Alpha one

ANNUAL REPORT 2007

Contents

Executive Summary

The past year was, for us, a year of promise and consolidation. It was a year of many achievements in the areas of detection, research and development. As with other years, everything did not work out exactly as planned. Some areas surpassed expectation and some fell a little short.

The detection programme made great strides in getting thirteen hospitals to sign up to it through their Research Ethics Committees. Two other hospitals have indicated their willingness to participate and their approvals are pending. As a result of the length of time it has taken with the approvals gained and pending there has been a delay in achieving the targets we set ourselves in testing for Alpha-1 Antitrypsin Deficiency.

As a result of our interaction with institutions, clinicians and laboratory personnel we have decided that our next priority for the programme is to obtain quality assurance and ultimately, full accreditation. This will take some time and may prove somewhat costly. However, we consider it essential to reinforce and enhance the bona fides of the programme and are proceeding with it as a matter of urgency. QA and accreditation will ensure that all work conforms to WHO standards for AATD testing which, interestingly, was established by us.

As a member of Medical Research Charities Group we were successful in securing research grants under the HRB/MRCG scheme. These grants together are for a three year period and are valued in total at €300,000.

Work has progressed very satisfactorily with the Alpha-1 Registry. We secured the services of an intern form Boston College to assist with the data entry for the Registry. We are very pleased to have a very professional dynamic patient registry.

During the year we also consolidated our patient support group. As a result of recent patient information days in Marino Institute of Education it became clear that patients were happy and anxious to form such a group as an integral part of the Alpha One Foundation. The group organised excellent awareness events with a horse ride from Mizen Head to Malin Head by John Hannan, the Women's Mini Marathon and several information meetings.

We continued our cooperation and active participation with other allied groups both nationally and internationally. We currently hold the Presidency of Alfa Europe. We also value our cooperation with the Department of Health and Children, the HSE and other statutory bodies.

The diligence and team work of our colleagues together with Prof. N.G. McElvaney, Kitty O'Connor, Tomás Carroll and Olwen Floyd ensured the success the past year. I thank them for their support.

Larry Warren, CEO.

Introduction

This is the third annual report from the Alpha One Foundation. The Annual Report provides an overview of the functionality of the Alpha One Foundation. Within the last year we have seen an increase in centres screening for Alpha-1, further development of our patient Registry and a successful patient-led awareness campaign.

In addition we have recruited a full time dedicated research assistant Olwen Floyd. This has increased our efficiency and has reduced the turn around time for results. Olwen has enabled us to improve techniques in measuring AAT levels and genotyping.

Our successful Awareness Campaign in June of this year was formulated by our patient support group. This involved the dedication and support of many patients and their families. We at the Alpha One Foundation would like to express our gratitude to everyone involved with this tremendous achievement.

We continue to have strategic alliances with advisory committees, organisational memberships, health care professionals, universities, foundations and pharmaceutical companies.

Our aim is to expand Alpha-1 detection, research and public awareness. The Alpha One Foundation is continuing to evaluate the most useful practices to promote early detection.

The Alpha One Foundation is striving for enhancement in levels of community awareness, contributions, partnership and research in order to reach our ultimate goal of curing Alpha-1.

Alpha-1 Screening Centres

COUNTY	HOSPITAL	CONSULTANT
Dublin	Beaumont Hospital	Prof N.G. McElvaney
	Mater Misericordiae Hospital	Dr Sean Gaine
	Tallaght Hospital	Dr Stephen Lane
	Peamount Hospital	Dr Stephen Lane
	St James's Hospital	Dr Finbar O'Connell
	Connolly Hospital, Blanchardstown	Dr John Faul
Westmeath	Midland Regional Hospital, Mullingar	Dr Aidan O'Brien
Louth	Our Lady of Lourdes Hospital, Drogheda	Dr John Kiely
Galway	University College Hospital, Galway	Dr Anton O' Regan
Cork	Cork University Hospital	Dr Charles Bredin
	Mercy University Hospital	Dr Neil Brennan Dr Terence O'Connor
Limerick	Midwestern Regional Hospital	Dr Thomas Peirce
Letterkenny	Letterkenny General Hospital	Dr Vera Keatings
Cavan	Cavan General Hospital	Dr James Hayes

AATD Targeted Detection Programme

Alpha-1 Antitrypsin deficiency (AATD) is an under diagnosed condition. It is estimated there are approximately 1,200 individuals with Alpha-1 Antitrypsin deficiency in Ireland, however less than five per cent of these have been identified. The World Health Organization (WHO) has recommended that individuals with chronic obstructive pulmonary disease (COPD) and asthma should be screened for AATD.

