the changes in lung density correlated significantly with changes in forced expiratory volume in one second (FEV_1) or changes in gas transfer (K_{CO}) in this study.

1.4.3.3 Exercise Capacity

Health status has been shown to correlate with exercise capacity in COPD patients with AATD (Dowson et al, 2001b). A strong association was seen between a high SGRQ activity score (worse activity) and a low distance covered in the ISWT (Spearman's correlation coefficient $=-0.62$, $p<0.001$). Other studies have shown a less strong relationship between health status measures and exercise capacity however this

relationship can vary depending on the subjects studied and the methods of assessment used. Alpha-1-antitrypsin deficiency patients often develop disease earlier than usual COPD and therefore have less comorbidity that accompanies aging, which may confound the relationship between exercise capacity and health status.

1.4.4 Changes in Health Status

Health status shows a demonstrable decline in COPD patients over time, although interventions may alter the rate of this decline. Patients in the ISOLDE study were randomised to receive either fluticasone propionate 500 µg twice daily or placebo for 3 years and health status was assessed every 6 months over this time using the St George's Respiratory Questionnaire (SGRQ) (Spencer et al, 2001). Health status declined progressively over the three years in a linear fashion. The placebo treated patients showed a decline in SGRQ Total score of 3.2 units per year (standard error 0.3) which is much greater than the annual decline of 0.12 units per year which seen in healthy people without COPD (Jones, 2001). The total SGRQ score has a clinically important threshold of 4 units and so the placebo treated COPD patients in this study showed a clinically significant decline in health status over 15 months. The fluticasone treated group in the ISOLDE study declined at a rate of 2.0 units per year (standard error 0.3) which was significant slower ($p=0.004$) than the placebo group but still equates to a clinically significant decline in these patients over a 2 year period.

1.4.5 Health Status and Exacerbations

Frequent exacerbations has been shown to relate to worse health status as assessed by the St George's Respiratory Questionnaire (SGRQ) (Seemungal et al, 1998). In this study, patients recorded the number of exacerbations of their COPD over a 12 month period: 32 patients had infrequent exacerbations (less than three) and 38 patients had frequent exacerbations (three or more), using the median number of exacerbations as the cut-off point. Patients who had frequent exacerbations over the previous 12 months showed significantly worse health status compared to patients with infrequent exacerbations ($p \leq 0.003$ for all components). This may suggest that strategies that lead to a reduction in the frequency of COPD exacerbations may improve well-being and quality of life.

1.4.6 Health Status and Therapeutic Interventions

1.4.6.1 Pharmacological Interventions

Many studies that investigate therapeutic interventions now used health status measurement as an additional outcome measure. Health status measures can be used to identify improvements in symptoms or general health in these studies which is illustrated by the examples listed in Table 1.

Table 1

Study	Therapy/Intervention	Effect on health status
(Burge et al, 2000)	<ul style="list-style-type: none">• 500µg twice daily fluticasone	<ul style="list-style-type: none">• SGRQ showed less decline compared to placebo
(Casaburi et al, 2002)	<ul style="list-style-type: none">• 18µg once daily tiotropium	<ul style="list-style-type: none">• SGRQ symptoms score improved (statistically and clinically) compared to placebo
(Guyatt et al, 1987b)	<ul style="list-style-type: none">• Inhaled salbutamol	<ul style="list-style-type: none">• Improvements in dyspnoea and fatigue domains of CRQ
(Jones et al, 1997)	<ul style="list-style-type: none">• 50µg twice daily salmeterol for 16 weeks• 100µg twice daily salmeterol for 16 weeks	<ul style="list-style-type: none">• SGRQ significantly improved (statistically and clinically) compared to placebo• No change in SGRQ compared to placebo
(Rossi et al, 2002)	<ul style="list-style-type: none">• Oral theophylline	<ul style="list-style-type: none">• Improvement in SGRQ Total score compared to baseline

Effect of pharmacological therapies on health status in COPD.

Furthermore the use of these instruments may also provide additional information as illustrated in the study by Jones et al (Jones et al, 1997). In this study, salmeterol use was associated with improvements in health status but these improvements were not seen in the patients who received the higher dose, as side effects from the drug were more prevalent and had an impact on health status.

1.4.6.2 Non-pharmacological Interventions

The effect of non-pharmacological interventions, such as pulmonary rehabilitation, on health status has been investigated widely in COPD patients. Lacasse et al have performed a meta-analysis of six randomised controlled studies of pulmonary rehabilitation and health related quality of life, most using the Chronic Respiratory

Questionnaire (Lacasse et al, 1996). Pulmonary rehabilitation in these studies was defined as exercise training for at least 4 weeks with or without psychological support, education or both and control groups in the studies received no rehabilitation. Significant improvements were seen in the dyspnoea and mastery domains of the CRQ in COPD patients who underwent pulmonary rehabilitation and these improvements are above the smallest difference perceived as important by the average patient.

1.5 Clinical Trials in Alpha-1-Antitrypsin Deficiency

Intravenous replacement of alpha-1-antitrypsin is a recognised treatment for deficient patients in the USA, Canada, Germany, Spain, Italy and Sweden. However clear evidence for the effectiveness of this treatment is absent, as there have been no conclusive controlled studies to demonstrate an influence on lung disease. Marketing and licence approval for the available intravenous therapies has been based on safety studies and biochemical data only. Furthermore intravenous treatment is often inconvenient for patients and alternative routes of drug administration, such as inhalation, have potential. It is hoped that any new product will be tested in a randomised controlled trial.

Another approach for new therapies in this condition is to specifically target the emphysematous lung. Hyaluronic acid has been shown to be protective against elastase-related damage in hamster models (Cantor et al, 1998). Moreover, reversal of pathological changes have been demonstrated in rat models of emphysema after administration of all-trans retinoic acid (Massaro et al, 1997). Such agents have potential as treatments for the whole COPD population. Evaluation of these therapies in

AATD patients has the benefit of a low incidence of co-morbidities as these patients tend to be younger than the usual COPD population.

Modification of native AAT is another strategy to consider. The molecular polymerisation of Z protein within the liver has been well described and interruption of this process by the insertion of peptides may lead to release of protein into the serum and restoration of the anti-elastase protection.

Finally genetic manipulation is another potential treatment option. Virus vectors have been used to insert the normal AAT gene into cells in animal models (Flotte, 2002). This approach in humans would improve serum levels of AAT but would not overcome the problems of polymerisation of abnormal protein. However gene repair technology will advance over the coming years and this may be an option in the future.

Clinical trials of any new treatments will need to use defined outcome measures to assess an objective response. The effects of the treatment on the frequency and severity of exacerbations will be important. Furthermore the quality of life of the patients should be investigated alongside symptoms to assess the clinical impact of the treatment. These measures could be used alongside pulmonary function testing and emphysema quantification by CT scan. In order to use these measures as outcomes for trials, we must have clear evidence of these parameters outside the trial setting. Lung function deterioration and CT scanning has been adequately studied. However the nature and effect of exacerbations in this condition, as well as accurate information on the health status of AATD patients, must be investigated. In addition, any factors that may influence health status over time should be studied. The purposes of the current project were to investigate these features in a large group of AATD subjects. Furthermore

comparisons of lung function and health status were made between sibling pairs with severe deficiency.

2 AIMS

The studies included in this thesis were performed to further define exacerbations, lung function and health status in patients with alpha-1-antitrypsin deficiency including sibling pairs. The aims were as follows:

- 1) To investigate the frequency, duration, timing and nature of exacerbations and to identify host factors that may be associated.
- 2) To investigate the impact of exacerbations on health related quality of life as measured by health status questionnaires.
- 3) To investigate the changes in lung function and health status in patients with severe alpha-1-antitrypsin deficiency over time.
- 4) To identify sibling pairs of patients with severe deficiency and to compare cross-sectional lung function and health status.
- 5) To study patients with discordant lung disease to identify factors that may influence the phenotypic expression of this disease.

3 GENERAL METHODS

3.1 Patient Selection and Diagnosis

3.1.1 Referral and Consent

Patients were recruited from the ADAPT (Antitrypsin Deficiency and Assessment Programme for Treatment) project where the United Kingdom registry for AATD is held. This programme started in May 1996 and is funded by Bayer Corporation, USA. Subjects are referred to the registry by other health care professionals, largely chest physicians, from the whole of the UK. At the initial consultation a full family history is taken and family screening of first degree relatives is discussed.

Assessment occurs annually and comprises collection of demographic data, clinical examination, pulmonary function testing, measurement of health status and computed tomography scanning where appropriate. The programme has been approved by South Birmingham Research Ethics Committee and by University Hospital Birmingham NHS Trust Research and Development Committee. Informed and written consent was obtained from all participating subjects and the consent form is included in Appendix 1.

3.1.2 Diagnosis

Serum alpha-1-antitrypsin levels were measured by immunoassay and all patients included in this work had a level of $<11\mu\text{M}$. All subjects also had PiZ phenotype identified by isoelectric focusing. Serum assays and phenotyping were both performed in a single laboratory in Heredilab, Salt Lake City, Utah, USA.

3.1.3 Demographic Data Collection

At baseline, a full clinical history was taken to include respiratory symptoms, smoking history and the presence of chronic bronchitis (Medical Research Council, 1965). Smoking exposure was quantified in pack years which were calculated as the number of cigarettes smoked per day, divided by 20, and then multiplied by the number of years of smoking. Patients were classified as index cases if their symptoms had led to the diagnosis of AATD and non-index if they were diagnosed through family screening. None of the subjects received alpha-1-antitrypsin replacement therapy.

3.2 Lung Function Testing

All lung function tests were performed at least four weeks after any exacerbation. Predicted values were calculated using the equations from the British Thoracic Society/Association of Respiratory Technicians and Physiologists guidelines for respiratory function (1994). All tests were performed according to standard operating procedure by trained respiratory physiologists within the Lung Investigation Unit, University Hospital Birmingham, Birmingham, UK.

3.2.1 Spirometry and Reversibility Testing

Spirometry was measured using wedge bellows (Vitalograph Ltd, Buckinghamshire, UK) and both forced and relaxed manoeuvres were performed. At the first visit each subject performed spirometry before and after nebulised 5mg β_2 -agonist and 500 μ g ipratropium bromide (Dowson et al, 2001b). Positive bronchodilator reversibility was taken to be an increase in FEV₁ of greater than 200ml and greater than 12% predicted

(Quanjer et al, 1993). At subsequent visits, spirometry was repeated after dual bronchodilatation and changes in lung function were derived from all post-bronchodilator values.

3.2.2 Lung Volume Measurements

Lung volume measurements were assessed using helium dilution as first described in 1949 (Meneely et al, 1949). The Benchmark system was used (Morgan Medical, Kent, UK). After correct positioning each patient was asked to breathe at tidal volumes and the initial concentration of helium was recorded. Following tidal expiration, the patient was connected to the helium circuit and continued to breathe tidally. The concentration of helium was measured every 30 seconds until stable. Finally the patient performed 2 relaxed expiratory residual volume/ inspiratory vital capacity manoeuvres followed by 2 relaxed expiratory vital capacity manoeuvres in sequence.

Static lung volumes including total lung capacity (TLC) and residual volume (RV) were calculated from the measurements taken. All volume measurements were subsequently corrected to Body Temperature and Pressure Saturated with water (BTPS) using the appropriate correction factor.

3.2.3 Gas Transfer Measurements

Gas transfer measurements were obtained by the single breath carbon monoxide method (Blakemore et al, 1957) using the Benchmark system (Morgan Medical, Kent, UK). After a small breath in, patients were requested to expire as far as possible to aim to reach residual volume. After full inspiration (to at least 90% of relaxed vital capacity)

patients were asked to hold their breath, without straining, for 10 seconds. This was followed by full expiration to collect both washout and sample gas.

Transfer factor (TL_{CO}) was calculated in units of mmol/min/kPa to represent the rate of uptake of gas per pressure gradient. The uptake of gas per unit of effective alveolar volume, K_{CO} , was also calculated.

3.3 Health Status Measurements

Two health status questionnaires were used to assess health-related quality of life. Data was recorded at each visit before lung function testing and prior to contact with a physician. No reference to previous answers was made during follow up visits.

3.3.1 St George's Respiratory Questionnaire

The St George's Respiratory Questionnaire (SGRQ) (Jones et al, 1992) was used as a disease specific tool with a high score indicating worse health status and a clinically important change in total score being greater than four points (Jones et al, 1997; Jones, 2002; Jones, 2002). This self-administered questionnaire consists of 50 items and is included in Appendix 2. Each questionnaire response has a unique empirically derived weight with the lowest possible weight as 0 for the best possible patient and the highest as 100 for the worst possible patient. Three component scores are calculated (symptoms, activity and impacts) as well as a total score. Each component score is calculated by dividing the summed weights for that component by the maximum possible weight for that component and is expressed as a percentage. The total score is calculated in a similar manner. Missing items in the symptoms component are treated as

negative answers. For blank answers in the activity and impacts components, the item is coded as missing and the weight for that item is subtracted from the total possible weight for that component and from the total weight.

3.3.2 Short-Form 36

The Short-Form 36 (SF36) was used as a generic health status questionnaire (Brazier et al, 1992). The SF36 is a standardised, self-administered tool that assesses eight components of health. These components can be combined to produce 2 summary scores (Physical and Mental). Missing items are calculated using an algorithm if the respondent answered at least half of the items within that component. Each component score is transformed into a percentage of the total possible score for that component. A low score indicates worse health status and to date no threshold for a clinically significant change has been validated. The SF36 is included in Appendix 3.

3.4 Computed Tomography

Computed Tomography (CT) scans of the chest were acquired using a General Electric Prospeed scanner (General Electric Medical Systems, Milwaukee, Wisconsin, USA). A high resolution protocol was employed and images were obtained at 10mm intervals with the patient lying supine and at full inspiration. All scans were reported by a single radiologist with a specialist interest in lung disease. The presence of emphysema was documented according to published guidelines (Naidich, 1991).

3.5 Statistics

Data were analysed using SPSS version 11. Parametric data are displayed as mean with standard error (SE) and non-parametric data as median with interquartile range (IQR). Repeated measures at two time points were compared using the Wilcoxon signed rank test. Subgroup comparisons were made using the Mann-Whitney U test for two groups and Jonckheere-Terpstra test for three and four groups. Pearson's coefficients were used for correlations between continuous variables and frequency comparisons were made using the χ^2 test. Binary logistic regression was used to test for independent predictors of exacerbations and stepwise linear regression to identify independent factors associated with changes in lung function parameters and health status. Statistical significance was accepted as $p < 0.05$.

Changes in lung function tests over three years were calculated using linear regression on the four measurements taken over that time and then converted into a change per year. Similar calculations were performed to obtain values for health status changes.

Linear regression was performed on a larger set of 408 PiZ patients to calculate the effect of smoking on FEV₁. From the resultant equation, a predicted value was calculated for each sibling based on the smoking history of the individual. The actual FEV₁ value measured was then compared to the predicted value to calculate a residual value for each patient: a low value indicating a worse FEV₁ than predicted.

3.6 Author Contribution

The current work was made possible through the work of all of the team members of the ADAPT project. Patient recruitment and clinical data collection was performed by me and other physicians working on the project. Lung function testing was performed by

the trained technicians from the Lung Investigation Unit. Nurse team members performed the health status questionnaires before any physician contact but I performed the subsequent health status calculations. I collated the clinical, exacerbation, pulmonary function and health status data to create new databases for the analyses in this thesis. All calculations and statistical analysis was performed solely by me, as was the writing of this thesis.

4 EXACERBATIONS AND HEALTH STATUS

4.1 Introduction

Although exacerbations in patients with usual COPD have been reasonably well documented, less is known about exacerbations in alpha-1-antitrypsin deficiency and the effect of exacerbations on health status and disease progression. In order to design and power relevant clinical trials in AATD, more detailed information concerning the number, nature, timing and consistency of exacerbations is required. It is also important to identify any host factors that may be associated with these episodes.

Health status questionnaires are frequently used to assess the quality of life of patients with Chronic Obstructive Pulmonary Disease (COPD). COPD is associated with reduced health related quality of life, which is related, in part, to FEV₁. However little is known about the change in health status over time and the relationship to changes in lung function.

Many clinical trials now include health status as a valid outcome measure and so an understanding of how exacerbations may affect health status and the change in health status over time is also important.

4.2 Methods

4.2.1 Subjects

The first 265 patients with PiZ deficiency recruited to the UK registry from 1996 were studied. Patients were assessed at an initial baseline visit and full demographic data was collected. Further assessment was made at 12 months for all patients. Ninety patients

were assessed annually for a further 2 years. One subject underwent lung transplantation during this time and was removed from the three year analysis. Two patients continued to smoke during this follow up period and were also removed from the analysis to maximise patient homogeneity.

4.2.2 Exacerbation Data

On visits, after initial baseline visit, details of the occurrence and nature of exacerbations of respiratory symptoms over the previous year were obtained during patient interview. The length and time of year of the first four episodes were noted from patient recall, with corroboration from diary cards where possible. Exacerbations were defined as acute episodes of worsening symptoms and were categorised according to the Anthonisen classification (Anthonisen et al, 1987). Type 1 exacerbations were defined as those with increased dyspnoea, sputum volume and sputum purulence. Type 2 exacerbations occurred when two of these symptoms were present and type 3 exacerbations when only one symptom was present. The duration of the exacerbation was taken as the length of time from the onset of worsening symptoms up to the last day before the patient was back to their usual state of health. Frequent exacerbations were defined as 3 or more episodes over a 12 month period. The incidence of exacerbations was documented annually for the patients who were followed for 3 years.

4.2.3 Lung Function Testing

Full lung function tests were performed at each annual visit as described in the general methods 3.2 (Section 3.2). All tests were performed at least 4 weeks after any exacerbation to minimise any temporary effect on these values.

Changes in lung function measurements over 3 years were calculated using linear regression on the four measurements taken for each individual over that time. This result was then converted into a change per annum.

4.2.4 Health Status

Health status measurements were made at each annual visit using the SGRQ and the SF-36. No reference to previous answers was made during data collection.

Annual changes were calculated from linear regression of the four values obtained over the three years.

4.3 Results

4.3.1 Subjects studied for 1 year

4.3.1.1 Baseline Characteristics

Of the 265 PiZ patients, 167 (63%) were male and 210 (79%) were index cases. One hundred and eighty four patients (69%) were ex-smokers and 17 (6%) continued to smoke during the first year. Chronic bronchitis was diagnosed in 110 (42%) patients. One hundred and forty six patients used inhaled steroids consistently throughout the initial 12 month period. Thirteen patients had steroid therapy changed during this 12 month period and were removed from the analysis related to the influence of inhaled corticosteroids. The baseline characteristics of the whole group are shown in Table 2.

Baseline characteristics for the whole patient group, patients with and without exacerbations over the first year and for the subgroup followed for 3 years. Data presented as mean \pm standard deviation or median (interquartile range). NS: not significant; * univariate comparison of those with and without exacerbations; # t test; † Chi squared test; all other statistical comparison uses Mann Whitney U test.

Table 2

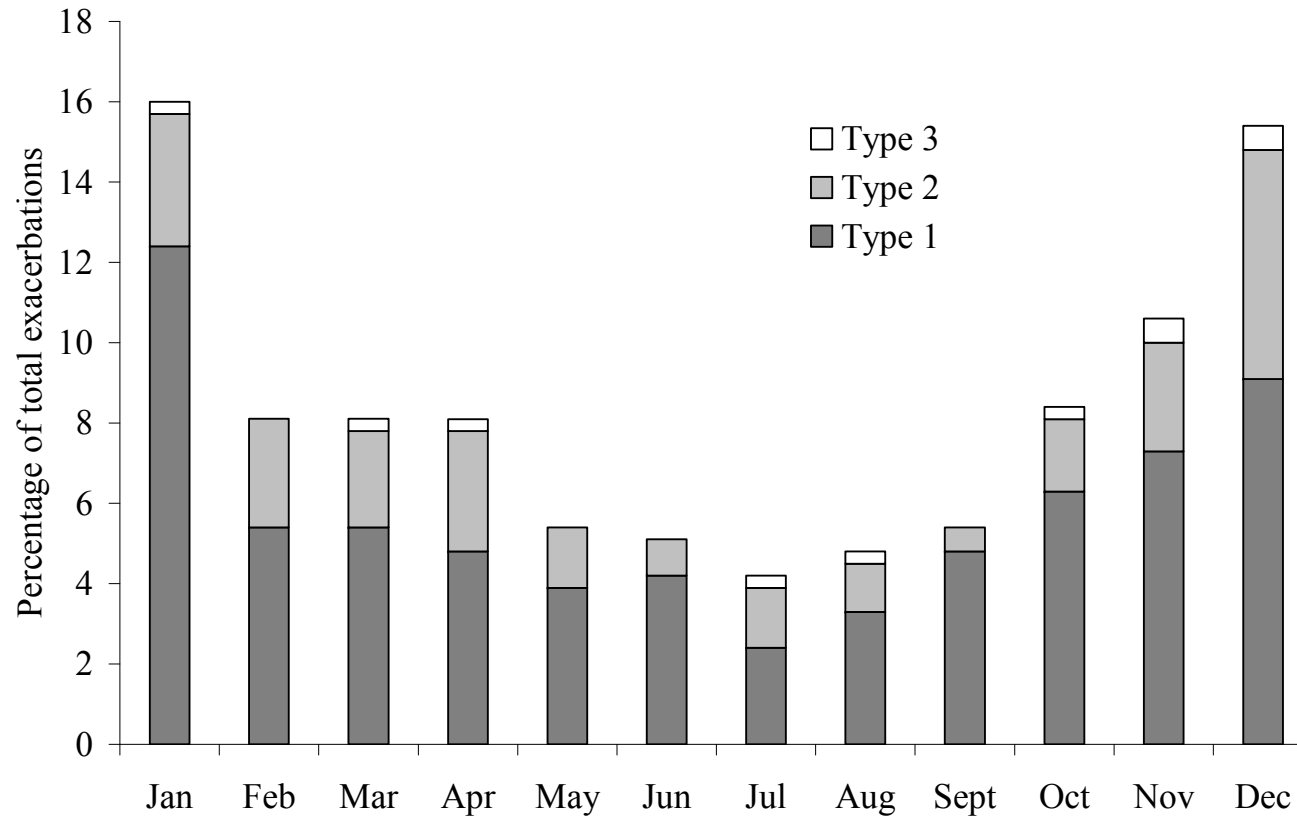
	All patients	Patients with no exacerbations over year 1	Patients with ≥1 exacerbation over year 1	p value*	Subgroup studied for 3 years
Number of subjects	265	123	142		87
Age (years)	49.9 ± 9.8	50.0 ± 11.1	49.9 ± 9.5	NS [#]	50.2 ± 9.3
Body mass index (kg/m²)	25.0 (22.9-27.8)	25.1 (23.2-27.8)	24.8 (22.2-27.8)	NS	25.0 (23.0-27.0)
Smoking history (pack years)	13.0 (1.4-24.0)	12.0 (0.5-22.8)	14.6 (4.0-26.0)	NS	17.5 (0-25.0)
Smoking status					
Current	17	8	9	NS [†]	0
Ex	187	84	103		65
Never	61	31	30		22
Gender					
Male	167	80	87	NS [†]	59
Female	98	43	55		28
Presentation					
Index	210	90	120	0.023 [†]	67
Non Index	55	33	22		20
Chronic bronchitis					
Yes	110	39	71	0.003 [†]	35
No	155	84	71		52
Inhaled steroid in year 1					
Yes	146	56	90	0.002 [†]	47
No	106	64	42		37
Altered	13	3	10		3
Bronchodilator reversibility					
Yes	65	23	42	0.034 [†]	23
No	198	100	98		63

4.3.1.2 Exacerbations

During the first year of study, exacerbations occurred in 142 subjects (54%) and 47 (18%) had frequent exacerbations as defined by three or more episodes (Seemungal et al, 1998). One patient described 30 exacerbations over twelve months and was excluded from the analysis. The median number of exacerbations for the whole group was 1 (IQR 0-2), although for those patients who experienced at least one exacerbation the median number was 2 (IQR 1-3). Eighty nine patients had type 1 exacerbations, 44 had type 2 exacerbations, 2 had both type 1 and type 2 exacerbations and 7 patients had type 3 exacerbations during this year.

Exacerbations were experienced throughout the year but were more frequent in the winter months, with 32% of episodes occurring in December and January as shown in Figure 1.

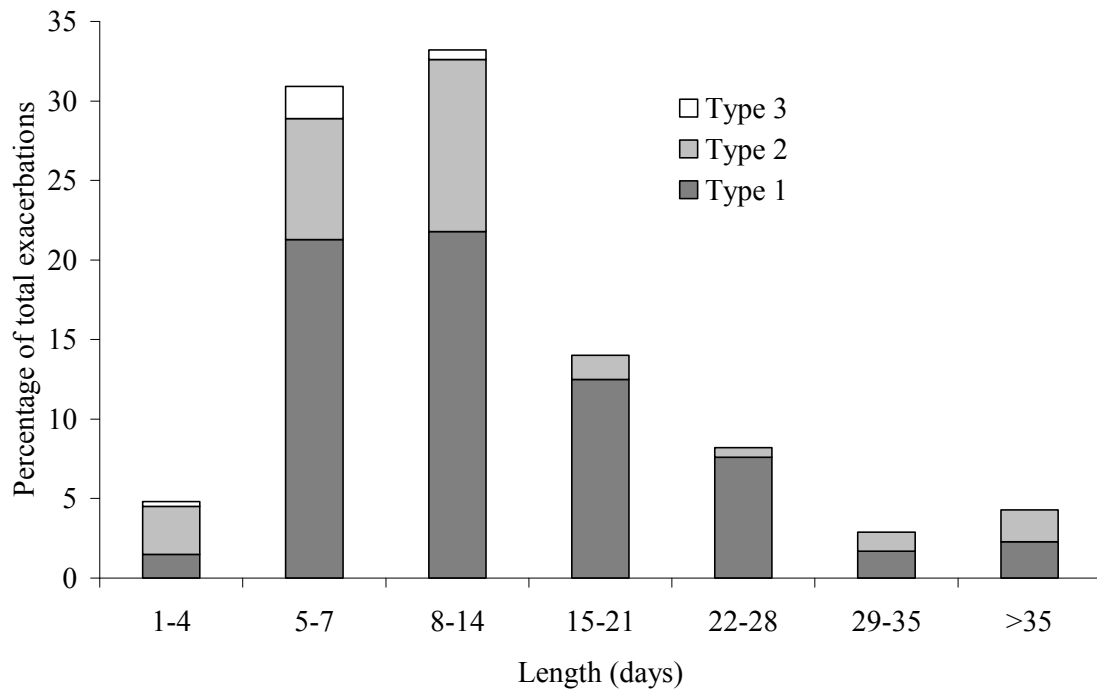
Figure 1



The proportion (%) of exacerbations occurring in each month of the year. Exacerbations are categorised by Anthonisen criteria into type 1 (dark grey), type 2 (light grey) and type 3 (white).

Episodes varied in length from 2 to more than 35 days as illustrated in Figure 2. The median length of each episode was 14.0 days (IQR 7-21).

Figure 2



The length of the exacerbations of symptoms is illustrated with the proportion of the total (%) shown for each time period. Exacerbations are categorised by Anthonisen criteria into type 1 (dark grey), type 2 (light grey) and type 3 (white).

4.3.1.3 Exacerbations and Baseline Characteristics

Exacerbations occurred more frequently in those patients with chronic bronchitis (1.7 ± 0.2 vs 1.2 ± 0.2 , χ^2 : $p < 0.001$). Index patients were more likely to have exacerbations compared to non-index patients (1.6 ± 0.2 vs 0.8 ± 0.2 , χ^2 : $p = 0.016$). There was no relationship between the number of exacerbations and age, gender or body mass index.

However, a weak correlation was seen between the number of pack years smoked and the number of exacerbations ($r=0.12$, $p=0.03$).

Furthermore, patients using inhaled steroids consistently throughout the twelve months had more exacerbations than those who were consistent non-users (χ^2 : $p=0.004$) as did those with bronchodilator reversibility (χ^2 : $p=0.042$).

Univariate comparisons of baseline characteristics of those with and without exacerbations are displayed in Table 2 (page 57).

4.3.1.4 Lung Function

Lung function data was obtained from 259 patients as 6 did not wish to undergo testing. Measurements ranged from normal to severe airflow obstruction and markedly impaired gas transfer. Data obtained at the start of the study and at twelve months are included for completion: baseline data are summarised in Table 3 alongside univariate comparisons of baseline lung function of those with and without exacerbations; Table 4 shows the lung function data after one year together with comparisons between those with and without exacerbations.

Table 3

	n	All patients	Patients with no exacerbations over year 1	Patients with ≥1 exacerbation over year 1	p value *
FEV₁ (L)	259	1.53 (1.02-2.45)	1.77 (1.11-2.93)	1.42 (0.98-2.16)	0.005
FEV₁ (% predicted)	259	46.2 (33.8-76.8)	55.2 (35.6-95.3)	43.3 (32.9-68.5)	0.013
VC (L)	259	4.13 (3.16-4.99)	4.28 (3.66-5.11)	3.97 (2.85-4.92)	0.049
VC (% predicted)	259	103.5 (91.4-117.3)	106.9 (95.6-119.9)	101.7 (87.6-115.9)	0.044
FEV₁/VC (%)	259	39.5 (28.2-58.8)	42.2 (28.4-73.8)	36.5 (27.3-52.4)	0.002
RV (L)	250	2.58 (2.04-3.24)	2.51 (2.00-3.16)	2.72 (2.18-3.42)	0.037
RV (% predicted)	250	129.7 (102.6-155.1)	126.1 (99.7-148.9)	137.4 (106.7-173.8)	0.008
TLC (L)	250	7.40 (6.06-8.40)	7.38 (6.20-8.54)	7.47 (5.91-8.37)	NS
TLC (% predicted)	250	119.0 (107.4-130.5)	118.4 (107.7-128.1)	119.1 (108.1-132.6)	NS
RV/TLC (%)	250	36.4 (29.4-43.0)	34.3 (28.0-41.5)	38.9 (32.4-45.5)	0.002
TL_{CO} (mmol/min/kPa)	256	6.37 (4.65-8.27)	6.60 (4.99-8.46)	5.86 (4.29-7.85)	0.035
TL_{CO} (% predicted)	256	68.0 (52.0-85.0)	72.0 (55.0-88.0)	63.6 (50.0-83.0)	0.055
K_{CO} (mmol/min/kPa/L)	256	1.08 (0.80-1.39)	1.12 (0.80-1.44)	1.00 (0.78-1.35)	NS
K_{CO} (% predicted)	256	67.9 (53.7-86.0)	69.0 (55.0-87.0)	66.2 (51.0-85.7)	NS

Lung function values at baseline for the whole patient group and patients with and without exacerbations over the first year. Data presented as mean ± standard error or median (interquartile range). NS: not significant; * univariate comparison of those with and without exacerbations using Mann Whitney U test.

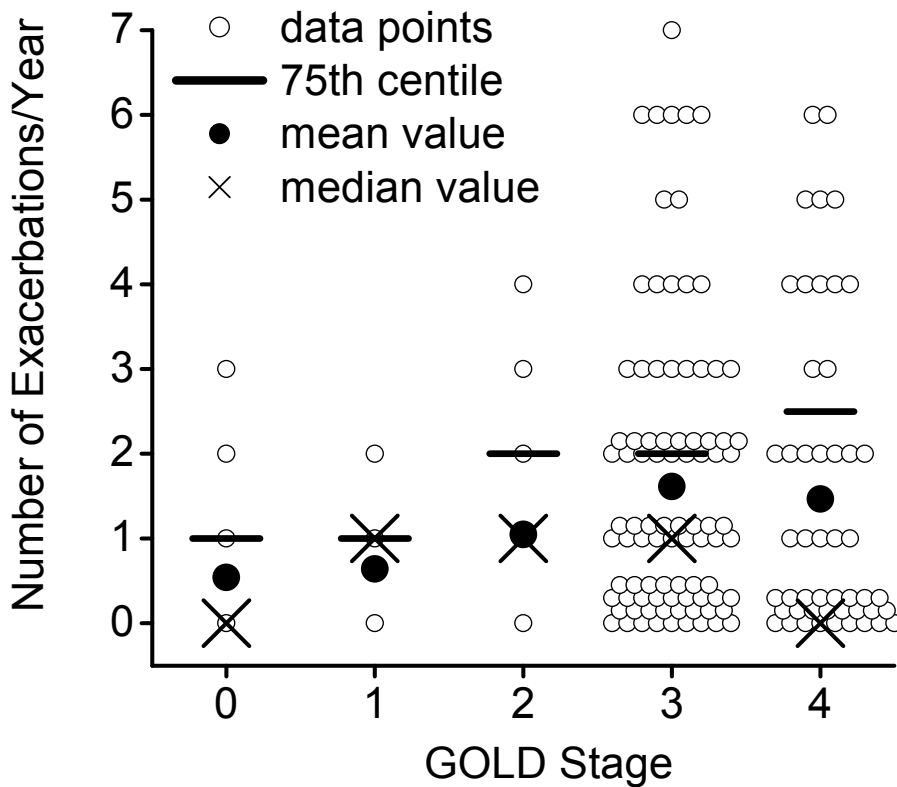
Table 4

	n	All patients	Patients with no exacerbations over year 1	Patients with ≥1 exacerbation over year 1	p value*
FEV₁ (L)	259	1.49 (0.97-2.31)	1.66 (1.04-2.78)	1.41 (0.96-2.06)	0.008
FEV₁ (% predicted)	259	46.6 (33.0-74.9)	52.6 (35.2-94.8)	43.0 (31.6-65.7)	0.014
VC (L)	259	4.08 (3.19-4.97)	4.42 (3.58-5.13)	3.79 (2.95-4.83)	0.005
VC (% predicted)	259	104.3 (90.7-118.5)	107.9 (97.3-122.3)	102.5 (86.3-113.1)	0.004
FEV₁/VC (%)	259	38.0 (27.8-57.9)	42.3 (26.9-73.3)	36.2 (28.1-49.1)	0.003
RV (L)	242	2.66 (2.02-3.28)	2.51 (1.90-3.28)	2.70 (2.19-3.32)	NS
RV (% predicted)	242	127.5 (103.2-162.9)	123.7 (96.0-150.0)	132.3 (107.7-170.2)	0.013
TLC (L)	242	7.24 (6.16-8.62)	7.20 (6.20-8.71)	7.26 (6.01-8.51)	NS
TLC (% predicted)	242	118.7 (107.7-131.0)	118.3 (107.1-130.0)	119.9 (108.0-131.5)	NS
RV/TLC (%)	242	37.5 (29.9-44.1)	34.2 (28.3-42.5)	38.8 (32.9-45.8)	0.004
TL_{CO} (mmol/min/kPa)	250	6.22 (4.57-8.08)	6.40 (4.66-8.25)	6.07 (4.42-7.93)	NS
TL_{CO} (% predicted)	250	66.0 (50.0-82.0)	68.0 (51.0-84.5)	65.0 (50.0-81.0)	NS
K_{CO} (mmol/min/kPa/L)	250	1.02 (0.79-1.37)	1.09 (0.80-1.38)	1.01 (0.78-1.32)	NS
K_{CO} (% predicted)	250	66.2 (52.0-84.0)	68.1 (53.0-85.1)	65.1 (52.0-83.0)	NS

Lung function values at year 1 for the whole patient group and patients with and without exacerbations over the first year. Data presented as mean ± standard error or median (interquartile range). NS: not significant; * univariate comparison of those with and without exacerbations using Mann Whitney U test.

Patients with more severe airflow obstruction, as defined by GOLD criteria (Pauwels et al, 2001), had more frequent exacerbations (Jonckheere-Terpstra : $p=0.002$) over the study period as demonstrated in Figure 3.

Figure 3



The bubble plot shows the number of exacerbations over the first 12 months in patients grouped according to their GOLD stage. Open circles indicate each data point. Solid circles show the mean for each stage, horizontal line is the 75th centile, and the cross indicates the median value. The lower 25th centile was zero for all stages. Significantly more exacerbations are seen in those patients with greater airflow obstruction (Jonckheere-Terpstra: $p=0.002$).

Binary logistic regression was performed using all of the patient demographic data, lung function data and smoking history. The presence of chronic bronchitis, bronchodilator reversibility and increased air trapping, as measured by RV/TLC, were all independent predictors of exacerbations (estimated $r^2=0.13$). For those patients with exacerbations, greater body mass index, female gender, increasing age and lower FEV₁ were all predictors of type 1 exacerbations (estimated $r^2=0.17$).

4.3.1.5 Health Status

Health status scores also showed a wide variation at baseline and are summarised in Table 5 together with univariate comparisons of those with and without exacerbations. Health status measurements at year 1 are shown in Table 6.

Table 5

	All patients	Patients with no exacerbations over year 1	Patients with ≥1 exacerbation over year 1	p value*
n	263	121	142	
SGRQ				
Total	51.6 (31.5-65.3)	45.3 (25.1-58.2)	55.4 (38.0-68.7)	<0.001
Symptoms	64.0 (44.1-78.8)	57.7 (36.8-72.6)	70.9 (53.6-83.8)	<0.001
Activity	66.2 (41.4-85.9)	59.5 (35.2-79.7)	72.8 (48.5-92.6)	<0.001
Impacts	36.8 (20.0-52.5)	31.8 (14.7-46.3)	41.5 (25.2-56.8)	<0.001
SF-36				
Physical	37.7 (28.9-45.9)	40.5 (32.6-49.7)	34.0 (26.7-42.5)	<0.001
Mental	53.2 (45.2-58.7)	53.3 (46.4-58.8)	53.1 (42.5-58.5)	NS

Health status scores at baseline for the whole patient group and patients with and without exacerbations over the first year. Data presented as mean ± standard error or median (interquartile range). NS: not significant; * univariate comparison of those with and without exacerbations using Mann Whitney U test.

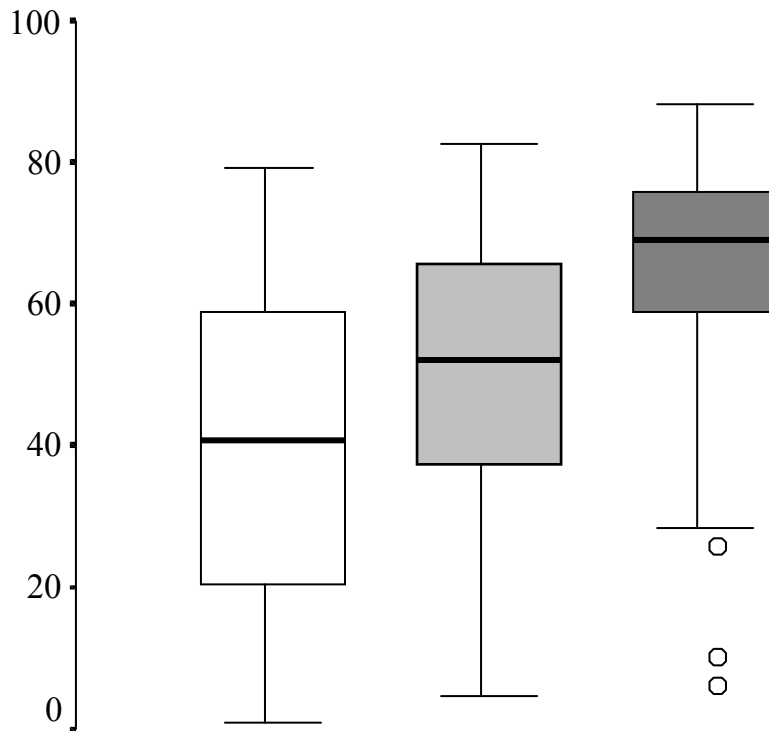
Table 6

	All patients	Patients with no exacerbations over year 1	Patients with ≥1 exacerbation over year 1	p value*
n	262	122	140	
SGRQ				
Total	49.5 (31.0-64.0)	42.0 (22.2-57.3)	56.4 (39.2-70.2)	<0.001
Symptoms	60.7 (38.8-75.0)	40.3 (20.1-61.6)	70.8 (59.0-81.7)	<0.001
Activity	66.2 (41.4-87.2)	57.3 (35.5-79.7)	73.0 (47.9-92.5)	<0.001
Impacts	36.4 (18.7-52.3)	28.2 (13.6-46.2)	40.9 (25.2-55.6)	<0.001
SF-36				
Physical	36.7 (28.3-45.4)	39.4 (31.2-48.7)	34.4 (26.2-43.8)	0.001
Mental	54.8 (47.5-59.2)	55.4 (48.6-59.2)	54.2 (46.0-59.3)	NS

Health status scores at year 1 for the whole patient group and patients with and without exacerbations over the first year. Data presented as mean ± standard error or median (interquartile range). NS: not significant; * univariate comparison of those with and without exacerbations using Mann Whitney U test.

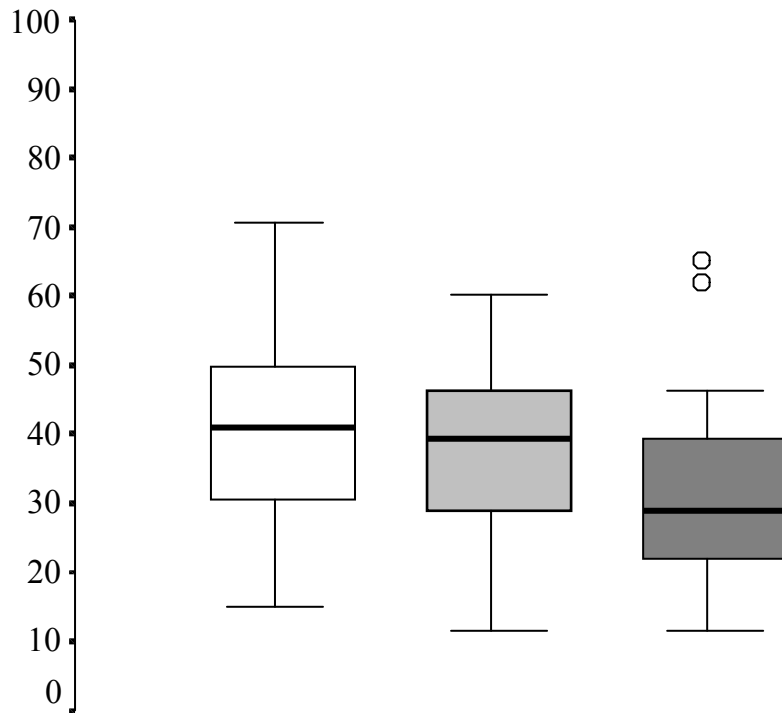
A clear relationship was seen between the number of exacerbations and SGRQ total score at year 1 ($r=0.41$, $p<0.001$) as shown in Figure 4. A correlation was also seen between the exacerbation number and SF36 physical score ($r=-0.33$, $p<0.001$) as seen in Figure 5.

Figure 4



The boxplots show SGRQ total score at month 12 in patients with no (white), infrequent (light grey) and frequent (dark grey) exacerbations. Thick black lines show the median values, boxes are interquartile ranges, whiskers show the total range and circles are outliers. Higher scores, indicating worse health status, are found in patients with infrequent exacerbations and even higher in those with frequent exacerbations (Jonckheere-Terpstra: $p < 0.001$) compared to those patients without episodes.

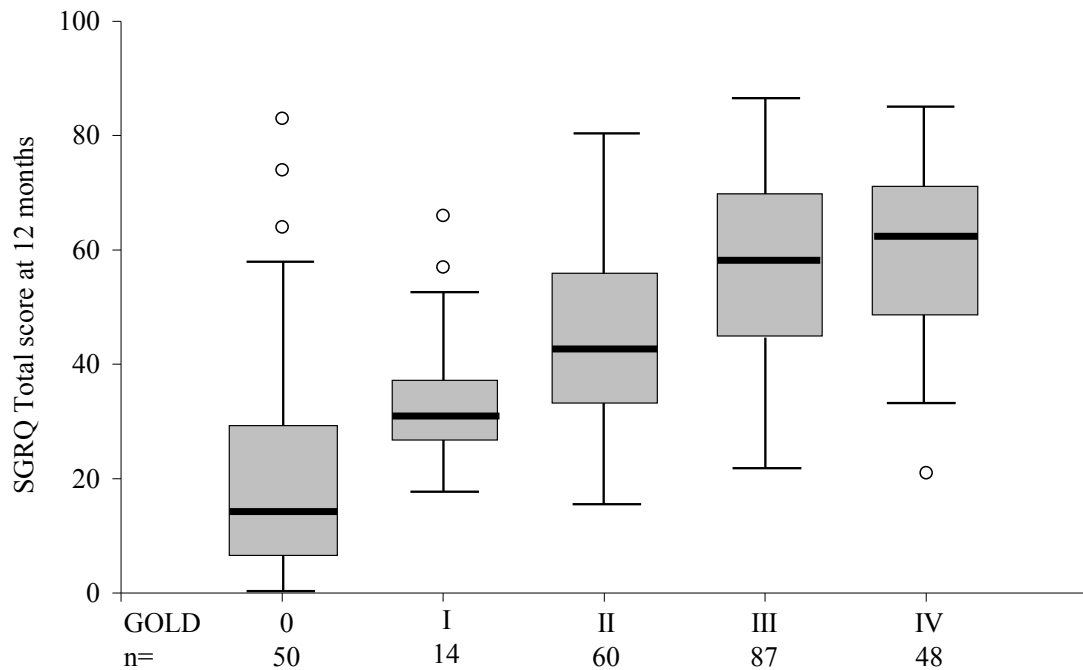
Figure 5



The boxplots show the SF36 physical score at month 12 in patients with no (white), infrequent (light grey) and frequent (dark grey) exacerbations. Thick black lines show the median values, boxes are interquartile ranges, whiskers show the total range and circles are outliers. Lower scores, indicating worse health status, are seen in patients with infrequent exacerbations and even lower in those with frequent exacerbations (Jonckheere-Terpstra: $p < 0.001$) compared to those without episodes.