The Targeted Detection Programme screens patients attending respiratory outpatient clinics in various hospitals for AATD. As per WHO guidelines, the majorities of patients screened suffer from chronic obstructive pulmonary disease (COPD) or poorly controlled non-responsive asthma. Other patient groups targeted include first-degree relatives of known AATD sufferers, and patients with cryptogenic liver disease.

DIAGNOSTIC METHODS

To diagnose AATD, 2.7mls of blood is collected in an EDTA tube from a suspected patient. The blood is centrifuged to isolate serum which is used in two different assays.

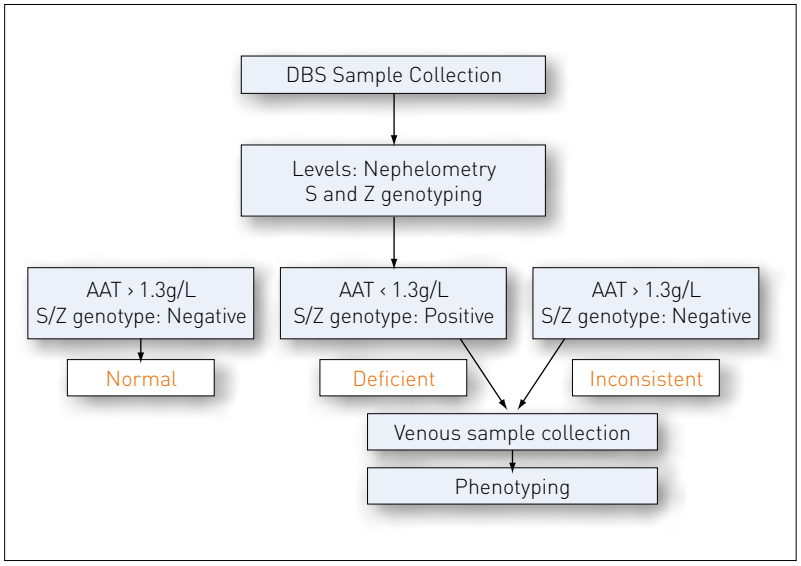

Fig. 1 — Diagnostic Algorithm

RADIAL IMMUNODIFFUSION

The first assay involves measuring the patients circulating AAT levels by RID (radial immunodiffusion). A small sample of serum is placed in a well of an agarose plate which is then left to stand at room temperature for two days. Proteins in the patient's serum react immunochemically with specific antibodies in the agarose gel of the plate. This leads to the formation of immune complexes which are visible as circular precipitin lines (radial immunodiffusion). The diameter of the precipitin ring is measured after 48 hours and is directly proportional to the concentration of the respective protein in the sample. Conversion to the corresponding protein concentration is calculated by means of a reference value table (see below).

(mm)	Alpha-1 Antitrypsin (Percent of Normal)	(g/l)
4.0	20	0.333
5.0	56	0.932
6.0	100	1.66

PHENOTYPING BY ISOELECTRIC FOCUSING

The second assay involves phenotyping by Isoelectric focussing (IEF). IEF separates molecules according to differences in their charge with each molecule migrating to a point in a pH gradient where it has no net charge. Another small serum sample is placed on a precast polyacrylamide gel, which is placed

on a Multiphor unit connected to a waterbath. A cathodic wick is placed at the bottom of the gel and an anodic wick at the top of the gel. The electrode lid is placed on top of the gel so that both electrodes are in direct contact with the anode and cathode wick. Twenty serum samples are placed on each gel along with four known standards (MM, MZ, ZZ and MS). The gel is focussed for 2 hours and then placed in Coomassie Blue stain for 1 hour. The stain is later decanted into a waste container. The gel is destained overnight and can be read the next day. The patterns on the gel are analysed by at least two independent observers and compared to the included reference samples (MM, MZ, SZ, ZZ) to confirm phenotype identification.

GENOTYPING:

We have recently we have implemented a DNA genotyping system which can detect two alleles (S and Z) responsible for >98% of all cases of AATD. After filling out a short questionnaire with each patient, our clinical research nurse uses a lancet to obtain a small blood sample which is collected on specially treated filter paper. The ease of sample collection and storage using this method has allowed for self-testing in the home. Information brochures on Alpha-1 and a stamped addressed envelope are supplied with each kit. The completed kit should be sent directly to the diagnostic laboratory in the RCSI Education & Research Centre in Beaumont Hospital, where the test is performed free of charge.

To isolate DNA, four circles of blood spot are placed in a small test tube with some nuclease-free water. The sample is vortexed for approximately 1 minute and the water discarded three times. A small quantity of buffer is then added to the sample which is heated for 30 minutes. This isolated template DNA can then be used to perform RT-PCR (Real-Time Polymerase Chain Reaction) using a Roche Lightcycler.