Patients with more severe airflow obstruction (GOLD criteria) had worse SGRQ health status scores and SF36 physical component scores (all $p < 0.001$). SGRQ Total scores are shown in Figure 6.

Figure 6



The boxplots show the SGRQ Total score at month 12 in patients grouped according to their GOLD stage. Thick black lines show median values, boxes are interquartile ranges, whiskers show the total range and circles indicate outliers. Significantly worse scores are seen in those patients with greater airflow obstruction (Jonckheere-Terpstra: $p < 0.001$).

Linear regression of SGRQ total scores at month 12 showed that lower FEV₁, number of exacerbations, increasing age, increase in pack year smoking history and the presence of chronic bronchitis were all independent factors associated with worse SGRQ total score and together accounted for 54% of the overall variability in the score.

4.3.2 Subjects Studied for 3 Years

The baseline characteristics of this subgroup are shown in Table 2 (page 55). Fifty nine patients (68%) were male, 67 (77%) were index cases and 65 (75%) were ex-smokers.

4.3.2.1 Lung Function Progression

The lung function data obtained at baseline and year 3 are summarised in Table 7.

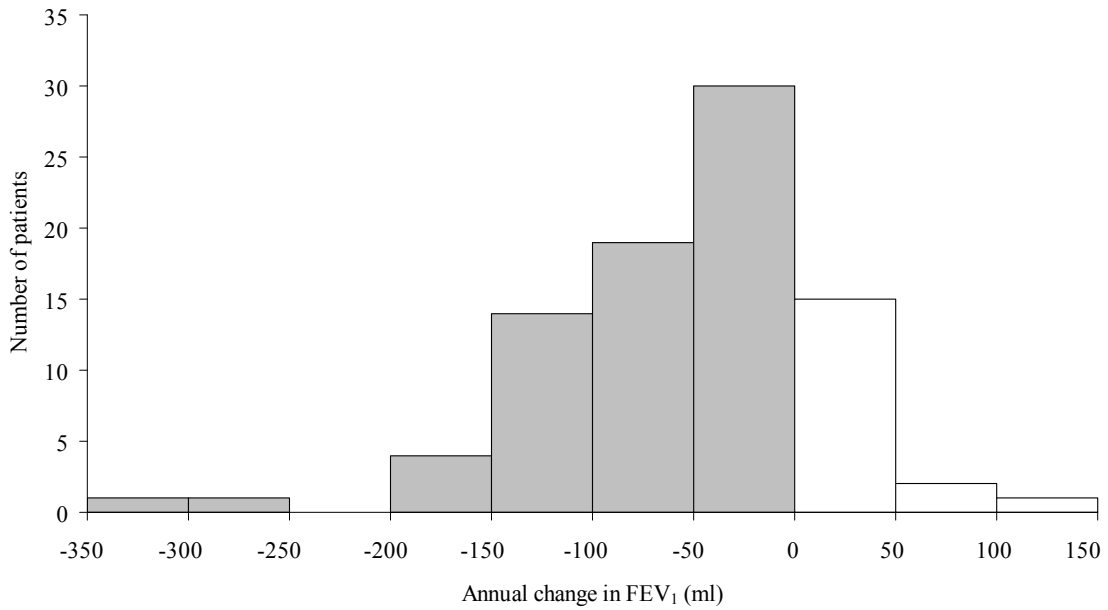
FEV₁ values showed a significant decline ($p < 0.001$) with a median change of -41ml/year (IQR -96 to -15) over 3 years which reflects an average change of 57ml/year (SE \pm 7.5). A frequency histogram showing annual change in absolute FEV₁ values is shown in Figure 7.

Table 7

	n	Base	Year 3	Annual change
FEV₁ (L)	87	1.57 (1.00-2.43)	1.32 (0.88-2.22)	-0.041 (-0.096 to -0.015)
FEV₁ (% predicted)	87	48.5 (33.1-87.8)	41.6 (31.1-81.5)	-1.0 (-2.5 to 0.1)
VC (L)	87	4.09 (3.25-4.83)	3.90 (3.29-4.71)	-0.052 (-0.120 to 0.006)
VC (% predicted)	87	105.3 (94.5-120.5)	106.2 (87.1-122.9)	-0.5 (-2.2 to 1.4)
FEV₁/VC (%)	87	40.8 (26.7-60.8)	38.2 (25.7-55.0)	-0.9 (-2.1 to 0.2)
RV (L)	81	2.52 (2.00-3.24)	2.70 (2.05-3.37)	0.064 (-0.034 to 0.172)
RV (% predicted)	81	126.5 (97.0-154.8)	133.7 (101.8-169.7)	1.7 (-3.3 to 7.9)
TLC (L)	81	7.28 (6.14-8.22)	7.21 (6.02-8.46)	0.029 (-0.117 to 0.148)
TLC (% predicted)	81	118.9 (104.7-132.1)	117.4 (105.8-134.2)	0.5 (-1.9 to 2.4)
RV/TLC (%)	81	35.5 (29.2-42.3)	39.5 (30.5-44.8)	0.6 (-0.3 to 2.0)
TL_{CO} (mmol/min/kPa)	87	6.24 (4.67-8.32)	5.39 (4.11-7.16)	-0.21 (-0.37 to -0.04)
TL_{CO} (% predicted)	87	66.0 (53.0-88.0)	61.0 (46.5-80.5)	-1.8 (-3.6 to -0.2)
K_{CO} (mmol/min/kPa/L)	87	1.06 (0.77-1.41)	0.99 (0.72-1.33)	-0.03 (-0.05 to 0)
K_{CO} (% predicted)	87	67.2 (53.3-91.8)	64.5 (46.5-85.8)	-2.0 (-3.7 to -0.7)

The lung function data at baseline and the end of year 3 are shown together with the change per year and the number of patients tested at the two time points. All values were post-bronchodilator measurements. The annual change was calculated from a linear regression analysis of four measurements over 3 years for each individual patient. All values shown are median values with interquartile range in parentheses.

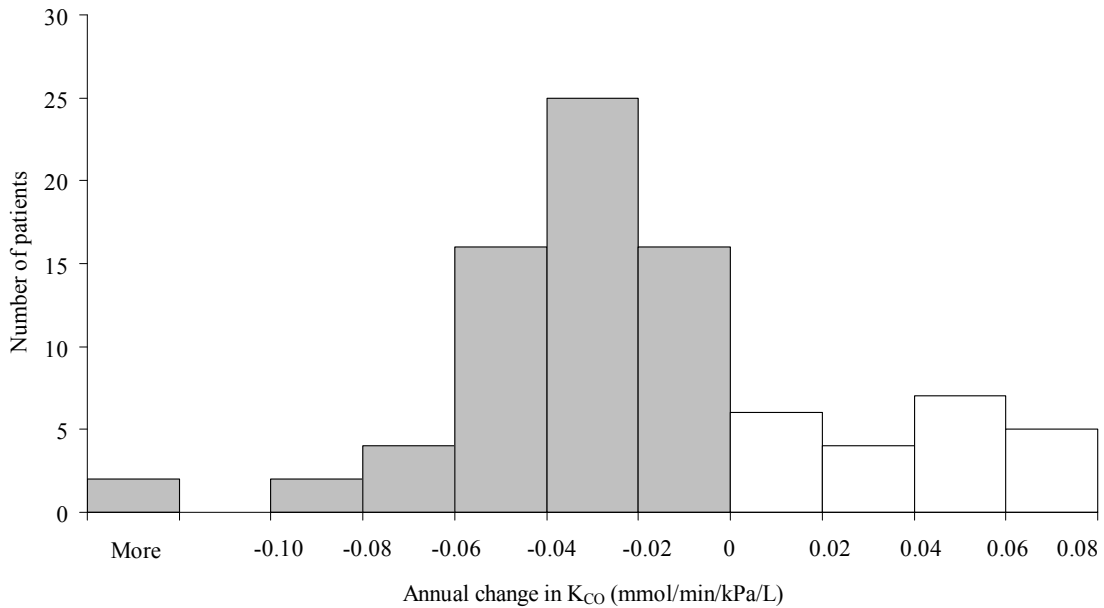
Figure 7



Frequency histogram of annual absolute change in FEV₁. Annual change was calculated from a linear regression analysis of four measurements over 3 years for each individual patient. Grey bars illustrate lung function decline and open bars represent measured improvement in FEV₁.

Residual volume measurements and air trapping (as measured by RV/TLC ratio) showed significant increases ($p < 0.001$) over the 3 year period whereas gas transfer measurements showed a significant decline ($p < 0.001$). A frequency histogram showing annual change in absolute K_{CO} values is shown in Figure 8.

Figure 8



Frequency histogram of annual absolute change in K_{CO}. Annual change was calculated from a linear regression analysis of four measurements over 3 years for each individual patient. Grey bars illustrate lung function decline and open bars represent measured improvement in K_{CO}.

4.3.2.2 Health Status Change

Health status values measured at baseline and year 3 are summarised in Table 8. Over the 3 year period, there was no significant change in SGRQ total score, activity score and impact score or in the SF36 scores. However, the SGRQ symptom score showed a significant improvement ($p=0.003$) over this period of time with a median change of -2.0 units/year (IQR -6.2 to +1.6).

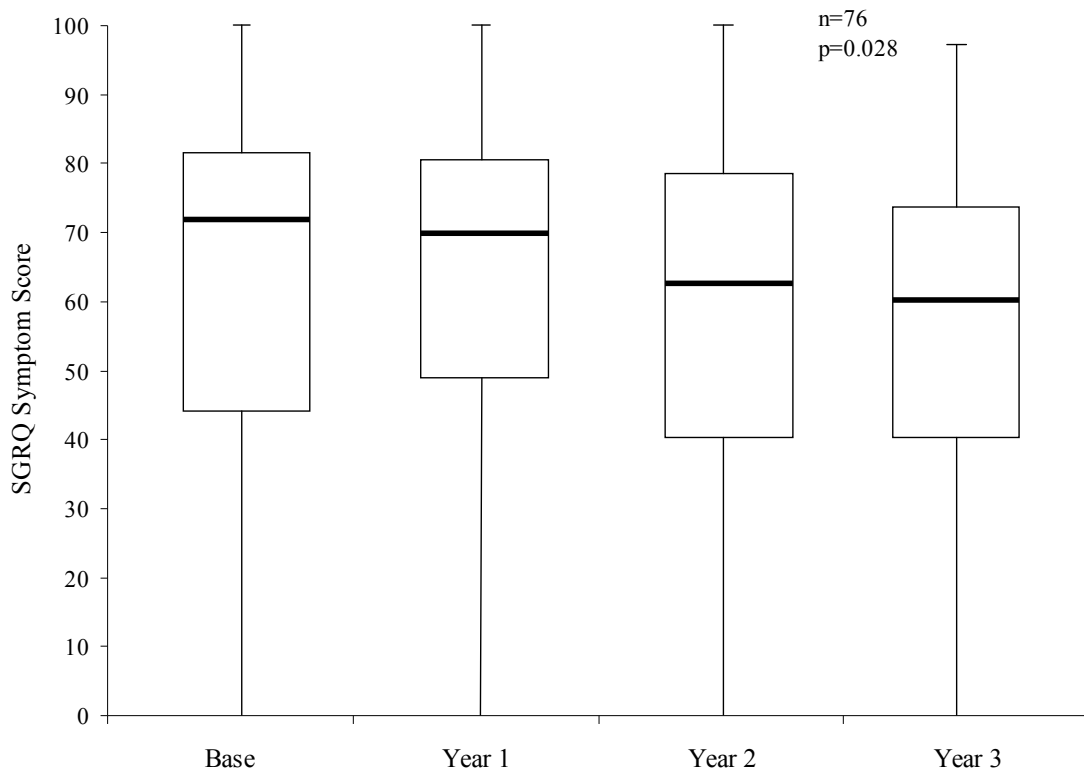
Table 8

	n	Base	Year 3	Annual Change
SGRQ				
Total	86	52.5 (27.8-66.6)	50.0 (29.3-67.7)	-0.5 (-2.2 to 1.4)
Symptoms	86	66.4 (37.5-84.3)	61.4 (37.9-74.3)	-2.0 (-6.2 to 1.6)
Activity	86	61.1 (35.5-86.6)	69.6 (36.2-92.5)	0.2 (-1.3 to 2.5)
Impacts	86	39.3 (17.7-54.5)	34.0 (16.2-54.0)	-0.1 (-2.4 to 1.9)
SF-36				
Physical	86	39.7 (28.8-47.6)	34.6 (29.1-47.6)	-0.4 (-2.2 to 1.1)
Mental	86	52.3 (42.6-57.8)	55.0 (46.7-59.3)	0.5 (-1.1 to 2.3)

The health status scores at baseline and the end of year 3 are shown together with the change per year and the number of patients tested at the two time points. The annual change was calculated from a linear regression analysis of four measurements over 3 years for each individual patient. All values shown are median values with interquartile range in parentheses.

This improvement in SGRQ symptom score was shown to be progressive over the three years in the 76 individuals who completed all four time points in the annual assessment as shown in Figure 9.

Figure 9



The boxplots show the SGRQ Symptom score at four annual time points in 76 patients. Thick black lines show median values, boxes are interquartile ranges and whiskers show the total range. Significant reduction in scores relating to improvement in symptoms is seen progressively year on year (Freidmans $p=0.028$).

4.3.2.3 Exacerbations over 3 Years

The mean number of exacerbations reported for all patients was 1.0 (± 0.2) in the first year, 1.3 (± 0.2) in year 2 and 1.2 (± 0.2) for year 3. The proportion of patients having exacerbations remained consistent over the three years and patients with exacerbations in the first year were more likely to continue having exacerbations in year 2 ($p=0.04$) and year 3 ($p<0.001$). At least one exacerbation had occurred in 50.6% of patients in the first year, 75.9% by year 2 and 80.5% by year 3. Frequent exacerbations (more than 2

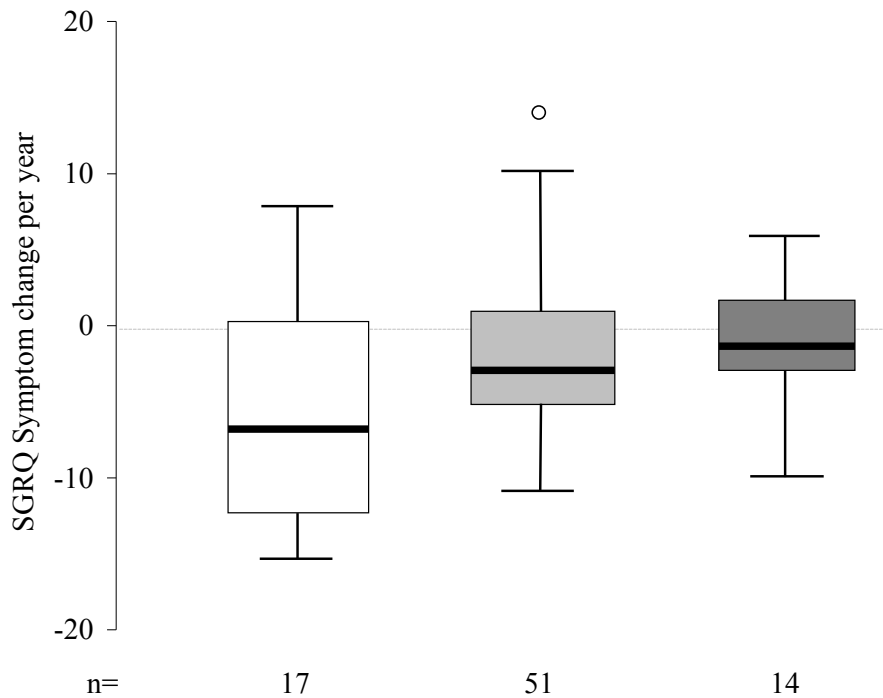
episodes per year) were seen in 12.6% of patients in the first year, 19.0% in year 2 and 13.8% in year 3.

4.3.2.4 Exacerbations, Changes in Lung Function and Changes in Health Status

Neither the presence nor the frequency of exacerbations over the 3 years showed a relationship to the absolute decline in FEV₁ or FEV₁ expressed as a % predicted. Furthermore, exacerbations did not have an effect on the decline in FEV₁ when patients were grouped according to baseline airflow obstruction (GOLD criteria). However, a weak but significant correlation was seen between frequency of exacerbations and decline in TL_{CO} as a % predicted ($r=-0.19$, $p=0.037$).

Greater improvement in SGRQ symptom score was seen in those patients who had infrequent or no exacerbations ($p=0.029$) (Figure 10).

Figure 10



The boxplots show the annual change in SGRQ symptom score in patients with no (white), infrequent (light grey) and frequent (dark grey) exacerbations. Thick black lines show the median values, boxes are interquartile ranges, whiskers show the total range and circles are outliers. Greater improvement is seen in patients with fewer exacerbations (Jonckheere-Terpstra: $p=0.029$).

When patients were grouped according to baseline airflow obstruction (GOLD criteria), no significant differences in the change in health status were seen between the groups.

Worsening SGRQ activity score was, however, related to the decline in FEV_1 ($r=-0.24$, $p=0.015$) and to decline in VC ($r=-0.23$, $p=0.017$). Finally, although the total score did not change, a significant correlation was also seen between the changes in SGRQ total score and increased air trapping as measured by the RV/TLC ratio ($r=0.20$, $p=0.037$).

4.3.2.5 *Linear Regression of Changes in Lung Function*

Linear regression showed that bronchodilator reversibility and lower body mass index at baseline were both independently associated with a greater decline in FEV₁ (% predicted) and together accounted for 14% of the overall variability in the decline. An increasing number of exacerbations was the only independent factor associated with a greater decline in TL_{CO} (% predicted) but accounted for only 5% of the variability in this decline.

4.4 Discussion

These results have demonstrated that exacerbations occur commonly in patients with AATD and a third of the patients with exacerbations (18% of the total group) had 3 or more episodes per year. The average number of exacerbations for the whole group was similar to that seen in non AATD COPD in general (Seemungal et al, 1998). The presence and frequency of exacerbations remained consistent from year to year and, although exacerbations occur throughout the year, nearly one third occurred in December and January. The median length of symptoms in these patients is 14 days which, despite being based largely on patient recall, is similar to the duration determined prospectively by diary cards in a group of subjects studied previously (Hill et al, 1999a). The length of these exacerbations is greater than the 7 days seen in usual COPD (Seemungal et al, 2000a) although the current study used different methods for data collection. Taken together, these data suggest that AATD alone does not predispose to exacerbations but may influence their resolution.

At the start of patient recruitment in 1996 there was no consensus about the definition of an exacerbation, which has subsequently been more precisely defined to include a

change in regular medication as has been suggested subsequent to the commencement of our study (Rodriguez-Roisin, 2000). Nevertheless, the overall frequency is similar to that seen in COPD in general, when this more rigorous definition is applied, suggesting that the data are equally valid.

There is a potential for recall bias when the patients were asked about their exacerbations during the previous year. This was minimised, where possible, by comparison with diary cards of symptoms. Patient recall data on exacerbations in this group of patients has also been corroborated with patient records in a previous more limited study that gave similar results (Dowson et al, 2002). Thus the current results are likely to be an accurate representation of the number and nature of exacerbations in AATD when obtained by this method. It should be considered however that the actual number of episodes may be higher than reported here. Studies on exacerbations in usual COPD using diary cards show that up to 49% of exacerbations may remain unrecognised or unreported by patients (Perera et al, 2007; Seemungal et al, 1998; Seemungal et al, 2000a). Thus any future studies should carefully consider how recall and report bias may underestimate these episodes.

The population used was an unselected group of subjects with severe AATD and thus the patients showed a range of lung function from normal to severe impairment.

The patients included in the 3 year analysis study were all non-smokers or ex-smokers and the small number of continuing smokers was excluded to maximise patient homogeneity. Thus this data cannot be extrapolated to patients with AATD who continue to smoke. Patients who continue to smoke may show a greater decline in lung function measurements and the effect that this has on health status is unknown.

Chronic mucus hypersecretion has been shown to be associated with increased likelihood of exacerbations in usual COPD (Miravittles et al, 2000) and in AATD (Dowson et al, 2002). In the current study, 42% of the patients had chronic bronchitis and these patients showed an increased frequency of exacerbations, confirming previous findings (Dowson et al, 2002).

Although index patients had more exacerbations than non-index patients in the univariate analysis, index status was not independently related to exacerbations in the multivariate analysis. Index patients had a greater smoking history and a lower baseline FEV₁ and patients with more severe airflow obstruction had more frequent exacerbations, which probably accounts for the initial association with index status. It should be noted that half of the patients in the most severe group as grouped by airflow obstruction had no exacerbations. This may be due to survivorship bias. Some of these more severely affected patients may not return to research programmes or may die during an exacerbation. Thus the data from subjects who do return for further testing may be from a “select” group who, perhaps, have remained free of exacerbations.

In addition, increased air trapping as measured by residual volume was also shown to be associated with exacerbations. The reason for this is unknown but increased air trapping would increase the work of breathing and may, therefore, lead to a greater awareness of slight pathophysiological changes that increase this work further and be reflected in the awareness of increased breathlessness. Alternatively, the exacerbations themselves may damage the small airways leading to an increase in air trapping. At present, it remains unknown whether this is a cause or effect but the observed relationship between FEV₁ decline and exacerbation frequency in usual COPD (Donaldson et al, 2002) is more supportive of a direct effect. It remains unknown why this is not reflected in a change in

FEV₁ in AATD. However there are changes in TL_{CO} which suggest that exacerbations do play a role in the impairment of lung physiology.

Bronchodilator reversibility was also associated with exacerbations and this may reflect a change in the reversible component of airways function during such episodes. In the current study, the immediate effect of an exacerbation on spirometry and reversibility was not investigated because of patient distance from the centre, but other studies have shown some correlation between symptoms of dyspnoea and a temporary reduction in peak flow measurements (Seemungal et al, 2000a).

Exacerbations were associated with the use of an inhaled steroid. However, the current study was not designed as an interventional study and this observation may reflect the practice of prescribing inhaled steroids more often in patients with frequent exacerbations or in those with lower lung function.

Exacerbations were shown to have an influence on health status scores which supports data obtained by other groups (Doll et al, 2002; Seemungal et al, 1998). For the SGRQ symptom score this reflects the fact that two of the component questions ask about “the number and length of attacks of chest trouble”. However, worse health status was also reflected in the other individual components and total score of the SGRQ as well as the components of the SF36 in those patients with exacerbations. This association was not due to a difference in lung function alone as exacerbations were found to have an additional independent effect on health status scores using regression analysis. In addition, other factors such as increasing age, chronic bronchitis, and increase in pack year smoking history as well as reduced FEV₁ were all shown to have independent effects on health status scores irrespective of exacerbations. This confirms that quality of life as measured by these questionnaires is affected by many factors other than

physiological impairment and that improvement in health status measures is potentially achievable by using several diverse therapeutic strategies. The suggestion that augmentation therapy reduces exacerbations (Lieberman, 2000) may therefore be an important therapeutic aim in patients with alpha-1-antitrypsin deficiency.

During previous work on a small number of subjects, Dowson et al (Dowson et al, 2001a) demonstrated that improvements in SGRQ symptom score occur in patients attending for review at the UK specialist clinic over 2 years. The current study shows that this improvement is progressive and is sustained in a larger group of patients over a longer period of time and reflects a change that is recognisable by the patients. This improvement is greater in patients with fewer exacerbations.

Of more importance, the overall health status did not decline despite major changes in lung function (mean FEV₁ decline of 171 ml over the 3 years). Miravittles et al have also shown an improvement in SGRQ symptom score in a cohort of non-AATD COPD patients despite worsening activity (Miravittles et al, 2004). Other data in usual COPD shows a decline in health status with an increase in SGRQ total score of 9.6 points over 3 years when the FEV₁ falls by 177 ml although a lesser change of 6.0 points occurs in patients on inhaled steroids with a mean FEV₁ decline of 150ml (Burge et al, 2000; Spencer et al, 2001). Since the patients in the current study showed a similar decline in FEV₁, it would be expected that the SGRQ should also change by at least this amount. Recently, Stolk et al have demonstrated a worsening of SGRQ score in 22 patients with AATD over 30 months with a mean change of 6.5 units (-2.9-17.5). The lack of significant change in overall health status in the current cohort, despite documented deterioration in lung function and activity may reflect as yet undefined benefits of the specialist clinic in which patients are seen on an annual basis in the UK. Inhaled steroid

treatment was not altered significantly in these patients over the study period. However other therapeutic measures, such as the use of long-acting β -agonists, an alternative management strategy for the treatment of infections and improved patient education may have played a role in the maintenance of health status over the 3 year period.

It is confirmed that lung physiology measurements deteriorate in these patients over time. The decline in spirometric values is not related to the frequency of exacerbations in the whole group or in patient subgroups defined by severity of FEV₁ impairment. This is contrary to the findings of Donaldson et al in usual COPD (Donaldson et al, 2002). However, other workers have suggested that only smokers are susceptible to an influence of exacerbations on FEV₁ decline (Kanner et al, 2001) and the patients in the 3 year study reported here were all ex-smokers or non-smokers. It is also possible that exacerbations have a less clear deleterious effect on FEV₁ in AATD patients than in usual COPD patients due to the increased ongoing inflammation in the stable state. The FEV₁ decline was, however, associated with greater bronchodilator reversibility at baseline as has been seen previously (Dowson et al, 2001a; Group, 1998) and this may be due to an increase in airway inflammation contributing to both airways hyperreactivity and subsequent structural changes in the airways.

An association of low BMI with FEV₁ decline is seen in the current study but it is not clear if this reflects an increased risk or represents a consequence of established disease. It has been suggested that a low BMI in men may increase the risk of development of COPD (Harik-Khan et al, 2002) but how this relates to patients with another risk factor such as AATD is not known.

The decline in TL_{CO} was associated with the number of exacerbations. The proposed pathogenic mechanism for the development of emphysema and decreased gas transfer in

AATD is related to an increased neutrophilic elastase burden along with reduced protection due to low alpha-1-antitrypsin levels. An increase in elastase challenge is seen during periods of exacerbations especially in AATD (Hill et al, 1999a) but this is only a feature of purulent exacerbations. In this study, the majority of exacerbations were classified as type 1, which, by definition, includes purulent sputum as a feature. Thus increased elastase activity should be present in most of these episodes and may lead to further tissue destruction and reduction in the gas transfer ability of the lungs. However, it should be noted that such episodes were not related to the decline in K_{CO} . The TL_{CO} reflects not only alveolar gas exchange but also regional gas distribution which will be influenced by small airways damage. Exacerbations are more likely to be related to infection and inflammation in the airways than in the alveoli. Thus, such episodes may have more effect on the small airways and any subsequent structural change would lead to altered distribution of ventilation thereby reducing the TL_{CO} but not the K_{CO} .

The patients in the current work were followed for 3 years. Although this length of time is comparable to that in many observational and interventional studies in COPD, it may be too short to reliably estimate changes in lung function. More reliable estimates of changes in lung function measures may be achieved by following patients for 10 or more years (Burrows et al, 1986; Lebowitz et al, 1975). Longer longitudinal studies can account for survey bias, learning effects and non-linearity of lung function change. However these studies can be difficult to achieve with large numbers of subjects and also may under-represent severe disease due to a survivor effect of less severe patients continuing in the study.

More recently other pulmonary function measures including operating lung volumes have been incorporated into COPD and exacerbation studies. Acute exacerbations are associated with lung hyperinflation as well as worsening airflow obstruction and measuring inspiratory capacity is one way to assess this. It has been shown that improvements in inspiratory capacity relate to reduction in dyspnoea during the recovery from exacerbation (Parker et al, 2005; Stevenson et al, 2005) and that inspiratory capacity improves at a faster rate than FEV₁ (Pinto-Plata et al, 2007). The relationship between inspiratory capacity and exacerbations over time remains unclear. Interpretations of individual pulmonary function measures may be difficult and hard to transpose into meaningful benefits for patients and so combined measures may be more useful. Cote et al have shown that exacerbations negatively impact on the BODE index (body mass index, obstruction, dyspnoea and exercise capacity) (Cote et al, 2007) and future studies may benefit from using these patient-centred outcomes.

Alpha-1-antitrypsin augmentation therapy may lessen the decline in lung function in AATD, as has been suggested by some workers (Wencker et al, 1994) . Lieberman has hypothesized that augmentation therapy may also reduce the frequency and severity of infections in AATD on the basis of patient opinion on the effect of their treatment (Lieberman, 2000). Prospective controlled studies are therefore needed to investigate these hypotheses further and future trials of antiprotease replacement should include exacerbations as an important outcome measure. If a reduction in exacerbations can be achieved with augmentation therapy, the data suggests it should also influence quality of life and decline in lung function, although not necessarily the decline in FEV₁. The current study provides a firm basis upon which to design, power and implement such trials.

5 SIBLINGS

5.1 Introduction

Adult patients are primarily diagnosed with alpha-1-antitrypsin deficiency after presentation to health services with respiratory symptoms (index patients). However the alpha-1-antitrypsin genes are inherited in an autosomal co-dominant fashion and thus siblings of an index PiZ patient, who share heterozygote parents, have a 1 in 4 chance of having the same genotype and may be detected by family screening (non-index subjects). Index patients have been shown to have more severe airflow obstruction than non-index patients (Silverman et al, 1989), although differences in these populations may be influenced by ascertainment bias and differences in smoking. Nevertheless, investigation of heterozygote relatives of PiZ individuals suggests that undetermined familial factors may contribute to lung disease expression in this disease (Silverman et al, 1990a; Silverman et al, 1990b).

Sibling pairs with this deficiency have not been widely studied although case reports of individual sibships have suggested that the lung disease of PiZ siblings may be discordant (Apprill et al, 1990; Stableforth, 1978). In this section of work a group of sixty sibling pairs have been studied. Comparisons of lung function data, health status measurements and CT scans were made between index and non-index siblings, as well as baseline demographics.

In addition, a larger group of patients were analysed to estimate the impact of smoking on spirometric values. A correction factor based on an individual's smoking history was devised and applied to the values obtained from the sibling pairs in order to determine whether any difference between pairs can be explained by a smoking effect.

Finally a subset of 6 pairs of non-index siblings was analysed separately to counteract any ascertainment bias.

5.2 Methods

5.2.1 Subjects

Sixty sibling pairs with severe alpha-1-antitrypsin deficiency (PiZ) were studied.

A full clinical history was taken to include respiratory symptoms, smoking history and the presence of chronic bronchitis as defined by MRC criteria (Medical Research Council, 1965). Patients were classified as index siblings if their symptoms had led to the diagnosis of alpha-1-antitrypsin deficiency being made and non-index patients if they were diagnosed through family screening of the index case. Six families contained more than one PiZ sibling identified through family screening and these non-index pairs were analysed separately to overcome any potential ascertainment bias.

5.2.2 Lung Function Testing

Lung function tests were performed as described in Section 3.2. Patients performed dynamic spirometry after dual bronchodilatation with nebulised β_2 -agonist and ipratropium bromide. Lung volume measurements were taken using helium dilution (Morgan Medical, Kent, UK) and gas transfer measurements by the single breath carbon monoxide method. One patient did not perform any lung function tests and thus data from 59 sibling pairs in total were analysed.

5.2.3 Health Status

Health status comparisons were made in the index/non-index pairs. The St George's Respiratory Questionnaire (SGRQ) was used as a disease specific tool with a high score indicating worse health status and was completed by 51 pairs. The generic Short-Form 36 (SF36) was also used with a low score indicating worse health status and 52 pairs completed this questionnaire.

5.2.4 CT scanning

The index/non-index pairs were also assessed using computed tomography (CT) scanning of the chest to identify the presence or absence of emphysema (Naidich, 1991). The scans were reported by a single radiologist. The quantity and distribution of emphysema was not analysed in this study. Seven patients from the whole group did not have a CT scan performed, most commonly because they had recently received a recent CT scan at another centre.

5.2.5 Smoking Correction Factor

Linear regression was performed on a larger set of 408 PiZ patients to calculate the effect of smoking on FEV₁. The FEV₁, as percent of predicted, was regressed against smoking expressed in pack years. From the resultant equation, a "new" predicted value was calculated for each sibling based on the smoking history of the individual. The measured FEV₁, as expressed as percent of predicted, was then compared to the "new" predicted value to calculate a residual value for each patient: a low value indicating a worse FEV₁ than predicted.

5.3 Results

5.3.1 Patient Characteristics

Patient characteristics of the 54 pairs of index and non-index siblings are shown in Table 9.

Table 9

	Index Sibling	Non-Index Sibling	p value
Age (years)	46.9 (41.4-53.6)	44.4 (37.1-52.9)	NS*
Body mass index (kg/m²)	24.6 (22.3-27.2)	26.7 (24.4-29.7)	0.016*
Smoking history (pack years)	14.4 (4.9-24.0)	7.2 (0-15.0)	NS*
Smoking status			
Current	7	9	NS [#]
Ex	37	29	
Never	10	16	
Gender			
Male	31	35	NS [#]
Female	23	19	
Chronic Bronchitis			
Yes	26	22	NS [#]
No	28	32	
Inhaled Steroid			
Yes	34	13	<0.001 [#]
No	20	41	
Emphysema on CT scan			
Yes	44	25	<0.001 [#]
No	5	27	

Patient characteristics of index and non-index siblings. Values given as median (IQR).* Wilcoxon signed rank test; # χ^2 test. CT scanning was not performed in five index and two non-index subjects.

There was no significant difference in age, gender, presence of chronic bronchitis or baseline smoking status between the index and non-index siblings. The index patients had smoked more than non-index cases as a whole group (14.4 pack years [IQR 4.9-24.0] vs 7.2 [IQR 0-15.0]; $p=0.039$, Mann-Whitney U), although this was not significant when the two groups were compared as pairs ($p=0.085$, Wilcoxon signed rank test).

Index patients had a median body mass index (BMI) of 24.6 (IQR 22.3-27.2) which was significantly lower than the BMI of non-index sibling at 26.7 (IQR 24.4-29.7) ($p=0.016$, Wilcoxon signed rank test). More index patients were prescribed an inhaled steroid compared to their non-index siblings ($p<0.001$, χ^2 test).

Analysis of the 101 patients who had undergone CT scanning showed a 4.0 fold increase in relative risk of having emphysema on the scan if the patient had ever smoked. Index siblings ($n=49$) were more likely to have evidence of emphysema on CT scan (44 with emphysema: 5 without) compared to non-index siblings ($n=52$) (25 with emphysema: 27 without) ($p<0.001$, χ^2 test).

5.3.2 Lung Function Data

5.3.2.1 Index-Non-index Pairs

Lung function measurements taken from the patients ranged from normal to severe airflow obstruction and markedly impaired gas transfer. Data are listed in Table 10.

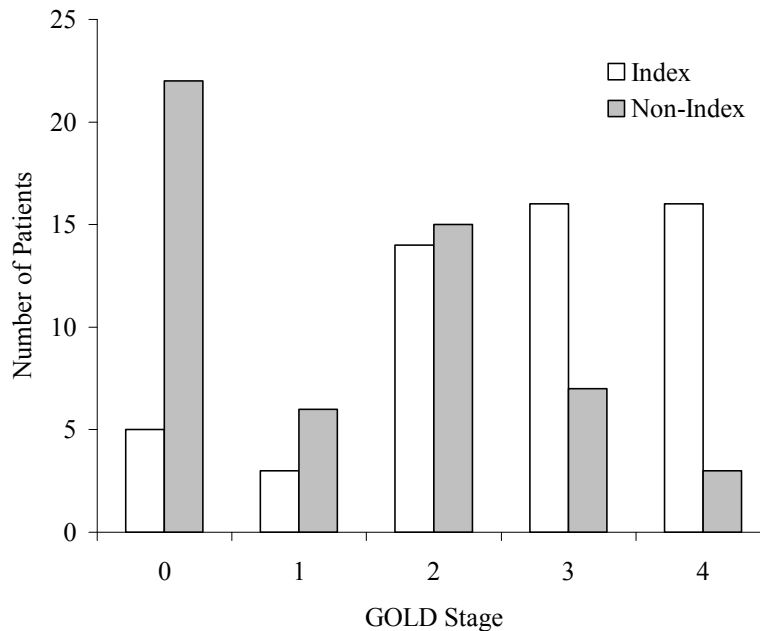
Table 10

	n	Index Sibling	Non-Index Sibling	p value*
FEV₁, (L)	53	1.31 (1.00-2.20)	2.34 (1.74-4.05)	<0.001
FEV₁ (% predicted)	53	42.4 (28.1-69.6)	82.9 (52.8-116.1)	<0.001
VC, (L)	53	3.80 (3.23-4.79)	4.43 (3.67-5.60)	0.003
VC (% predicted)	53	99.4 (83.8-113.7)	110.0 (97.6-124.4)	0.007
FEV₁/VC (%)	53	37.8 (28.3-50.0)	63.5 (43.9-80.4)	<0.001
RV, (L)	51	2.71 (2.23-3.59)	2.03 (1.73-2.70)	0.002
RV (% predicted)	51	136.9 (110.5-194.6)	109.6 (89.6-129.4)	0.001
TLC, (L)	51	7.45 (5.74-8.32)	7.12 (6.06-8.07)	NS
TLC (% predicted)	51	119.3 (110.3-133.4)	114.2 (107.0-114.2)	0.033
RV/TLC, (%)	51	39.9 (29.7-48.7)	31.7 (24.9-41.9)	<0.001
TL_{CO}, (mmol/min/kPa)	50	5.85 (4.38-8.43)	7.79 (6.77-10.07)	<0.001
TL_{CO} (% predicted)	50	62.1 (51.0-85.5)	84.5 (72.5-105.0)	<0.001
K_{CO}, (mmol/min/kPa/L)	50	1.09 (0.76-1.34)	1.40 (1.10-1.61)	<0.001
K_{CO} (% predicted)	50	69.1 (48.3-87.4)	82.8 (70.9-101.3)	<0.001
Bronchodilator reversibility				
Yes	52	18	11	NS [#]
No		34	41	

Lung function data of index and non-index siblings with paired statistical comparison of the 2 groups. Values given as median (IQR). * Wilcoxon signed rank test, # χ^2 test.

The number of patients in each group as categorised by GOLD stage (Fabbri et al, 2003) is illustrated in Figure 11.

Figure 11

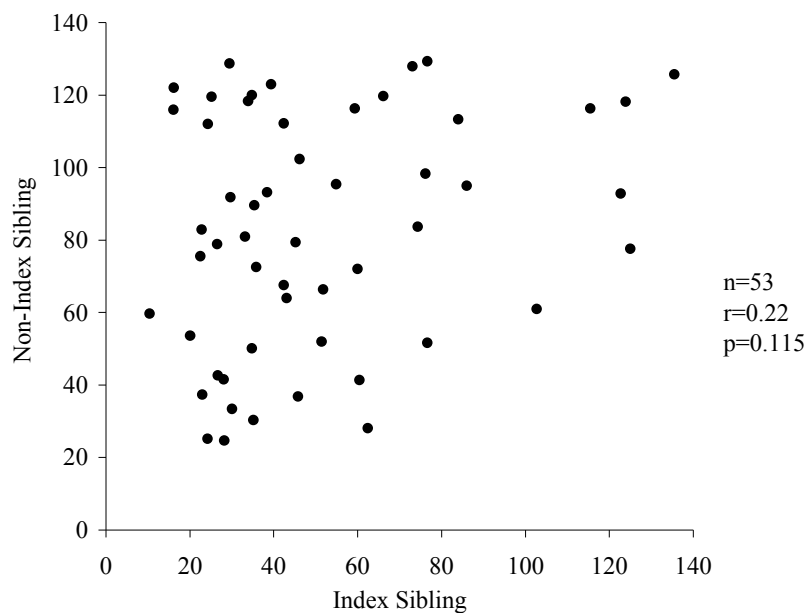


The numbers of index (white) and non-index siblings (grey) are shown categorised by GOLD stage (Fabbri et al, 2003). A significant proportion of non-index patients have airflow obstruction but this is less severe than the index cases.

Thirty one (58%) of the siblings diagnosed through family screening had evidence of airflow obstruction on testing ($FEV_1/FVC < 70\%$) and 25 (47%) patients in this group had an FEV_1 which was less than 80% predicted. However, paired comparisons between the two groups showed that the index siblings had a significantly lower FEV_1 , lower carbon monoxide transfer factor (TL_{CO}), lower gas transfer per unit volume (K_{CO}) and greater residual volume/total lung capacity ratio (RV/TLC) than their siblings (all $p < 0.001$). Index siblings also showed higher RV and TLC expressed as % predicted than their relatives ($p = 0.001$ and $p = 0.033$ respectively). There was no significant difference in bronchodilator reversibility between the pairs.

There was no correlation between the FEV₁ (Figure 12), VC or RV values (% predicted) of the index and non-index siblings.

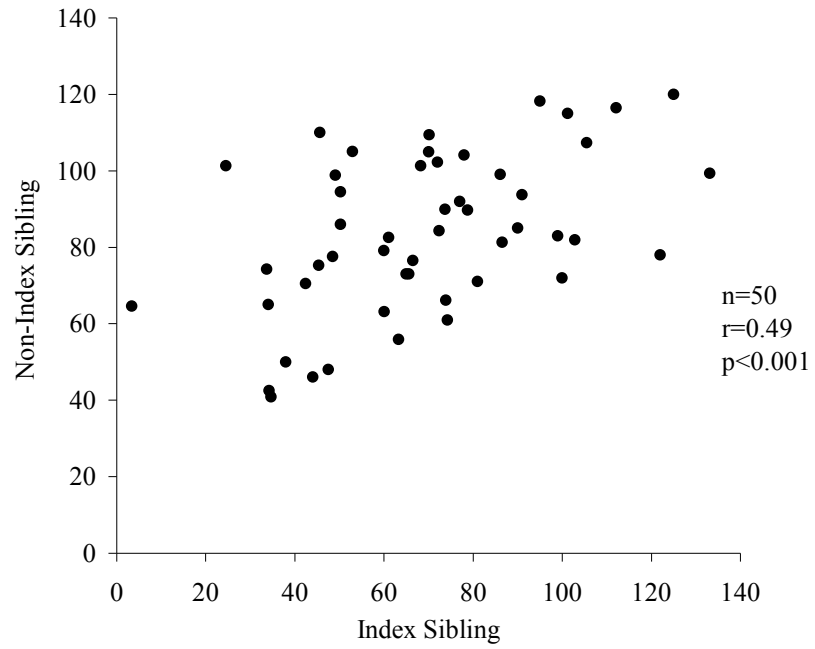
Figure 12



The scatter plot shows the FEV₁ of the sibling pairs expressed as % predicted. No correlation exists between the index and non-index siblings.

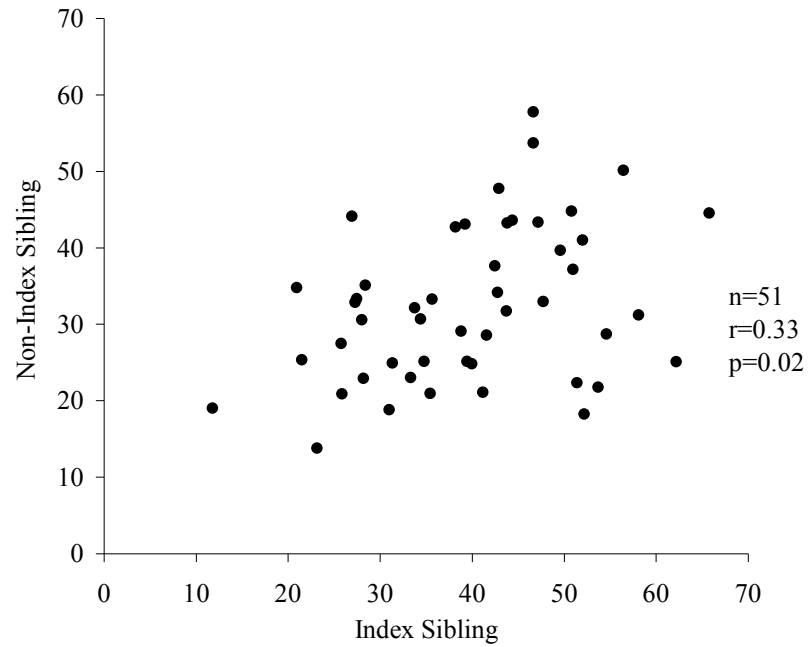
However the 2 groups showed a significant correlation of K_{CO} (r=0.49, p<0.001) (Figure 13), TL_{CO} (r=0.47, p=0.001), TLC (r=0.34, p=0.017) % predicted values and RV/TLC ratios (r=0.33, p=0.02) (Figure 14). One patient had a markedly low gas transfer due to a combination of emphysema and thromboembolic disease but removal of this outlying subject from the analysis did not alter the significance of the correlation of gas transfer values between the pairs.

Figure 13



The scatter plot shows the K_{CO} of the sibling pairs expressed as % predicted. A significant correlation is seen between the index and non-index siblings.

Figure 14

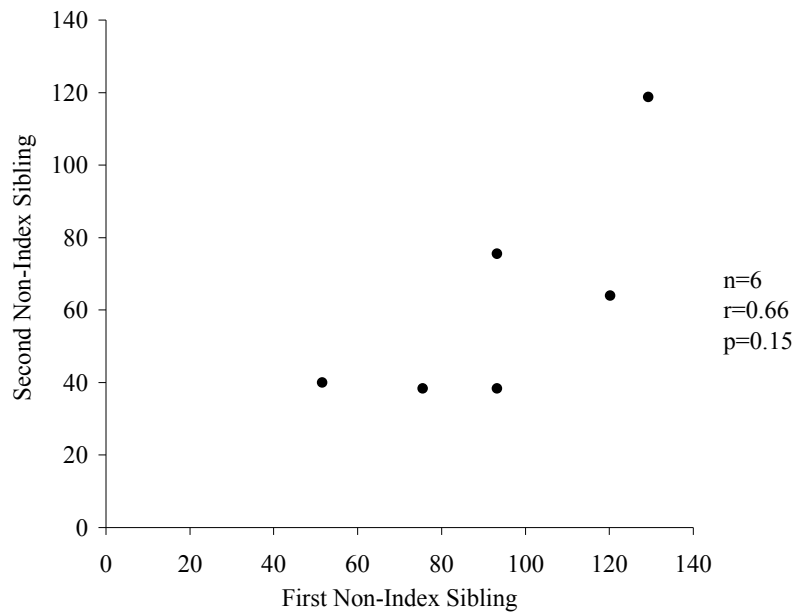


The scatter plot shows the RV/TLC ratio of the sibling pairs. A significant correlation is seen between the index and non-index siblings.

5.3.2.2 Non-index-Non-Index Pairs

Six pairs of non-index siblings were analysed separately. There was no significant correlation between these pairs for FEV₁ (Figure 15) or gas transfer (p=0.152 and p=0.551 respectively).

Figure 15



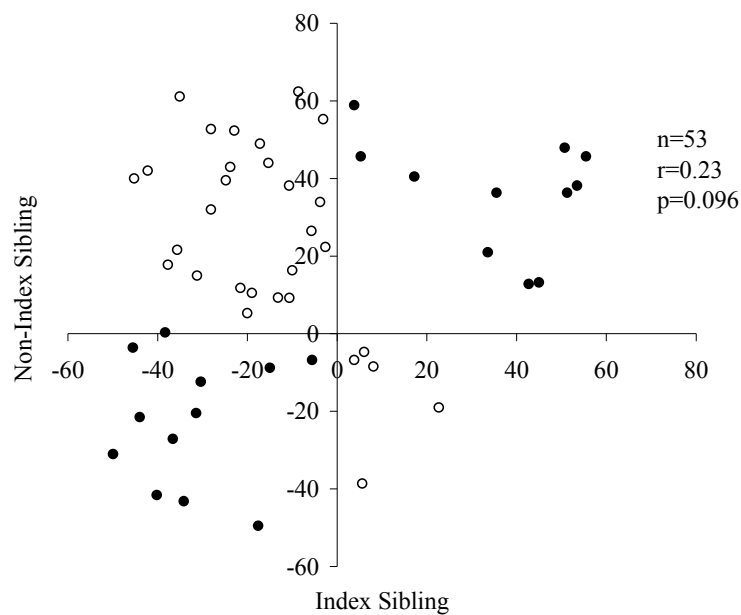
The scatter plot shows the FEV₁ of the non-index sibling pairs expressed as % predicted. No significant correlation is seen between the siblings identified through family screening.

5.3.2.3 Correction for Smoking

Linear regression showed that the number of pack years smoked was a significant variable associated with FEV₁ in a core group of 408 PiZ patients. The linear equation estimated an average reduction in FEV₁ of 1.2% of the predictive value per pack year smoked. This figure was used to calculate a predicted FEV₁ for each sibling based on their personal smoking history. This calculated value was subtracted from the measured FEV₁ (% predicted) value to obtain a residual figure. Comparison of these residual figures between siblings showed no significant correlation (Figure 16). Thirty sibling pairs showed “discordance” with one sibling having a worse FEV₁ than predicted and

the other a better FEV₁ than predicted after correction for smoking (illustrated by open circles in Figure 16).

Figure 16



The scatter plot shows the residual FEV₁ value of the sibling pairs. The residual FEV₁ was calculated by subtracting the predicted FEV₁ for each individual (based on the smoking history) from the measured FEV₁. Negative values show a measured FEV₁ that is worse than predicted and positive values a value that is better than predicted based on the smoking history. No significant correlation is seen between the index and non-index siblings after this correction for smoking differences. Discordant sibling pairs are shown by open circles and concordant siblings by closed circles.

5.3.3 Health Status Data

Health status scores also showed a wide variation and index patients had worse SGRQ scores and SF36 Physical scores than their siblings (all $p < 0.001$). Health status scores are shown in Table 11.

Table 11

	n	Index Sibling	Non-Index Sibling	p value*
SGRQ	51			
Total		53.6 (39.8-69.4)	34.0 (11.5-55.1)	<0.001
Symptoms		75.7 (61.0-86.1)	50.9 (23.8-73.6)	<0.001
Activity		72.8 (53.5-92.5)	47.3 (11.9-72.8)	<0.001
Impact		39.9 (28.5-54.2)	25.9 (5.8-46.1)	<0.001
SF-36	52			
Physical		38.1 (24.6-44.3)	45.1 (35.0-53.5)	<0.001
Mental		53.4 (43.3-58.7)	53.3 (49.1-58.8)	NS

Health Status scores of index and non-index siblings with paired statistical comparison of the 2 groups. Values given as median (IQR). * Wilcoxon signed rank test.

5.4 Discussion

The current work investigates sibling pairs with severe alpha-1-antitrypsin deficiency. The data shows that siblings identified through family screening of a proband also have a significant risk of lung disease. However the degree of impairment in these cases is on average less severe than that seen in the index patients.

It is possible that ascertainment bias may have influenced the current results. It is not surprising that index patients show significant disease, as these patients have usually been tested for AATD after presentation with respiratory symptoms and signs. It is

known that many people with AATD remain undiagnosed and that restricted testing for the condition influences the detection rate and types of patients diagnosed (Hill et al, 1999b). Thus some selection bias will have influenced the current study population. However the results are fully representative of the patients that present in respiratory practice.

Index siblings had lower body mass index, higher inhaled corticosteroid use, and worse health status than their non-index siblings. All of these measures probably reflect the more severe disease seen in the index cases. This is consistent with previous studies comparing groups of unpaired index patients and non-index subjects identified by other methods (Silverman et al, 1989).

There may also be some selection bias in the non-index siblings who are identified, despite the current practice to offer testing to all siblings of index cases that present to the UK registry. PiZ relatives who experience symptoms may perhaps be more likely to accept the offer for testing than PiZ relatives who remain symptom-free, which may explain the high incidence of disease in the identified siblings. Forty seven percent of the non-index PiZ siblings in this study had a $FEV_1 < 80\%$ predicted which is greater than the 29% of affected cases seen in siblings of usual COPD patients (McCloskey et al, 2001). Moreover, this high incidence of lung disease provides further support for screening in these families to enable smoking prevention or cessation advice to be given to susceptible family members, as well as management of exacerbations and symptoms where appropriate (American Thoracic Society et al, 2003).

An important observation is that no correlation in FEV_1 values is seen between the two groups even when values were adjusted for differences in personal smoking history. Furthermore, this discordance was also seen in the pairs of non-index siblings identified

by family screening who would not be affected by ascertainment bias, although the study numbers were small. Taken together, these results suggest that there is another factor or factors that vary between these sibling pairs that influences the susceptibility of lung disease. This could be a factor that increases the risk of one sibling or indeed a factor that has a protective effect on the less severely affected sibling. Genome scanning and linkage studies in usual COPD patients have identified genetic loci that may influence spirometric measures and lung function change (Gottlieb et al, 2001; Joost et al, 2002; Wilk et al, 2003). These or other genetic modifiers may also influence the risk of COPD development in AATD and these “discordant” siblings would be an important group to investigate further through molecular studies with this concept in mind.

On the other hand, this study has shown that gas transfer values do correlate between the pairs, indicating physiological concordance for this measure despite differences in spirometric values. This may suggest that the different phenotypes of parenchymal disease and airway disease may be affected by alternate subsets of genes which influence the underlying pathological changes. Alternatively, the parenchymal disease may be more closely related to the alpha-1-antitrypsin deficiency itself and less affected by modifier genes. These hypotheses provide a sound background for further genetic investigation of sibling pairs with AATD in the future.

Smoking remains the most important risk factor for the development and progression of lung disease in AATD (Seersholm et al, 1995; Seersholm et al, 1998) and some of the putative genes that influence lung function may do so by altering the susceptibility to smoke. Silverman et al have shown that the increased risk of airflow obstruction in relatives of early-onset usual COPD patients is restricted to smokers alone (Silverman et al, 1998). Whether this is also the case in AATD patients is unclear, although some

lifetime non-smokers do develop airflow obstruction (Janus et al, 1985; Seersholm et al, 1998). It would be of importance to determine whether the same is true of other physiological changes such as gas transfer.

In summary, the current work emphasises that other factors other than cigarette smoke contribute to the development of COPD or its phenotype in AATD patients and their families. These factors may include other genes, environmental exposures and gene-environment interactions. Sibling pairs with this condition, particularly those with discordant disease or concordant parenchymal disease, are an ideal group of patients to further investigate the influence of other candidate genes.

6 DISCORDANCE IN LUNG FUNCTION IN PATIENTS WITH AATD

6.1 Introduction

Alpha-1-antitrypsin deficiency (AATD) is associated with the development of early-onset emphysema especially in patients who smoke. Airflow obstruction and impairment of gas transfer are often seen together but this is not universal and even patients with severe airflow obstruction and prominent emphysema may have normal diffusion capacity as measured by gas transfer, indicating ‘discordant’ lung function (Guest et al, 1992; Wilson et al, 2000). These published reports relate to limited numbers of patients and so the true incidence of this phenotype is unknown. It is also unclear whether these patients with discordant lung function have different health status than those patients with both impaired gas transfer and airflow obstruction, so called ‘concordant’ lung function. The environmental and/or genetic factors that contribute to these alternate phenotypes remain unclear. Thus this study aimed to assess the frequency of physiological discordance in severe AATD and to investigate any factors which might be associated with the development of discordant disease.

6.2 Methods

Three hundred and nine patients with severe AATD (PiZ phenotype) were studied. All the patients included had measured airflow obstruction with a reduced FEV₁ (below 80 % predicted) after dual bronchodilatation. Demographic details including full smoking history were collected at a baseline visit.

6.2.1 Lung Function Testing

Lung function tests were performed as described in Section 3.2. Patients performed dynamic spirometry after bronchodilatation with nebulised β_2 -agonist and ipratropium bromide. Gas transfer was measured using the single breath carbon monoxide method.

6.2.2 Health Status

Three hundred and seven patients had health status assessment using established questionnaires. The St George's Respiratory Questionnaire (SGRQ) was used as a disease specific tool with a high score indicating worse health status. The generic Short-Form 36 (SF36) was also used with a low score indicating worse health status.

6.3 Results

6.3.1 Patient Characteristics

Fifty six patients (18%) had a K_{CO} measurement of greater than 80 % predicted despite a reduced FEV_1 and were classified as “discordant” and 253 patients (82%) had “concordant” physiology with both reduced K_{CO} and FEV_1 . Patient characteristics of these two groups are shown in Table 12.

Table 12

	K_{CO} (% predicted)		p value*
	>80	<80	
n=	56	253	
Age (Years)	51.4 ± 7.5	51.1 ± 9.5	NS [#]
Smoking (pack years)	15.8 (1.0-25.0)	19.0 (9.5-28.0)	NS
Body mass index (kg/m²)	26.4 (24.5-28.6)	24.5 (22.1-27.1)	p=0.001
Gender			
Male	36	167	NS [†]
Female	20	86	
Chronic Bronchitis			
Yes	29	106	NS [†]
No	27	147	
Index			
Yes	47	228	NS [†]
No	9	25	
FEV₁ (% Predicted)	43.9 (32.6-59.8)	39.1 (28.1-53.1)	p=0.022
FEV₁/VC (%)	42.3 (36.1-54.6)	31.9 (24.0-40.1)	P<0.001

Characteristics and FEV₁ of discordant and concordant patients. Data is shown as mean ± standard deviation or median (interquartile range). NS: not significant; * univariate comparison of the two groups; # t test; † Chi squared test; all other statistical comparison used Mann Whitney U test.

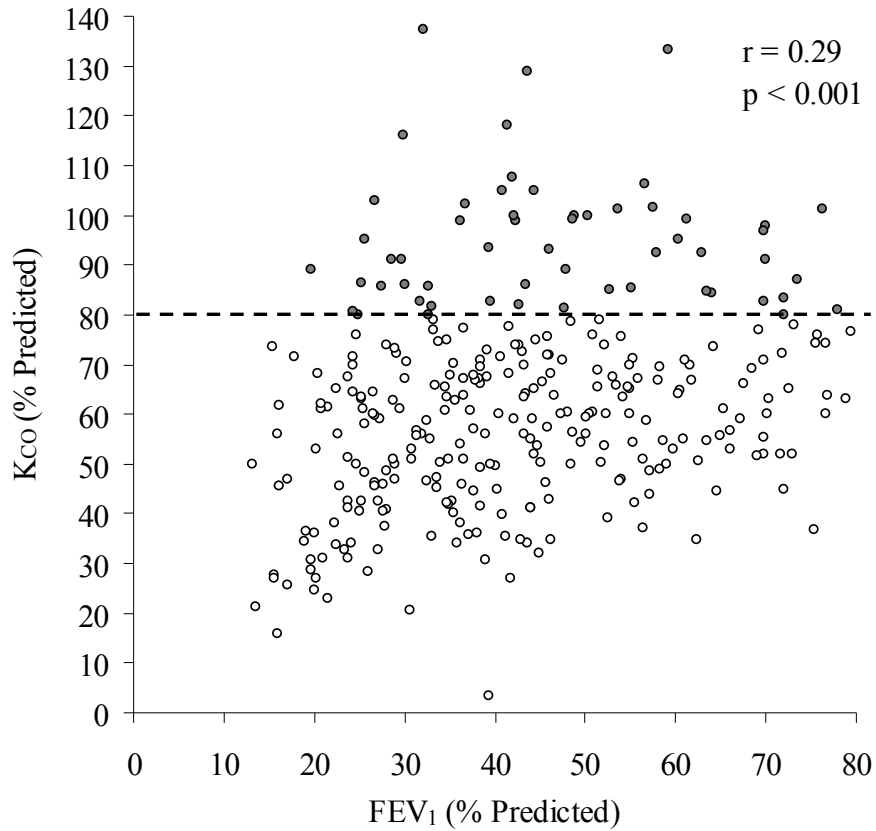
There was no difference in the age or pack years smoked between the two groups although there was a higher proportion of never smokers in the discordant group (p=0.006). Patients with discordant physiology had a higher body mass index (BMI) (26.4; IQR 24.5-28.6 vs 24.5; IQR 22.1-27.1: p=0.001) than concordant patients. The

frequency of chronic bronchitis, index status (identified through lung disease rather than family screening) and gender were not different between the two groups.

6.3.2 Lung Function Data

The relationship between FEV_1 and K_{CO} expressed as percent predicted is illustrated in Figure 17.

Figure 17



The scatter plot shows the FEV₁ and K_{CO} of the study group expressed as percent predicted. Subjects with discordant physiology (airflow obstruction but preserved gas transfer) are shown by closed circles and those with concordant physiology (airflow obstruction and impaired gas transfer) by closed circles. A significant correlation is seen between the two variables with a correlation coefficient (r) of 0.29.

Patients with discordant physiology had a higher FEV₁ % predicted (43.9; IQR 32.6-59.8 vs 39.1; IQR 28.1-53.1: p=0.022) than concordant patients.

6.3.3 Health Status Data

The health status data is summarised in Table 13.

Table 13

	K_{CO} (% predicted)		p value*
	>80	<80	
n	56	253	
SGRQ			
Total	55.1 (36.9-68.5)	55.0 (44.5-67.1)	NS
Symptoms	77.9 (49.0-85.8)	68.4 (54.0-79.5)	NS
Activity	67.0 (47.9-82.8)	73.7 (56.9-92.5)	NS
Impact	40.8 (25.0-56.2)	41.8 (28.9-54.0)	NS
SF-36			
Physical	34.5 (26.7-42.3)	34.2 (28.0-41.1)	NS
Mental	55.4 (48.7-58.2)	53.2 (44.3-59.1)	NS

Health Status scores of discordant and concordant groups with statistical comparison. Values given as median (IQR). NS: not significant.* Mann Whitney U test.

No significant difference in health status was seen for either of the questionnaires used or their domains between the discordant and concordant groups.

6.3.4 Predictors of Discordant Disease

Binary logistic regression showed that the only independent predictors for discordant disease were never smoking and higher BMI.

6.4 Discussion

This study confirms that almost many patients with severe alpha-1-antitrypsin deficiency have no impairment of gas transfer despite having evidence of airflow obstruction. These significant numbers of discordant patients are more likely to be never smokers and have a higher body mass index than patients with concordant physiological impairment. Despite these features, discordant patients had the same degree of impairment of health status as patients with both airflow obstruction and impaired gas transfer in this condition.

Routine measurement of gas transfer often includes a correction for the haemoglobin level particularly if it is markedly reduced or elevated. Other factors are known to influence the result including height gender and ethnicity but these are often not accounted for. The patient's weight and body mass index may also affect the result. One recent study (Saydain et al, 2004) found that patients with a high TL_{CO} had a higher body mass index than control subjects of comparable age and height. Furthermore, this positive correlation between body mass index persisted when the values were corrected for measured alveolar volume. Although the Saydain study was investigating subjects with increased diffusion capacity, the relationship between gas transfer and BMI supports the results seen in this current study. Greater body weight may be associated with a higher blood volume and cardiac output which in turn would increase capillary blood volume and increase gas transfer during testing. However we have taken the value of 80% predicted as the cut off for K_{CO} in this study. It is therefore possible that the patients in the current study with "preserved K_{CO} " may actually have a reduced value compared to their own "supranormal" baseline value. This stresses the

importance of viewing isolated one-off measurements in context, as a single value may not reflect the severity of gas exchange impairment in one individual.

The discordant patients in the current study were more likely to be never smokers and so this may have led to less severe parenchymal disease. Indeed these patients also had significantly less airflow obstruction. The lower body mass index seen in the concordant group may be a reflection of more severe underlying disease. Despite this no significant difference in health status was seen between the two groups.

Another possibility is that these two groups of patients may have different phenotypic expression of parenchymal disease. In usual COPD the emphysema described classically affects the upper zones of the lungs (Thurlbeck, 1963). Klein et al have described COPD patients with impaired gas transfer but relatively preserved airflow (Klein et al, 1992), which is the opposite to the pattern described here. This may suggest that the pattern and distribution of emphysema may influence impairment seen in physiological testing. Parr et al performed quantitative CT scanning in a group of 102 patients with severe AATD and emphysema and found that 37% of subjects had greater involvement in the upper regions (Parr et al, 2004). This is in contrast to the basal distribution of emphysema in AATD reported prior to the advent of CT (Gishen et al, 1982; Mazodier et al, 1971). In addition, Parr et al found that subjects with basal-distributed emphysema showed greater impairment of FEV₁ but less impairment of gas exchange than patients with apical- distributed disease despite being matched for total volume of emphysema and for age. These differences may be explained by the differences in ventilation and perfusion ratio that are seen in the lungs even in health. The ventilation/perfusion ratio is more than four times greater in the apices compared to the lung bases (West, 1992). It may be that patients with basal emphysema are able to

maintain effective gas exchange due to increased perfusion of relatively normal lung in the upper zones. However patients with apical disease are unlikely to be able to affect a further increase in the already well perfused bases.

This study has used an arbitrary level of abnormality as 80% predicted. However this level does not define the lower limit of normal and some patients with values below this level will have normal lung function (Pellegrino et al, 2005). The value of 80% was chosen as an arbitrary cut off in the absence of firm guidance on the optimum method of expressing the severity of lung function. This cut off point may artificially distort the presented data as many more patients than listed will have normal gas transfer. Thus the proportion of patients with “discordant” disease is likely to be higher than the numbers presented here.

It is unclear whether the discordant and concordant groups of patients in this study are at different points in the natural history and expression of AATD or if they represent discrete phenotypic subsets. One study on selective CT quantification showed that upper zone analysis was related to all-cause and respiratory mortality (Dawkins et al, 2003). This may relate to more severe disease progressing from the bases to the apices. However it may also represent differences in gas exchange related to emphysema distribution. Why this has no influence on health status and how this affects disease progression needs further study.

Further investigation including genetic characterisation, as well as longer term follow up, of these heterogeneous subsets of AATD patients will improve our understanding of the natural history of this condition. In particular modifier genes may influence both emphysema distribution and progression and this should be the basis for future studies.

7 GENERAL DISCUSSION

The current body of work investigates patients selected from the UK registry of alpha-1-antitrypsin deficiency in Birmingham. One limitation of such studies which use patient registries and cohorts is the ascertainment bias of the subjects studied. In the UK registry of AATD, 76% of PiZ patients have been identified following investigation of respiratory symptoms or disease and 19% through family screening. This is similar to the proportions reported in the National Heart Lung and Blood Institute (NHLBI) registry (McElvaney et al, 1997). In the current registry, only 3% of patients have been identified as a result of liver disease, although it is likely that greater numbers of such patients are identified and treated elsewhere in the UK.

These groups of patients are not fully representative of the whole population of people with this deficiency, as recruitment through chest physicians means that symptomatic patients are more likely to be enrolled and studied. Population screening is not widely performed although one large cohort study is ongoing in Sweden with subjects identified through screening at birth (Piitulainen et al, 2002). However the subjects in this Swedish study are still less than thirty years of age and so much of the data on the natural history of the condition through adult life remains to be collected and studied.

Many people with AATD within the UK population will not have symptoms nor will have significant lung function impairment and currently these people are often only detected through family screening (Silverman et al, 1989). Furthermore even symptomatic individuals will only be identified if the screening test is done and this is influenced by local and national practice. Many patients experience a delay of up to 10 years between the onset of symptoms and diagnosis (Stoller et al, 1994). Thus any

descriptive data obtained through registry studies on the natural history of the condition cannot be translated to the whole PiZ population, many of whom are not recognised as having AATD. However this does not mean that such studies are less useful. Indeed it is unlikely that testing for AATD will be introduced into the whole population until a definitive treatment is established and so the current targeted methods of identification of these individuals will remain. A single centre registry has many advantages for study of these patients ensuring consistent measurements, investigation and treatment. It is likely that in the future such specialist centres for the investigation and treatment of AATD will be disseminated throughout the UK.

Another type of selection bias that may affect studies involving patient registries is the survivor effect, as patients who remain in the study population may retain a certain degree of health in order to continue their attendance. This is particularly the case if patients have to travel a great distance to the specialist centre.

Alpha-1-antitrypsin replacement is currently not licensed for treatment in the UK. However, intravenous replacement is a recognised treatment for deficient patients in other countries including the USA, Canada, Germany, Spain, Italy and Sweden. Currently there is no clear evidence for the effectiveness of this treatment, as there have been no conclusive controlled studies to demonstrate an influence on lung disease. Studies have adequately demonstrated that weekly or monthly infusions of AAT are safe and can increase plasma and lung fluid levels of AAT (Hubbard et al, 1988; Wewers et al, 1987). However it is less clear whether this treatment holds any clinical benefit as, to date, any trials have been underpowered or not adequately controlled. Clinical trials of any new treatments will need to use defined outcome measures to

assess an objective response. The effects of the treatment on the frequency and severity of exacerbations will be important. Furthermore the quality of life of the patients should be investigated alongside symptoms to assess the clinical impact of the treatment. These measures could be used together with pulmonary function testing and emphysema quantification by CT scan. In order to use these measures as outcomes for trials, we must have clear data on the reproducibility and progression of these parameters in the natural history of the disease.

The current standard method for monitoring disease progression in COPD and for monitoring the effect of interventions is to measure pulmonary function, in particular FEV₁. Spirometry has the advantage of a wide evidence base and is readily available, inexpensive and relatively simple to perform under supervision of a trained technician. However this measurement can be variable and the accuracy of the value obtained is dependent on many factors. Furthermore pulmonary function measurements do not directly translate into clinically important values for patients and so other outcome measures, such as exacerbations and health status, should be included in any interventional trials.

In order for these measurements to be included into trial outcomes accurate information is required on the nature and effect of exacerbations in this condition and the effect on the health status of patients. Hence the first aims of the current study were to investigate these features in a large cohort of AATD patients. The frequency, duration, timing and nature of exacerbations were studied as well as host factors that may be associated with them. Furthermore the impact of exacerbations on health related quality of life as measured by questionnaire was also measured.

The results showed that exacerbations occur frequently in patients with AATD and the number of exacerbations for the whole group was similar to that seen in non AATD COPD in general (Seemungal et al, 1998). Nearly one third of exacerbations occurred in December and January and the presence and frequency of exacerbations was generally consistent year on year. The median length of symptoms in these patients is 14 days which is greater than the 7 days seen in usual COPD (Seemungal et al, 2000a) although the current study used different methods for data collection. Patients with chronic bronchitis showed an increased frequency of exacerbations, confirming previous findings (Dowson et al, 2002).

Exacerbations were shown to have an influence on health status scores which supports data obtained by other groups (Doll et al, 2002; Seemungal et al, 1998). Furthermore this association was shown not to be due to a difference in lung function alone as exacerbations were found to have an additional independent effect on health status scores using regression analysis. Increasing age, chronic bronchitis, and increase in pack year smoking history as well as reduced FEV₁ were all shown to have independent effects on health status scores irrespective of exacerbations.

Despite major changes in lung function over the 3 year study period health status did not decline. It is possible that this is due to benefits of being seen in a specialist clinic for alpha-1-antitrypsin deficiency.

A further aim of the current work was to investigate the changes in lung function and health status in patients with severe alpha-1-antitrypsin deficiency over time. Lung physiology measurements were shown to deteriorate in these patients over time. However the decline in spirometric values was not related to the frequency of

exacerbations in the whole group or in subgroups according to airflow obstruction. This is contrary to the findings in patients with usual COPD (Donaldson et al, 2002). The current study did however show that the number of exacerbations was associated with decline in gas transfer.

These study data provide a firm basis upon which to design, power and implement clinical trials in alpha-1-antitrypsin deficiency using exacerbations and health status as outcome measures.

Cigarette smoking is the most important environmental risk factor for COPD and yet only around 15% of smokers develop clinically significant disease (Fletcher et al, 1977). Genetic factors are also important in the development of the disease as identified in alpha-1-antitrypsin deficiency. Other genetic factors are likely to also influence disease and this is supported by the observation of higher incidence of airflow obstruction in first-degree relatives of patients with usual COPD (Kueppers et al, 1977). Other studies of families with early-onset COPD show lower FEV₁ values in relatives of probands despite similar age and smoking history (Silverman et al, 1998). These same or other host factors may also influence the expression of the disease within the individual and the progression of the disease over time. The Silverman study showed that the increased risk for airflow obstruction was limited to current or ex-smokers, with no risk of reduced FEV₁ in lifelong non-smokers. This supports the hypothesis of a genotype-environment interaction which is defined as a non-additive contribution of gene and environment towards the clinical phenotype. Thus the two influences confer a different level of risk than that expected by simply adding each effect together.

These gene-environment interactions may also be important in the expression of disease in alpha-1-antitrypsin deficiency. There has been little evidence to support genetic modifiers in families with more than one homozygote member although studies of heterozygote relatives using pulmonary function and respiratory questionnaire do suggest familial aggregation (Silverman et al, 1989; Silverman et al, 1990b). Furthermore parents of diseased PiZ individuals were more likely to report a history of emphysema than parents of unaffected PiZ individuals in the same study, which is consistent with COPD aggregation in families.

Sibling pairs with this deficiency have not been widely studied although case reports of individual sibships have suggested that the lung disease of PiZ siblings may be discordant (Apprill et al, 1990; Stableforth, 1978). This led to a further aim of the current work to identify sibling pairs of patients with severe deficiency and to compare cross-sectional lung function data and health status measurements between index and non-index siblings.

The current study shows that there is no correlation in FEV₁ values between the index siblings and non-index siblings, even when values were adjusted for differences in personal smoking history. Furthermore, this discordance was also seen in the pairs of non-index siblings identified by family screening who would not be affected by ascertainment bias, although the study numbers were small. Taken together, these results suggest that there is another factor or factors that vary between these sibling pairs that influences the susceptibility of lung disease. This could be a factor that increases the risk of one sibling or indeed a factor that has a protective effect on the less severely affected sibling. It is more likely that there are a number of factors at play.

In contrast to the spirometry results, this study has shown that gas transfer values do correlate between the sibling pairs, indicating physiological concordance for this measure. This may suggest that the different phenotypes of parenchymal disease and airway disease may be affected by alternate subsets of genes which influence the underlying pathological changes. Alternatively, the parenchymal disease may be more closely related to the alpha-1-antitrypsin deficiency itself and less affected by modifier genes. These hypotheses provide a sound background for further genetic investigation of sibling pairs with AATD in the future.

The final part of the current work set out to investigate the presence of physiological discordance in a larger set of individuals with AATD. It was found that significant numbers, almost one fifth of patients, with severe alpha-1-antitrypsin deficiency have no impairment of gas transfer despite having evidence of airflow obstruction. These discordant patients are more likely to be never smokers and also have a higher body mass index than patients with concordant physiological impairment. However despite preservation of gas transfer values, discordant patients show the same degree of impairment of health status as patients with airflow obstruction and impaired gas transfer in this condition.

COPD is a complex disease and a variety of phenotypes are expressed even within a well-defined population such as the current study subjects. Thus it is likely that there are a number of genes and genotype-environment interactions that have additive, synergistic or even protective effects. These complex interactions should be accounted for when interpreting the results of genetic studies.

Some genetic loci associated with spirometric measures and lung function change have already been identified in patients with usual COPD (Gottlieb et al, 2001; Joost et al, 2002; Wilk et al, 2003). These or other genetic modifiers may also influence the risk of COPD development in AATD. Genome wide linkage and association studies in families of PiZ individuals, similar to those performed in usual COPD, may identify other putative genes in the future and the “discordant” sibling pairs identified in the current work would be an ideal group for investigation with this concept in mind. Careful selection of a study population containing well characterised families with disparate lung function would improve the potential for such work to identify other genetic factors that are associated with phenotype. Furthermore study of subsets of PiZ individuals with differing patterns of lung function impairment may also yield results. These studies may lead to identification of putative genes that modify and influence the development and progression of lung disease in AATD subjects. Any positive findings may then be studied further in COPD patients without severe AAT deficiency. This may lead to improved understanding of the pathogenesis of lung disease in patients with and without alpha-1-antitrypsin deficiency.

8 REFERENCES

Guidelines for the measurement of respiratory function: recommendations of the British Thoracic Society and the Association of Respiratory Technicians and Physiologists. *Respir Med* 1994;**88**:165-194.

American Thoracic Society, European Respiratory Society. Standards for the Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency. *Am J Respir Crit Care Med* 2003;**168**:818-900.

Anderson HR, Spix C, Medina S, Schouten JP, Castellsague J, Rossi G. Air pollution and daily admissions for chronic obstructive pulmonary disease in 6 European cities: results from the APHEA project. *Eur.Respir.J.* 1997;**10**:1064-1071.

Anthonisen NR, Manfreda J, Warren CP, Hershfield ES, Harding GK, Nelson NA. Antibiotic therapy in exacerbations of chronic obstructive pulmonary disease. *Ann.Intern.Med* 1987;**106**:196-204.

Apprill M, North ML, Mirhom R, Weitzenblum E. Alpha-1-antitrypsin deficiency in a large sibship. *Respiration* 1990;**57**:310-312.

Ball P, Harris JM, Lowson D, Tillotson G, Wilson R. Acute infective exacerbations of chronic bronchitis. *QJM.* 1995;**88**:61-68.

Beatty K, Bieth J, Travis J. Kinetics of association of serine proteinases with native and oxidized alpha-1-proteinase inhibitor and alpha-1-antichymotrypsin. *J Biol.Chem.* 1980;**255**:3931-3934.

Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax* 2000;**55**:114-20.

Billingsley GD, Walter MA, Hammond GL, Cox DW. Physical mapping of four serpin genes: alpha 1-antitrypsin, alpha 1-antichymotrypsin, corticosteroid-binding globulin, and protein C inhibitor, within a 280-kb region on chromosome I4q32.1. *Am J Hum.Genet.* 1993;**52**:343-353.

Black LF, Kueppers F. alpha1-Antitrypsin deficiency in nonsmokers. *Am.Rev.Respir.Dis.* 1978;**117**:421-428.

Blakemore WS, Forester RE, Morton JW, Ogilvie C. A standardized breath holding technique for the clinical measurement of the diffusing capacity of the lung for carbon monoxide. *J Clin Invest* 1957;**36**:1-17.

Brazier JE, Harper R, Jones NM, O'Cathain A, Thomas KJ, Usherwood T, Westlake L. Validating the SF-36 health survey questionnaire: new outcome measure for primary care. *B.M.J.* 1992;**305**:160-4.

Burge PS, Calverley PM, Jones PW, Spencer S, Anderson JA, Maslen TK. Randomised, double blind, placebo controlled study of fluticasone propionate in patients with moderate to severe chronic obstructive pulmonary disease: the ISOLDE trial. *B.M.J.* 2000;**320**:1297-1303.

Burrows B, Knudson RJ, Cline MG, Lebowitz MD. Quantitative relationships between cigarette smoking and ventilatory function. *Am Rev.Respir Dis.* 1977;**115**:195-205.

Burrows B, Lebowitz MD, Camilli AE, Knudson RJ. Longitudinal changes in forced expiratory volume in one second in adults. Methodologic considerations and findings in healthy nonsmokers. *Am Rev.Respir Dis.* 1986;**133**:974-980.

Cantor JO, Cerreta JM, Armand G, Turino GM. Aerosolized hyaluronic acid decreases alveolar injury induced by human neutrophil elastase. *Proc.Soc.Exp.Biol.Med* 1998;**217**:471-475.

Casaburi R, Mahler DA, Jones PW, Wanner A, San PG, ZuWallack RL, Menjoge SS, Serby CW, Witek T, Jr. A long-term evaluation of once-daily inhaled tiotropium in chronic obstructive pulmonary disease. *Eur Respir J* 2002;**19**:217-224.

Celli BR, Barnes PJ. Exacerbations of chronic obstructive pulmonary disease. *Eur.Respir J* 2007;**29**:1224-1238.

Cichy J, Potempa J, Travis J. Biosynthesis of alpha1-proteinase inhibitor by human lung-derived epithelial cells. *J Biol.Chem.* 1997;**272**:8250-8255.

Cote CG, Dordelly LJ, Celli BR. Impact of COPD exacerbations on patient-centered outcomes. *Chest* 2007;**131**:696-704.

Cuvelier A, Muir JF, Hellot MF, Benhamou D, Martin JP, Benichou J, Sesboue R. Distribution of alpha(1)-antitrypsin alleles in patients with bronchiectasis. *Chest* 2000;**117**:415-419.

Dawkins PA, Dowson LJ, Guest PJ, Stockley RA. Predictors of mortality in alpha1-antitrypsin deficiency. *Thorax* 2003;**58**:1020-1026.

Dewan NA, Rafique S, Kanwar B, Satpathy H, Ryschon K, Tillotson GS, Niederman MS. Acute exacerbation of COPD: factors associated with poor treatment outcome. *Chest* 2000;**117**:662-71.

Dirksen A, Dijkman JH, Madsen F, Stoel B, Hutchison DC, Ulrik CS, Skovgaard LT, Kok-Jensen A, Rudolphus A, Seersholm N, Vrooman HA, Reiber JH, Hansen NC, Heckscher T, Viskum K, Stolk J. A randomized clinical trial of alpha(1)-antitrypsin augmentation therapy. *Am J Respir Crit Care Med* 1999;**160**:1468-72.

Doll H, Grey-Amante P, Duprat-Lomon I, Sagnier PP, Thate-Waschke I, Lorenz J, Rychlik R, Pfeil T. Quality of life in acute exacerbation of chronic bronchitis: results from a German population study. *Respir.Med.* 2002;**96**:39-51.

Donaldson GC, Seemungal T, Jeffries DJ, Wedzicha JA. Effect of temperature on lung function and symptoms in chronic obstructive pulmonary disease. *Eur Respir J* 1999;**13**:844-849.

Donaldson GC, Seemungal TA, Bhowmik A, Wedzicha JA. Relationship between exacerbation frequency and lung function decline in chronic obstructive pulmonary disease. *Thorax* 2002;**57**:847-852.

Donaldson GC, Wilkinson TM, Hurst JR, Perera WR, Wedzicha JA. Exacerbations and time spent outdoors in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2005;**171**:446-452.

Dowson LJ, Guest PJ, Stockley RA. Longitudinal changes in physiological, radiological, and health status measurements in alpha(1)-antitrypsin deficiency and factors associated with decline. *Am J Respir Crit Care Med* 2001a;**164**:1805-9.

Dowson LJ, Guest PJ, Stockley RA. The relationship of chronic sputum expectoration to physiologic, radiologic, and health status characteristics in alpha(1)-antitrypsin deficiency (PiZ). *Chest* 2002;**122**:1247-1255.

Dowson LJ, Newall C, Guest PJ, Hill SL, Stockley RA. Exercise capacity predicts health status in alpha(1)-antitrypsin deficiency. *Am J Respir Crit Care Med* 2001b;**163**:936-41.

Dubois AB, Botelho SY, Bedell GN, Marshall R, Comroe JHJ. A rapid plethysmographic method for measuring thoracic gas volume: comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects. *J Clin. Invest* 1956;**35**:322-326.

Eden E, Hammel J, Rouhani FN, Brantly ML, Barker AF, Buist AS, Fallat RJ, Stoller JK, Crystal RG, Turino GM. Asthma features in severe alpha 1-antitrypsin deficiency: experience of the National Heart, Lung, and Blood Institute Registry. *Chest* 2003;**123**:765-771.

Elliott PR, Bilton D, Lomas DA. Lung polymers in Z alpha 1-antitrypsin deficiency-related emphysema. *Am J Respir Cell Mol. Biol.* 1998;**18**:670-674.

Eriksson S. Pulmonary emphysema and alpha-1-antitrypsin deficiency. *Acta Med Scand* 1963;**175**:197-205.

Evald T, Dirksen A, Keittelmann S, Viskum K, Kok-Jensen A. Decline in pulmonary function in patients with alpha 1-antitrypsin deficiency. *Lung* 1990;**168 Suppl**:579-85.

Fabbri LM, Hurd SS, for the GOLD Scientific Committee. Global Strategy for the Diagnosis, Management and Prevention of COPD: 2003 update. *Eur Respir J* 2003;**22**:1-2.

Fagerhol MK, Hauge HE. Serum Pi types in patients with pulmonary diseases. *Acta Allergol.* 1969;**24**:107-114.

Fletcher C, Peto R. The natural history of chronic airflow obstruction. *Br. Med. J.* 1977;**1**:1645-1648.

Flotte TR. Recombinant adeno-associated virus gene therapy for cystic fibrosis and alpha(1)-antitrypsin deficiency. *Chest* 2002;**121**:98S-102S.

Foreman MG, Demeo DL, Hersh CP, Reilly JJ, Silverman EK. Clinical Determinants of Exacerbations in Severe, Early-onset COPD. *Eur. Respir J* 2007.

Gadek JE, Fells GA, Crystal RG. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science* 1979;**206**:1315-1316.

Garcia-Aymerich J, Tobias A, Anto JM, Sunyer J. Air pollution and mortality in a cohort of patients with chronic obstructive pulmonary disease: a time series analysis. *J Epidemiol Community Health* 2000;**54**:73-74.

Gevenois PA, Yernault JC. Can computed tomography quantify pulmonary emphysema? *Eur Respir J* 1995;**8**:843-848.

Gishen P, Saunders AJ, Tobin MJ, Hutchison DC. Alpha 1-antitrypsin deficiency: the radiological features of pulmonary emphysema in subjects of Pi type Z and Pi type SZ: a survey by the British Thoracic Association. *Clin Radiol.* 1982;**33**:371-377.

Gompertz S, Bayley DL, Hill SL, Stockley RA. Relationship between airway inflammation and the frequency of exacerbations in patients with smoking related COPD. *Thorax* 2001;**56**:36-41.

Gore JM, Brophy CJ, Greenstone MA. How well do we care for patients with end stage chronic obstructive pulmonary disease (COPD)? A comparison of palliative care and quality of life in COPD and lung cancer. *Thorax* 2000;**55**:1000-1006.

Gottlieb DJ, Wilk JB, Harmon M, Evans JC, Joost O, Levy D, O'Connor GT, Myers RH. Heritability of longitudinal change in lung function. The Framingham study. *Am J Respir Crit Care Med* 2001;**164**:1655-9.

Greenberg SB, Allen M, Wilson J, Atmar RL. Respiratory viral infections in adults with and without chronic obstructive pulmonary disease. *Am.J.Respir.Crit Care Med.* 2000;**162**:167-173.

Gross P, PFITZER EA, TOLKER E, BABYAK MA, KASCHAK M. Experimental emphysema: its production with papin in normal and silicotic rats. *Arch.Environ.Health* 1965;**11**:50-58.

Group TA-ADRS. Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. *Am J Respir Crit Care Med* 1998;**158**:49-59.

Guest PJ, Hansell DM. High resolution computed tomography (HRCT) in emphysema associated with alpha-1-antitrypsin deficiency. *Clin Radiol.* 1992;**45**:260-266.

Guyatt GH, Berman LB, Townsend M, Pugsley SO, Chambers LW. A measure of quality of life for clinical trials in chronic lung disease. *Thorax* 1987a;**42**:773-778.

Guyatt GH, Townsend M, Pugsley SO, Keller JL, Short HD, Taylor DW, Newhouse MT. Bronchodilators in chronic air-flow limitation. Effects on airway function, exercise capacity, and quality of life. *Am Rev Respir Dis*. 1987b;**135**:1069-1074.

Halbert RJ, Isonaka S, George D, Iqbal A. Interpreting COPD prevalence estimates: what is the true burden of disease? *Chest* 2003;**123**:1684-1692.

Harik-Khan RI, Fleg JL, Wise RA. Body mass index and the risk of COPD. *Chest* 2002;**121**:370-6.

Hill AT, Campbell EJ, Bayley DL, Hill SL, Stockley RA. Evidence for excessive bronchial inflammation during an acute exacerbation of chronic obstructive pulmonary disease in patients with alpha(1)-antitrypsin deficiency (PiZ). *Am J Respir Crit Care Med* 1999a;**160**:1968-75.

Hill AT, Campbell EJ, Hill SL, Bayley DL, Stockley RA. Association between airway bacterial load and markers of airway inflammation in patients with stable chronic bronchitis. *Am J Med* 2000;**109**:288-95.

Hill AT, Campbell EJ, Ward AM, Stockley RA. Chronic obstructive pulmonary disease, with and without alpha-1-antitrypsin deficiency: management practices in the U.K. *Respir Med* 1999b;**93**:481-90.

Hubbard RC, Crystal RG. Alpha-1-antitrypsin augmentation therapy for alpha-1-antitrypsin deficiency. *Am J Med* 1988;**84**:52-62.

Hubbard RC, Fells G, Gadek J, Pacholok S, Humes J, Crystal RG. Neutrophil accumulation in the lung in alpha 1-antitrypsin deficiency. Spontaneous release of leukotriene B4 by alveolar macrophages. *J Clin Invest* 1991;**88**:891-7.

Hurst JR, Wedzicha JA. The biology of a chronic obstructive pulmonary disease exacerbation. *Clin Chest Med* 2007;**28**:525-536.

Hutchison DC. Alpha 1-antitrypsin deficiency in Europe: geographical distribution of Pi types S and Z. *Respir Med* 1998;**92**:367-377.

Janoff A, Carp H, Lee DK, Drew RT. Cigarette smoke inhalation decreases alpha 1-antitrypsin activity in rat lung. *Science* 1979;**206**:1313-1314.

Janus ED, Phillips NT, Carrell RW. Smoking, lung function, and alpha 1-antitrypsin deficiency. *Lancet* 1985;**1**:152-154.

Jeppsson JO. Amino acid substitution Glu leads to Lys alpha1-antitrypsin PiZ. *FEBS Lett.* 1976;**65**:195-197.

Jones PW. Health status measurement in chronic obstructive pulmonary disease. *Thorax* 2001;**56**:880-7.

Jones PW. Interpreting thresholds for a clinically significant change in health status in asthma and COPD. *Eur.Respir J.* 2002;**19**:398-404.

Jones PW, Bosh TK. Quality of life changes in COPD patients treated with salmeterol. *Am J Respir Crit Care Med* 1997;**155**:1283-9.

Jones PW, Quirk FH, Baveystock CM, Littlejohns P. A self-complete measure of health status for chronic airflow limitation. The St. George's Respiratory Questionnaire. *Am Rev Respir Dis* 1992;**145**:1321-7.

Jones PW, Willits LR, Burge PS, Calverley PM. Disease severity and the effect of fluticasone propionate on chronic obstructive pulmonary disease exacerbations. *Eur.Respir.J.* 2003;**21**:68-73.

Joost O, Wilk JB, Cupples LA, Harmon M, Shearman AM, Baldwin CT, O'Connor GT, Myers RH, Gottlieb DJ. Genetic loci influencing lung function: a genome-wide scan in the Framingham Study. *Am J Respir Crit Care Med* 2002;**165**:795-9.

Kanner RE, Anthonisen NR, Connett JE. Lower Respiratory Illnesses Promote FEV(1) Decline in Current Smokers But Not Ex-Smokers with Mild Chronic Obstructive Pulmonary Disease. Results from the lung health study. *Am J Respir Crit Care Med* 2001;**164**:358-364.

King MA, Stone JA, Diaz PT, Mueller CF, Becker WJ, Gadek JE. Alpha 1-antitrypsin deficiency: evaluation of bronchiectasis with CT. *Radiology* 1996;**199**:137-141.

Klein JS, Gamsu G, Webb WR, Golden JA, Muller NL. High-resolution CT diagnosis of emphysema in symptomatic patients with normal chest radiographs and isolated low diffusing capacity. *Radiology* 1992;**182**:817-821.

Koj A, Regoeczi E, Toews CJ, Leveille R, Gauldie J. Synthesis of antithrombin III and alpha-1-antitrypsin by the perfused rat liver. *Biochim.Biophys.Acta* 1978;**539**:496-504.

Kueppers F, Miller RD, Gordon H, Hepper NG, Offord K. Familial prevalence of chronic obstructive pulmonary disease in a matched pair study. *Am J Med* 1977;**63**:336-342.

Kuwano K, Matsuba K, Ikeda T, Murakami J, Araki A, Nishitani H, Ishida T, Yasumoto K, Shigematsu N. The diagnosis of mild emphysema. Correlation of computed tomography and pathology scores. *Am Rev Respir Dis* 1990;**141**:178.

Lacasse Y, Wong E, Guyatt GH, King D, Cook DJ, Goldstein RS. Meta-analysis of respiratory rehabilitation in chronic obstructive pulmonary disease. *Lancet* 1996;**348**:1115-1119.

Laurell CB, Eriksson S. The electrophoretic alpha1 globulin pattern of serum in alpha1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963;**15**:132-140.

Lebowitz MD, Knudson RJ, Burrows B. Tucson epidemiologic study of obstructive lung diseases. I: Methodology and prevalence of disease. *Am J Epidemiol.* 1975;**102**:137-152.

Lieberman J. Heterozygous and homozygous alpha-antitrypsin deficiency in patients with pulmonary emphysema. *N.Engl.J Med* 1969;**281**:279-284.

Lieberman J. Augmentation therapy reduces frequency of lung infections in antitrypsin deficiency: a new hypothesis with supporting data. *Chest* 2000;**118**:1480-5.

Lieberman J, Winter B, Sastre A. Alpha 1-antitrypsin Pi-types in 965 COPD patients. *Chest* 1986;**89**:370-373.

Liou TG, Campbell EJ. Nonisotropic enzyme--inhibitor interactions: a novel nonoxidative mechanism for quantum proteolysis by human neutrophils. *Biochemistry* 1995;**34**:16171-7.

Lomas DA, Evans DL, Stone SR, Chang WS, Carrell RW. Effect of the Z mutation on the physical and inhibitory properties of alpha 1-antitrypsin. *Biochemistry* 1993;**32**:500-508.

Lopez AD, Murray CC. The global burden of disease, 1990-2020. *Nat.Med* 1998;**4**:1241-1243.

Mahler DA, Faryniarz K, Tomlinson D, Colice GL, Robins AG, Olmstead EM, O'Connor GT. Impact of dyspnea and physiologic function on general health status in patients with chronic obstructive pulmonary disease. *Chest* 1992;**102**:395-401.

Malerba M, Radaeli A, Ceriani L, Tantucci C, Grassi V. Airway hyperresponsiveness in a large group of subjects with alpha1-antitrypsin deficiency: a cross-sectional controlled study. *J Intern.Med.* 2003;**253**:351-358.

Malhotra A, Peiffer AP, Ryuji DT, Elsner T, Kanner RE, Leppert MF, Hasstedt SJ. Further evidence for the role of genes on chromosome 2 and chromosome 5 in the inheritance of pulmonary function. *Am J Respir Crit Care Med* 2003;**168**:556-561.

Massaro GD, Massaro D. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. *Nat.Med* 1997;**3**:675-677.

Matheson MC, Benke G, Raven J, Sim MR, Kromhout H, Vermeulen R, Johns DP, Walters EH, Abramson MJ. Biological dust exposure in the workplace is a risk factor for chronic obstructive pulmonary disease. *Thorax* 2005;**60**:645-651.

Mayer AS, Stoller JK, Bucher BB, James RA, Sandhaus RA, Newman LS. Occupational exposure risks in individuals with PI*Z alpha(1)-antitrypsin deficiency. *Am.J.Respir.Crit Care Med.* 2000;**162**:553-558.

Mazodier P, Orell SR, Siken L, Svanborg N. [Constitutional deficiency of alpha 1 antitrypsin and panlobular emphysema (report of 24 autopsies)]. *J Fr.Med Chir Thorac.* 1971;**25**:5-32.

McCloskey SC, Patel BD, Hinchliffe SJ, Reid ED, Wareham NJ, Lomas DA. Siblings of patients with severe chronic obstructive pulmonary disease have a significant risk of airflow obstruction. *Am J Respir Crit Care Med* 2001;**164**:1419-24.

McElvaney NG, Stoller JK, Buist AS, Prakash UB, Brantly ML, Schluchter MD, Crystal RD. Baseline characteristics of enrollees in the National Heart, Lung and Blood Institute Registry of alpha 1-antitrypsin deficiency. Alpha 1-Antitrypsin Deficiency Registry Study Group. *Chest* 1997;**111**:394-403.

McGuire A, Irwin DE, Fenn P, Gray A, Anderson P, Lovering A, MacGowan A. The excess cost of acute exacerbations of chronic bronchitis in patients aged 45 and older in England and Wales. *Value.Health* 2001;**4**:370-375.

Medical Research Council. Definition and classification of chronic bronchitis for clinical and epidemiological purposes. A report to the Medical Research Council by their Committee on the Aetiology of Chronic Bronchitis. *Lancet* 1965;**1**:775-779.

Meneely GR, Kaltreider NL. The volume of lung determined by helium dilution. *J Clin Invest* 1949;**28**:129-139.

Miravittles M, Ferrer M, Pont A, Zalacain R, Alvarez-Sala JL, Masa F, Vereza H, Murio C, Ros F, Vidal R. Effect of exacerbations on quality of life in patients with chronic obstructive pulmonary disease: a 2 year follow up study. *Thorax* 2004;**59**:387-395.

Miravittles M, Guerrero T, Mayordomo C, Sanchez-Agudo L, Nicolau F, Segu JL. Factors associated with increased risk of exacerbation and hospital admission in a cohort of ambulatory COPD patients: a multiple logistic regression analysis. The EOLO Study Group. *Respiration* 2000;**67**:495-501.

Monso E, Rosell A, Bonet G, Manterola J, Cardona PJ, Ruiz J, Morera J. Risk factors for lower airway bacterial colonization in chronic bronchitis. *Eur.Respir.J.* 1999;**13**:338-342.

Monso E, Ruiz J, Manterola J, Fiz J, Morera J, Ausina V. Bacterial infection in chronic obstructive pulmonary disease: a study of stable and exacerbated outpatients using the protected specimen brush. *Am J Respir Crit Care Med* 1995;**152**:1316-1320.

Mornex JF, Chytil-Weir A, Martinet Y, Courtney M, LeCocq JP, Crystal RG. Expression of the alpha-1-antitrypsin gene in mononuclear phagocytes of normal and alpha-1-antitrypsin-deficient individuals. *J Clin.Invest* 1986;**77**:1952-1961.

Morrison HM, Kramps JA, Burnett D, Stockley RA. Lung lavage fluid from patients with alpha 1-proteinase inhibitor deficiency or chronic obstructive bronchitis: anti-elastase function and cell profile. *Clin.Sci (Lond)* 1987;**72**:373-381.

Mossberg B, Afzelius B, Camner P. Mucociliary clearance in obstructive lung diseases. Correlations to the immotile cilia syndrome. *Eur.J.Respir.Dis.Suppl* 1986;**146**:295-301.

Naidich DP. High-resolution computed tomography of cystic lung disease. *Semin Roentgenol* 1991;**26**:151-174.

National Heart Lung and Blood Institute. The definition of emphysema. Report of a National Heart, Lung, and Blood Institute, Division of Lung Diseases workshop. *Am Rev Respir Dis*. 1985;**132**:182-185.

Novoradovsky A, Brantly ML, Waclawiw MA, Chaudhary PP, Ihara H, Qi L, Eissa NT, Barnes PM, Gabriele KM, Ehrmantraut ME, Rogliani P, Moss J. Endothelial nitric oxide synthase as a potential susceptibility gene in the pathogenesis of emphysema in alpha1-antitrypsin deficiency. *Am J Respir Cell Mol Biol*. 1999;**20**:441-447.

O'Brien C, Guest PJ, Hill SL, Stockley RA. Physiological and radiological characterisation of patients diagnosed with chronic obstructive pulmonary disease in primary care. *Thorax* 2000;**55**:635-642.

Office for National Statistics. Mortality Statistics: cause. *series DH2* 2004;**No. 30**:-142.

Ogushi F, Fells GA, Hubbard RC, Straus SD, Crystal RG. Z-type alpha 1-antitrypsin is less competent than M1-type alpha 1-antitrypsin as an inhibitor of neutrophil elastase. *J Clin Invest* 1987;**80**:1366-1374.

Oxman AD, Muir DC, Shannon HS, Stock SR, Hnizdo E, Lange HJ. Occupational dust exposure and chronic obstructive pulmonary disease. A systematic overview of the evidence. *Am Rev Respir Dis* 1993;**148**:38-48.

Palmer LJ, Cledon JC, Chapman HA, Speizer FE, Weiss ST, Silverman EK. Genome-wide linkage analysis of bronchodilator responsiveness and post-bronchodilator spirometric phenotypes in chronic obstructive pulmonary disease. *Hum Mol Genet*. 2003;**12**:1199-1210.

Parker CM, Voduc N, Aaron SD, Webb KA, O'Donnell DE. Physiological changes during symptom recovery from moderate exacerbations of COPD. *Eur Respir J* 2005;**26**:420-428.

Parmar JS, Mahadeva R, Reed BJ, Farahi N, Cadwallader KA, Keogan MT, Bilton D, Chilvers ER, Lomas DA. Polymers of alpha(1)-antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. *Am J Respir Cell Mol Biol*. 2002;**26**:723-730.

Parr DG, Stoel BC, Stolk J, Stockley RA. Pattern of emphysema distribution in alpha1-antitrypsin deficiency influences lung function impairment. *Am J Respir Crit Care Med*. 2004;**170**:1172-1178.

Pauwels RA, Buist AS, Calverley PM, Jenkins CR, Hurd SS. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease. NHLBI/WHO Global Initiative for Chronic Obstructive Lung Disease (GOLD) Workshop summary. *Am.J.Respir Crit Care Med* 2001;**163**:1256-1276.

Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R, Coates A, van der Grinten CP, Gustafsson P, Hankinson J, Jensen R, Johnson DC, MacIntyre N, McKay R, Miller MR, Navajas D, Pedersen OF, Wanger J. Interpretative strategies for lung function tests. *Eur.Respir J* 2005;**26**:948-968.

Perera WR, Hurst JR, Wilkinson TM, Sapsford RJ, Mullerova H, Donaldson GC, Wedzicha JA. Inflammatory changes, recovery and recurrence at COPD exacerbation. *Eur.Respir J* 2007;**29**:527-534.

Perlmutter DH, Daniels JD, Auerbach HS, Schryver-Kecskemeti K, Winter HS, Alpers DH. The alpha 1-antitrypsin gene is expressed in a human intestinal epithelial cell line. *J Biol.Chem.* 1989;**264**:9485-9490.

Piitulainen E, Eriksson S. Decline in FEV1 related to smoking status in individuals with severe alpha1-antitrypsin deficiency (PiZZ). *Eur.Respir.J.* 1999;**13**:247-251.

Piitulainen E, Sveger T. Effect of environmental and clinical factors on lung function and respiratory symptoms in adolescents with alpha1-antitrypsin deficiency. *Acta Paediatr.* 1998a;**87**:1120-1124.

Piitulainen E, Sveger T. Respiratory symptoms and lung function in young adults with severe alpha(1)-antitrypsin deficiency (PiZZ). *Thorax* 2002;**57**:705-708.

Piitulainen E, Tornling G, Eriksson S. Effect of age and occupational exposure to airway irritants on lung function in non-smoking individuals with alpha 1-antitrypsin deficiency (PiZZ). *Thorax* 1997;**52**:244-248.

Piitulainen E, Tornling G, Eriksson S. Environmental correlates of impaired lung function in non-smokers with severe alpha 1-antitrypsin deficiency (PiZZ). *Thorax* 1998b;**53**:939-943.

Pinto-Plata VM, Livnat G, Girish M, Cabral H, Masdin P, Linacre P, Dew R, Kenney L, Celli BR. Systemic cytokines, clinical and physiological changes in patients hospitalized for exacerbation of COPD. *Chest* 2007;**131**:37-43.

Quanjer PH, Tammeling GJ, Cotes JE, Pedersen OF, Peslin R, Yernault JC. Lung volumes and forced ventilatory flows. Report Working Party Standardization of Lung Function Tests, European Community for Steel and Coal. Official Statement of the European Respiratory Society. *Eur.Respir.J.Suppl* 1993;**16**:5-40.

Rodriguez-Roisin R. Toward a consensus definition for COPD exacerbations. *Chest* 2000;**117**:398S-401S.

Rossi A, Kristufek P, Levine BE, Thomson MH, Till D, Kottakis J, Della CG. Comparison of the efficacy, tolerability, and safety of formoterol dry powder and oral, slow-release theophylline in the treatment of COPD. *Chest* 2002;**121**:1058-1069.

Saetta M, Di Stefano A, Maestrelli P, Turato G, Ruggieri MP, Roggeri A, Calcagni P, Mapp CE, Ciaccia A, Fabbri LM. Airway eosinophilia in chronic bronchitis during exacerbations. *Am J Respir Crit Care Med* 1994;**150**:1646-1652.

Sanders C, Nath PH, Bailey WC. Detection of emphysema with computed tomography. Correlation with pulmonary function tests and chest radiography. *Invest Radiol* 1988;**23**:262-266.

Saydain G, Beck KC, Decker PA, Cowl CT, Scanlon PD. Clinical significance of elevated diffusing capacity. *Chest* 2004;**125**:446-452.

Seemungal T, Stockley R, Calverley P, Hagan G, Wedzicha JA. Investigating New Standards for Prophylaxis in Reduction of Exacerbations - The INSPIRE Study Methodology. *COPD*. 2007;**4**:177-183.

Seemungal TA, Donaldson GC, Bhowmik A, Jeffries DJ, Wedzicha JA. Time course and recovery of exacerbations in patients with chronic obstructive pulmonary disease. *Am.J.Respir.Crit Care Med*. 2000a;**161**:1608-1613.

Seemungal TA, Donaldson GC, Paul EA, Bestall JC, Jeffries DJ, Wedzicha JA. Effect of exacerbation on quality of life in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 1998;**157**:1418-22.

Seemungal TA, Harper-Owen R, Bhowmik A, Jeffries DJ, Wedzicha JA. Detection of rhinovirus in induced sputum at exacerbation of chronic obstructive pulmonary disease. *Eur.Respir.J*. 2000b;**16**:677-683.

Seersholm N, Kok-Jensen A. Clinical features and prognosis of life time non-smokers with severe alpha 1-antitrypsin deficiency. *Thorax* 1998;**53**:265-268.

Seersholm N, Kok-Jensen A, Dirksen A. Decline in FEV1 among patients with severe hereditary alpha 1-antitrypsin deficiency type PiZ. *Am.J Respir.Crit Care Med.* 1995;**152**:1922-1925.

Seersholm N, Wencker M, Banik N, Viskum K, Dirksen A, Kok-Jensen A, Konietzko N. Does alpha1-antitrypsin augmentation therapy slow the annual decline in FEV1 in patients with severe hereditary alpha1-antitrypsin deficiency? Wissenschaftliche Arbeitsgemeinschaft zur Therapie von Lungenerkrankungen (WATL) alpha1-AT study group. *Eur Respir J* 1997;**10**:2260-3.

Senior RM, Tegner H, Kuhn C, Ohlsson K, Starcher BC, Pierce JA. The induction of pulmonary emphysema with human leukocyte elastase. *Am Rev.Respir Dis.* 1977;**116**:469-475.

Shin MS, Ho KJ. Bronchiectasis in patients with alpha 1-antitrypsin deficiency. A rare occurrence? *Chest* 1993;**104**:1384-1386.

Silverman EK, Chapman HA, Drazen JM, Weiss ST, Rosner B, Campbell EJ, O'Donnell WJ, Reilly JJ, Ginns L, Mentzer S, Wain J, Speizer FE. Genetic epidemiology of severe, early-onset chronic obstructive pulmonary disease. Risk to relatives for airflow obstruction and chronic bronchitis. *Am J Respir Crit Care Med* 1998;**157**:1770-8.

Silverman EK, Mosley JD, Palmer LJ, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, Province MA, Rao DC, Reilly JJ, Ginns LC, Speizer FE, Weiss ST. Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes. *Hum Mol Genet* 2002a;**11**:623-32.

Silverman EK, Palmer LJ, Mosley JD, Barth M, Senter JM, Brown A, Drazen JM, Kwiatkowski DJ, Chapman HA, Campbell EJ, Province MA, Rao DC, Reilly JJ, Ginns LC, Speizer FE, Weiss ST. Genomewide linkage analysis of quantitative spirometric phenotypes in severe early-onset chronic obstructive pulmonary disease. *Am J Hum Genet* 2002b;**70**:1229-39.

Silverman EK, Pierce JA, Province MA, Rao DC, Campbell EJ. Variability of pulmonary function in alpha-1-antitrypsin deficiency: clinical correlates. *Ann Intern Med* 1989;**111**:982-91.

Silverman EK, Province MA, Campbell EJ, Pierce JA, Rao DC. Variability of pulmonary function in alpha-1-antitrypsin deficiency: residual family resemblance beyond the effect of the Pi locus. *Hum Hered* 1990a;**40**:340-55.

Silverman EK, Province MA, Rao DC, Pierce JA, Campbell EJ. A family study of the variability of pulmonary function in alpha 1-antitrypsin deficiency. Quantitative phenotypes. *Am Rev Respir Dis* 1990b;**142**:1015-21.

Silverman EK, Weiss ST, Drazen JM, Chapman HA, Carey V, Campbell EJ, Denish P, Silverman RA, Celedon JC, Reilly JJ, Ginns LC, Speizer FE. Gender-related differences in severe, early-onset chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2000;**162**:2152-8.

Spencer S, Calverley PM, Sherwood Burge P, Jones PW. Health status deterioration in patients with chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2001;**163**:122-8.

Stableforth DE. Lung function in alpha-1-antitrypsin deficient sisters. *Br.J.Dis.Chest* 1978;**72**:125-130.

Stevenson NJ, Walker PP, Costello RW, Calverley PM. Lung mechanics and dyspnea during exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2005;**172**:1510-1516.

Stewart A.L, Ilays R.D., Ware J.E. The MOS short-form general health survey. *Med Care* 1988;**26**:724-735.

Stockley RA, Bayley D, Hill SL, Hill AT, Crooks S, Campbell EJ. Assessment of airway neutrophils by sputum colour: correlation with airways inflammation. *Thorax* 2001;**56**:366-72.

Stockley RA, Bayley DL, Unsal I, Dowson LJ. The effect of augmentation therapy on bronchial inflammation in alpha1-antitrypsin deficiency. *Am J Respir Crit Care Med* 2002;**165**:1494-8.

Stockley RA, O'Brien C, Pye A, Hill SL. Relationship of sputum colour to nature and outpatient management of acute exacerbations of COPD. *Chest* 2000;**117**:1638-1645.

Stolk J, Ng WH, Bakker ME, Reiber JH, Rabe KF, Putter H, Stoel BC. Correlation between annual change in health status and computer tomography derived lung density in subjects with alpha1-antitrypsin deficiency. *Thorax* 2003;**58**:1027-1030.

Stoller JK, Smith P, Yang P, Spray J. Physical and social impact of alpha 1-antitrypsin deficiency: results of a survey. *Cleve.Clin J Med.* 1994;**61**:461-467.

Sunyer J, Saez M, Murillo C, Castellsague J, Martinez F, Anto JM. Air pollution and emergency room admission for chronic obstructive pulmonary disease: a 5-year study. *Am J Epidemiol* 1993;**137**:701-705.

Sunyer J, Schwartz J, Tobias A, Macfarlane D, Garcia J, Anto JM. Patients with chronic obstructive pulmonary disease are at increased risk of death associated with urban particle air pollution: a case-crossover analysis. *Am J Epidemiol* 2000;**151**:50-56.

Sveger T. Liver disease in alpha1-antitrypsin deficiency detected by screening of 200,000 infants. *N.Engl.J.Med.* 1976;**294**:1316-1321.

Tashkin DP, Clark VA, Coulson AH, Simmons M, Bourque LB, Reems C, Detels R, Sayre JW, Rokaw SN. The UCLA population studies of chronic obstructive respiratory disease. VIII. Effects of smoking cessation on lung function: a prospective study of a free-living population. *Am Rev.Respir Dis.* 1984;**130**:707-715.

Taylor DC, Cripps AW, Clancy RL. A possible role for lysozyme in determining acute exacerbation in chronic bronchitis. *Clin Exp.Immunol.* 1995;**102**:406-416.

The Alpha-1-Antitrypsin Deficiency Registry Study Group. Survival and FEV1 decline in individuals with severe deficiency of alpha1-antitrypsin. *Am J Respir Crit Care Med* 1998;**158**:49-59.

Thurlbeck WM. The incidence of pulmonary emphysema, with observations on the relative incidence and spatial distribution of various types of emphysema. *Am Rev.Respir Dis.* 1963;**87**:206-215.

Thurlbeck WM, Muller NL. Emphysema: definition, imaging, and quantification. *Am J Roentgenol* 1994;**163**:1017-1025.

Tobin MJ, Cook PJ, Hutchison DC. Alpha 1 antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z. A survey by the British Thoracic Association. *Br.J Dis.Chest* 1983;**77**:14-27.

Wencker M, Denker J, Konietzko N. Serial measurements of FEV1 over 12 years in a patient with alpha-1-protease inhibitor deficiency: influence of augmentation therapy and infections. *Respiration* 1994;**61**:195-8.

Wencker M, Fuhrmann B, Banik N, Konietzko N. Longitudinal follow-up of patients with alpha(1)-protease inhibitor deficiency before and during therapy with IV alpha(1)-protease inhibitor. *Chest* 2001;**119**:737-44.

West JB. Gravity and pulmonary blood flow distribution. *J Appl. Physiol* 1992;**73**:2201-2202.

Wewers MD, Casolaro MA, Sellers SE, Swayze SC, McPhaul KM, Wittes JT, Crystal RG. Replacement therapy for alpha 1-antitrypsin deficiency associated with emphysema. *N Engl J Med* 1987;**316**:1055-62.

White AJ, Gompertz S, Bayley DL, Hill SL, O'Brien C, Unsal I, Stockley RA. Resolution of bronchial inflammation is related to bacterial eradication following treatment of exacerbations of chronic bronchitis. *Thorax* 2003;**58**:680-685.

Wilk JB, DeStefano AL, Arnett DK, Rich SS, Djousse L, Crapo RO, Leppert MF, Province MA, Cupples LA, Gottlieb DJ, Myers RH. A genome-wide scan of pulmonary function measures in the National Heart, Lung, and Blood Institute Family Heart Study. *Am J Respir Crit Care Med* 2003;**167**:1528-1533.

Wilkinson TM, Donaldson GC, Hurst JR, Seemungal TA, Wedzicha JA. Early therapy improves outcomes of exacerbations of chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2004;**169**:1298-1303.

Wilson JS, Galvin JR. Normal diffusing capacity in patients with PiZ alpha(1)-antitrypsin deficiency, severe airflow obstruction, and significant radiographic emphysema. *Chest* 2000;**118**:867-71.

Woolhouse IS, Bayley DL, Stockley RA. Sputum chemotactic activity in chronic obstructive pulmonary disease: effect of alpha(1)-antitrypsin deficiency and the role of leukotriene B(4) and interleukin 8. *Thorax* 2002;**57**:709-714.

Zhu J, Qiu YS, Majumdar S, Gamble E, Matin D, Turato G, Fabbri LM, Barnes N, Saetta M, Jeffery PK. Exacerbations of Bronchitis: bronchial eosinophilia and gene expression for interleukin-4, interleukin-5, and eosinophil chemoattractants. *Am J Respir Crit Care Med* 2001;**164**:109-116.

APPENDIX 1

CONSENT FORM FOR PARTICIPATION IN RESEARCH

Assessment for Alpha₁ Antitrypsin Deficiency

What is the study about?

You or a member of your family has been identified as having alpha₁ antitrypsin deficiency. This is an inherited condition that is believed to increase the risk of development of lung health problems. However very little detailed information has been collected on the way this deficiency affects patients and some studies have suggested that lung disease may run in families even without the deficiency. It is likely however that the deficiency highlights the tendency to develop these diseases and when present will make them worse.

We wish to learn as much as possible about the deficiency and its relationship to lung disease and for this reason invite you to participate in our alpha₁ antitrypsin deficiency assessment programme.

What will I have to do?

In broad terms you will undergo all the routine questioning, examination and tests that we normally undertake when assessing somebody who presents with lung disease. However we hope to do this more carefully and in more detail than is routinely carried out by your own doctor or specialist.

We will ask many questions about your past and present symptoms, health and wellbeing. In addition you will be examined thoroughly to determine the presence of signs related to lung disease. After this screening programme you will be asked to perform some lung function testing which assesses how your lungs work and their ability to take oxygen in and out of your body. We may also perform a specialised CT scan of your lungs (if you have not had one) which is a very sensitive technique of detecting damage that has occurred. Finally we will ask you to provide one blood sample and if you have a cough productive of sputum we will arrange for you to collect this over several hours on one day before coming to see us.

Once all these tests have been performed we will be able to determine whether you have lung disease related to alpha₁ antitrypsin deficiency. This will be explained to you and any modifications in your treatment that are indicated will be communicated to both yourself and your own doctor.

It is our general clinical routine to follow patients with established lung disease on a long-term basis. Patients are usually seen once every 4-6 months to assess their wellbeing and follow any progress in the condition. If you have alpha₁ antitrypsin deficiency we would wish to see you once a year to assess your symptoms, clinical signs and repeat the extensive lung function tests and the CT scan to confirm the extent or any progression of your disease will be carried out every 2 years. After the first year the lung function may be repeated less frequently (2 or 3 yearly) depending on whether these are changing or are stable.

NOTE The CT scan exposes you to a small degree of radiation – about the equivalent of 6 months background radiation in the UK. Although this dose is safe (it is the same as a single x-ray of the abdomen), it is important that you inform us if you are likely to be pregnant as we will not carry out the test in these circumstances.

What are the benefits?

The major purpose of the study is to find out as much as possible about the lung disease associated with alpha₁ antitrypsin deficiency. This will provide the background information that enables us to design studies to assess the role of alpha₁ antitrypsin replacement therapy in both the short and long term. The investigations that we undertake will allow us to advise upon the degree of lung disease that you have and simple measures that you can undertake with your current treatment in order to try and stabilize the lung disease. In addition the breathing tests that we will perform will help us to optimize your current treatment in order to improve your breathlessness where possible.

What are the risks?

All the investigations that are taking place are entirely routine, used in the assessment of patients with lung disease. As such they are repeated on many occasions in the same patient without any adverse effects. The only minor problem that is likely to occur is a slight degree of bruising in some patients when they have their blood taken.

What are the alternatives?

There are currently no alternatives to finding the information that is required other than the assessment programme outlined above.

What happens if I do not wish to take part?

If you do not wish to participate in the assessment programme or the short term follow up programme this will be fully understood. Your own general practitioner, or the consultant chest physician who normally looks after you, will be informed of the diagnosis and provided with advice on how to assess and manage your follow up along the lines outlined above. If for any reason they or you require further advice from us in the future we will be only too pleased to see you. It is important to emphasize that your overall management by your doctor will not be affected by your decision.

What happens to the information?

The important information that we collect over the next year or two will be written up as a report and submitted to medical journals so that other doctors may read about the problem. The research information obtained from the samples that we have collected will be the basis for future studies on the role of alpha₁ antitrypsin replacement therapy which we hope to start within the next 12-18 months. Neither your name or any details relating to you personally will be released to any other person outside the research programme.

Who is taking part?

All subjects that we identify with alpha₁ antitrypsin deficiency and in some cases members of their family will be asked to take part. At present we know of over 800 such patients as yourself and it is likely that there are several thousand similar people in the West Midlands alone. It is hoped over the next year or two to identify and assess all people with the deficiency in the Midlands.

What if something goes wrong?

The question largely relates to clinical trials and at present that is not part of the assessment programme. If you develop new symptoms for any reason during the assessment day this will be in the presence of a doctor who will take any steps that are necessary to help you. In between visits to the assessment centre your own doctor will be largely responsible for your care but (depending on where you live) we may collaborate with your doctor and help by seeing you if you become unwell.

What happens at the end of a study?

At the end of the study we will have learnt a lot about alpha₁ antitrypsin deficiency. If you remain part of the programme for assessment we will be having regular meetings for all patients to attend to discuss how the assessment programme is going and how our understanding is developing. If for any reason you are unable to attend we will be keeping in touch with you by letter and newsletter to inform you of the progress of the programme.

What if I have more questions or do not understand something?

The doctors, co-ordinator and nurse involved in the alpha₁ antitrypsin assessment programme will happily answer your questions on any occasion when you visit. If questions arise between visits you will be able to contact the centre and either speak to somebody at that time or arrange to do so if for any reason if it is inconvenient.

What happens now if I decide to take part?

If you decide to take part in the programme now we will arrange an appointment in the not too distant future for you to come to the assessment centre for the investigations outlined above. This will be arranged to suit everybody's convenience and all the assessment will be completed where possible on a single visit.

What happens if I change my mind during the study?

If you change your mind during the study, it is important that you notify the assessment centre. This will enable any investigations that have been organized or visits to be cancelled. Your decision will be passed on to both your own doctor and where appropriate your own specialist in order that they can arrange for appropriate appointments to monitor your progress. Providing you are agreeable we would like to contact your own doctor or specialist from time to time in order to find out how you are progressing. However if in the future you once again decide to join the programme, we would be only too pleased to see you.

[Contact details]

CONSENT FORM FOR PARTICIPATION IN RESEARCH

Assessment for Alpha₁ Antitrypsin Deficiency

Please Initial

I.....
..... voluntarily agree to participate in the alpha₁
antitrypsin deficiency assessment programme.

Yes	No

I have been given a full explanation of the programme and
read the patient information sheet and have had all my
questions answered and agreed to cooperate where possible
with this programme.

Yes	No

I understand that that if I suffer from any unexpected
problems that it may be important to contact both my own
doctor and the staff at the alpha-1-antitrypsin resource centre.

Yes	No

I understand fully that I am free to withdraw from the
programme at any time without giving a reason and that this
will not adversely affect my future management.

Yes	No

Signed (Patient).....

Date

Signed (Witness).....

Date.....

APPENDIX 2

THE ST. GEORGE'S HOSPITAL RESPIRATORY QUESTIONNAIRE

This questionnaire is designed to help us learn much more about how your breathing is troubling you and how it affects your life. We are using it to find out which aspects of your illness cause you most problem, rather than what the doctors and nurses think your problems are.

Please read the instructions carefully and ask if you do not understand any thing. Do not spend too long deciding about your answers.

Name: _____ Date: _____

I.D. no. _____

Age: _____
Male/Female

Sex:

Before completing the rest of the questionnaire, please tick in the circle to show how you describe your current health

- Very Good
- Good
- Fair
- Poor
- Very Poor

PART 1

QUESTIONS ABOUT HOW MUCH CHEST TROUBLE YOU HAVE HAD OVER THE LAST YEAR.
PLEASE TICK IN ONE BOX FOR EACH QUESTION.

	most days a week	several days a week	a few days a month	only with chest infections	not at all
1) Over the last year, I have coughed:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2) Over the last year, I have brought up phlegm (sputum):	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3) Over the last year, I have had shortness of breath:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4) Over the last year, I have had attacks of wheezing:	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5) During the last year, how many severe or very unpleasant attacks of chest trouble have you had:					
			more than 3 attacks		<input type="checkbox"/>
			3 attack		<input type="checkbox"/>
			2 attacks		<input type="checkbox"/>
			1 attack		<input type="checkbox"/>
			no attacks		<input type="checkbox"/>
6) How long did the worst attack of chest trouble last: (Go to Question 7 if you had no severe attacks)					
			a week or more		<input type="checkbox"/>
			3 or more days		<input type="checkbox"/>
			1 or 2 days		<input type="checkbox"/>
			less than a day		<input type="checkbox"/>
7) Over the last year, in an average week, how many good days (with little chest trouble) have you had:					
			no good days		<input type="checkbox"/>
			1 or 2 good days		<input type="checkbox"/>
			3 or 4 good days		<input type="checkbox"/>
			nearly every day is good		<input type="checkbox"/>
			every day is good		<input type="checkbox"/>
8) If you have a wheeze, is it worse in the morning:					
			no		<input type="checkbox"/>
			yes		<input type="checkbox"/>

PART 2

SECTION 1:

HOW WOULD YOU DESCRIBE YOUR CHEST CONDITION?
(PLEASE TICK IN ONE BOX ONLY)

- | | |
|-----------------------------------|--------------------------|
| the most important problem I have | <input type="checkbox"/> |
| causes me quite a lot a problems | <input type="checkbox"/> |
| causes me a few problems | <input type="checkbox"/> |
| causes no problem | <input type="checkbox"/> |

IF YOU HAVE EVER HAD PAID EMPLOYMENT, PLEASE TICK ONE OF THESE:

- | | |
|--|--------------------------|
| my chest trouble made me stop work altogether | <input type="checkbox"/> |
| my chest trouble interferes with my work or made me change my work | <input type="checkbox"/> |
| my chest trouble does not affect my work | <input type="checkbox"/> |

SECTION 2:

QUESTIONS ABOUT WHAT ACTIVITIES USUALLY MAKE YOU FEEL BREATHLESS THESE DAYS. FOR EACH ITEM, PLEASE TICK IN THE BOX FOR EITHER TRUE OR FALSE AS IT APPLIES TO YOU

- | | | |
|-------------------------------|-------------------------------|--------------------------------|
| Sitting or lying still | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Getting washed or dressed | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Walking around the home | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Walking outside on the level | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Walking up a flight of stairs | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Walking up hills | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Playing sports or games | <input type="checkbox"/> True | <input type="checkbox"/> False |

SECTION 3:

SOME MORE QUESTIONS ABOUT YOUR COUGH AND BREATHLESSNESS THESE DAYS. FOR EACH ITEM, PLEASE TICK IN THE BOX FOR EITHER TRUE OR FALSE AS IT APPLIES TO YOU

- | | | |
|---|-------------------------------|--------------------------------|
| My cough hurts | <input type="checkbox"/> True | <input type="checkbox"/> False |
| My cough makes me tired | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I am breathless when I talk | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I am breathless when I bend over | <input type="checkbox"/> True | <input type="checkbox"/> False |
| My cough or breathing disturbs my sleep | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I get exhausted easily | <input type="checkbox"/> True | <input type="checkbox"/> False |

SECTION 4:

QUESTIONS ABOUT OTHER EFFECTS THAT YOUR CHEST TROUBLE MAY HAVE ON YOU THESE DAYS. FOR EACH ITEM, PLEASE TICK IN THE BOX FOR EITHER TRUE OR FALSE AS IT APPLIES TO YOU

- | | | |
|--|-------------------------------|--------------------------------|
| My cough or breathing is embarrassing in public | <input type="checkbox"/> True | <input type="checkbox"/> False |
| My chest trouble is a nuisance to my family, friends or neighbours | <input type="checkbox"/> True | <input type="checkbox"/> False |

- | | | |
|---|-------------------------------|--------------------------------|
| I get afraid or panic when I cannot get my breath | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I feel that I am not in control of my chest problem | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I do not expect my chest to get any better | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I have become frail or an invalid because of my chest | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Exercise is not safe for me | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Everything seems too much of an effort | <input type="checkbox"/> True | <input type="checkbox"/> False |

SECTION 5:

QUESTIONS ABOUT YOUR MEDICATION. IF YOU ARE RECEIVING NO MEDICATION GO STRAIGHT TO SECTION 6. TO COMPLETE THIS SECTION PLEASE TICK IN THE BOX FOR EITHER TRUE OR FALSE AS IT APPLIES TO YOU

- | | | |
|---|-------------------------------|--------------------------------|
| My medication does not help me very much | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I get embarrassed using my medication in public | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I have unpleasant side effects from my medication | <input type="checkbox"/> True | <input type="checkbox"/> False |
| My medication interferes with my life a lot | <input type="checkbox"/> True | <input type="checkbox"/> False |

SECTION 6:

THESE ARE QUESTIONS ABOUT HOW YOUR ACTIVITIES MIGHT BE AFFECTED BY YOUR BREATHING. FOR EACH QUESTION, PLEASE TICK TRUE IF ONE OR MORE OF THE PARTS OF THE QUESTION APPLIES TO YOU BECAUSE OF YOUR BREATHING. OTHERWISE TICK FALSE.

- | | | |
|--|-------------------------------|--------------------------------|
| I take a long time to get washed or dressed | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I cannot take a bath or shower, or I take a long time | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I walk slower than other people, or I stop for rests | <input type="checkbox"/> True | <input type="checkbox"/> False |
| Jobs such as housework take a long time,
or I have to stop for rests | <input type="checkbox"/> True | <input type="checkbox"/> False |
| If I walk up one flight of stairs,
I have to go slowly or stop | <input type="checkbox"/> True | <input type="checkbox"/> False |
| If I hurry or walk fast, I have to stop or slow down | <input type="checkbox"/> True | <input type="checkbox"/> False |
| My breathing makes it difficult to do things such as
walk up hills, carrying things up stairs, light gardening
such as weeding, dance, play bowls or play golf | <input type="checkbox"/> True | <input type="checkbox"/> False |
| My breathing makes it difficult to do things such as
carry heavy loads, dig the garden or shovel snow,
jog or walk at 5 miles per hour, play tennis or swim | <input type="checkbox"/> True | <input type="checkbox"/> False |
| My breathing makes it difficult to do things such as
very heavy manual work, run, cycle, swim fast
or play competitive sports | <input type="checkbox"/> True | <input type="checkbox"/> False |

SECTION 7:

WE WOULD LIKE TO KNOW HOW YOUR CHEST TROUBLE USUALLY AFFECTS YOUR DAILY LIFE. PLEASE TICK EITHER TRUE OR FALSE (REMEMBER THAT TRUE ONLY APPLIES TO YOU

IF YOU CANNOT DO SOMETHING BECAUSE OF YOUR BREATHING)

- | | | |
|---|-------------------------------|--------------------------------|
| I cannot play sports or games | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I cannot go out for entertainment or recreation | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I cannot go out of the house to do the shopping | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I cannot do housework | <input type="checkbox"/> True | <input type="checkbox"/> False |
| I cannot move far from my bed or chair | <input type="checkbox"/> True | <input type="checkbox"/> False |

HERE IS A LIST OF OTHER ACTIVITIES THAT YOUR CHEST TROUBLE MAY PREVENT YOU DOING. (YOU DO NOT HAVE TO TICK THESE, THEY ARE JUST TO REMIND YOU OF WAYS IN WHICH YOUR BREATHLESSNESS MAY AFFECT YOU):

- GOING FOR WALKS OR WALKING THE DOG
- DOING THINGS AT HOME OR IN THE GARDEN
- SEXUAL INTERCOURSE
- GOING OUT TO CHURCH, OR PLACE OF ENTERTAINMENT
- GOING OUT IN BAD WEATHER OR INTO SMOKY ROOMS
- VISITING FAMILY OR FRIENDS OR PLAYING WITH CHILDREN

PLEASE WRITE IN ANY OTHER IMPORTANT ACTIVITIES THAT YOUR CHEST TROUBLE MAY STOP YOU DOING:

NOW, WOULD YOU TICK IN THE BOX (ONE ONLY) WHICH YOU THINK BEST DESCRIBES HOW YOUR CHEST AFFECTS YOU:

- | | |
|---|--------------------------|
| It does not stop me doing anything I would like to do | <input type="checkbox"/> |
| It stops me doing one or two things I would like to do | <input type="checkbox"/> |
| It stops me doing most of the things I would like to do | <input type="checkbox"/> |
| It stops me doing everything I would like to do | <input type="checkbox"/> |

Copyright reserved
[Contact details]

APPENDIX 3

THE SF-36™ HEALTH SURVEY

INSTRUCTIONS: this survey asks for your views about your health. This information will help you keep track of how you feel and how well you are able to do your usual activities. If you are unsure about how to answer a question, please give the best answer you can.

Question 1

In general, would you say your health is:

- | | |
|-----------|--------------------------|
| Excellent | <input type="checkbox"/> |
| Very Good | <input type="checkbox"/> |
| Good | <input type="checkbox"/> |
| Fair | <input type="checkbox"/> |
| Poor | <input type="checkbox"/> |

Question 2

Compared to **ONE YEAR AGO**, how would you rate your health in general **now**?

- | | |
|---------------------------------------|--------------------------|
| Much better than one year ago | <input type="checkbox"/> |
| Somewhat better now than one year ago | <input type="checkbox"/> |
| About the same as one year ago | <input type="checkbox"/> |
| Somewhat worse now than one year ago | <input type="checkbox"/> |
| Much worse now than one year ago | <input type="checkbox"/> |

Question 3

The following items are about activities you might do during a typical day. **Does your health now limit** you in these activities? If so, how much?

	Yes, limited a lot	Yes, limited a little	No, not limited at all
a) Vigorous activities such as running, lifting heavy objects, participating in strenuous sports?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b) Moderate activities , such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c) Lifting or carrying groceries?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d) Climbing several flights of stairs?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
e) Climbing one flight of stairs?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
f) Bending, kneeling or stooping?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
g) Walking more than a mile ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
h) Walking half a mile ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
i) Walking 100 yards ?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
j) Bathing or dressing yourself?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Question 4

During the **past 4 weeks**, have you had any of the following problems with your work or other regular activities **as a result of your physical health?**

	Yes	No
a) Cut down on the amount of time you spent on work or other activities?	<input type="checkbox"/>	<input type="checkbox"/>
b) Accomplished less than you would like?	<input type="checkbox"/>	<input type="checkbox"/>
c) Were limited in the kind of work or other activities?	<input type="checkbox"/>	<input type="checkbox"/>
d) Had difficulty performing the work or other activities (for example it took extra effort)?	<input type="checkbox"/>	<input type="checkbox"/>

Question 5

During the **past 4 weeks**, how much time have you had any of the following problems with your work or other regular daily activities **as a result of any emotional problems?**

	Yes	No
a) Cut down on the amount of time you spent on work or other activities?	<input type="checkbox"/>	<input type="checkbox"/>
b) Accomplished less than you would like?	<input type="checkbox"/>	<input type="checkbox"/>
c) Didn't do work or other activities as carefully as usual?	<input type="checkbox"/>	<input type="checkbox"/>

Question 6

During the **past 4 weeks**, to what extent have your physical health or emotional problems interfered with your normal social activities with family, neighbours or groups?

- Not at all
- Slightly
- Moderately
- Quite a bit
- Extremely

g) Did you feel worn out?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
h) Have you been a happy person?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
i) Did you feel tired?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Question 10

During the **past 4 weeks**, how much of the time has your **physical health or emotional problems** interfered with your social activities (like visiting friends, relatives etc)?

- All of the time
- Most of the time
- Some of the time
- A little of the time
- None of the time

Question 11

How **TRUE** or **FALSE** is **each** of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a) I seem to get ill more easily than other people	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
b) I am as healthy as anybody I know	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
c) I expect my health to get worse	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
d) My health is excellent	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

APPENDIX 4

Papers and Abstracts published in relation to this thesis

Clinical Manifestations and Natural History of Alpha-1-Antitrypsin Deficiency
Needham M and Stockley RA. *Thorax* May 2004; **59(5)**:441-445.

Review Article

Exacerbations in Alpha-1-Antitrypsin deficiency
Needham M and Stockley RA. *Eur Respir J* 2005; **25(6)**:992-1000.

Research Paper

Exacerbations of Symptoms in Alpha-1-Antitrypsin Deficiency in the UK
Needham M, Bates DM, Wilkins HM and Stockley RA
European Respiratory Society Meeting, Stockholm 2002

Abstract

Health Status Changes in Patients with Alpha-1-Antitrypsin Deficiency
Needham M, Wilkins HM and Stockley RA
European Respiratory Society Meeting, Stockholm 2002

Abstract

The Influence of BMI on CT densitometry in Emphysema
Parr DG, Needham M, Stoel B, Stolk J and Stockley RA
British Thoracic Society Meeting, London 2002

Abstract

Sibling Pairs with Alpha-1-Antitrypsin Deficiency
Needham M, Parr DG, Hill SL and Stockley RA
European Respiratory Society Meeting, Vienna 2003

Abstract

Health Status Changes in Alpha-1-Antitrypsin Deficiency (PiZ phenotype) over 3 years
Needham M, Griffiths DM, Wilkins HM and Stockley RA
European Respiratory Society Meeting, Vienna 2003

Abstract

Discordance of Airflow Obstruction and Impaired Gas Transfer in Alpha-1-Antitrypsin Deficiency
Needham M and Stockley RA
British Thoracic Society Meeting, London 2003

Abstract