Each reaction mixture contains template DNA, Magnesium Chloride, primers, HybProbe pairs, enzyme and water. Each reaction mixture is placed in a 20µL glass capillary and RT-PCR is performed in a Roche Lightcycler using cycling conditions adapted from Rodriguez et al (2002).

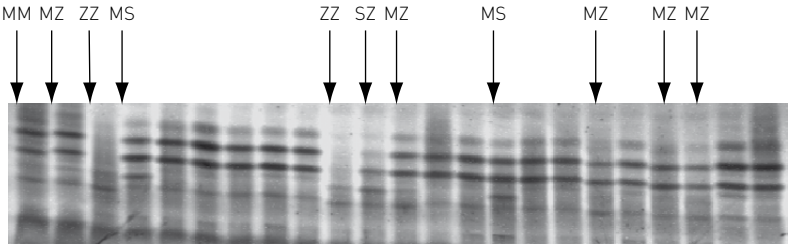

Fig. 2 — Typical Isoelectric Focussing Gel (pH 4-5)

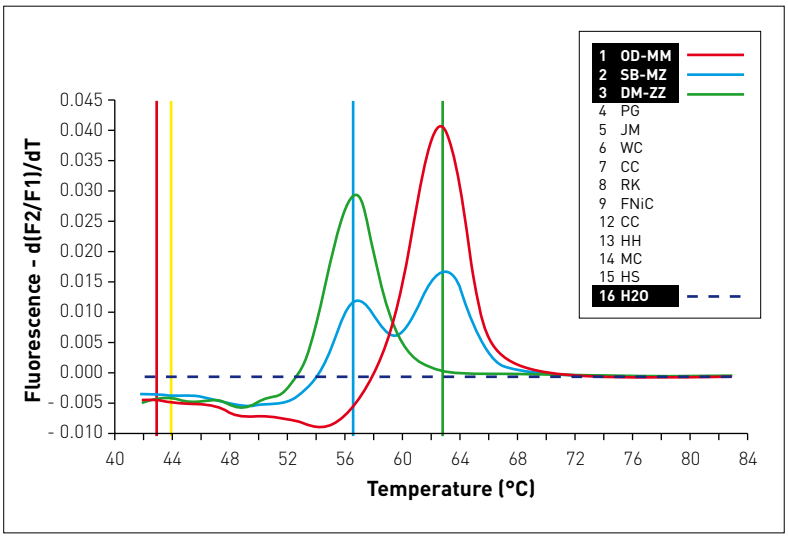

Fig. 3a — DBS MM, MZ, and ZZ standards highlighted in a Z assay on the Roche LightCycler with melt curve analysis.

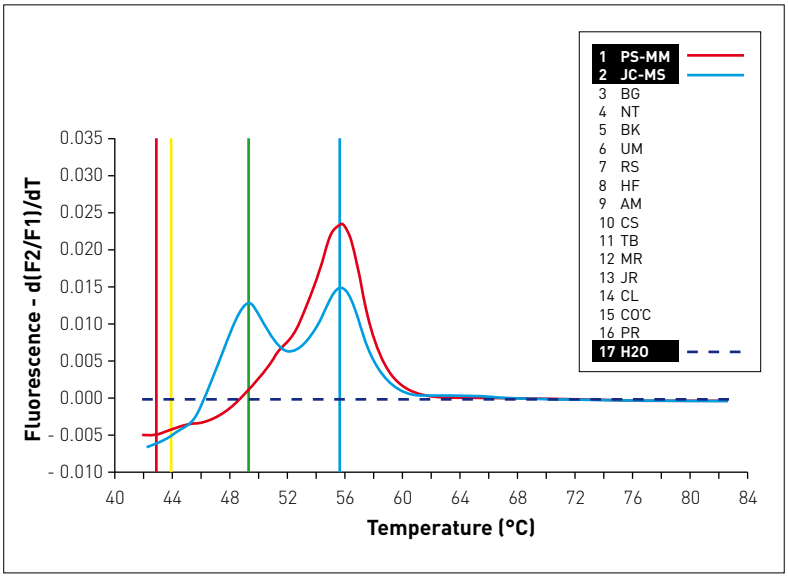

Fig. 3b — A typical S assay with DBS MM and MS standards on the Roche LightCycler.

After amplification, melting curves are generated by denaturation of the reaction. This melt curve analysis allows identification of the different genotypes on the basis of the temperatures at which they melt. Two major alleles (S and Z), responsible for >98% of all cases of Alpha-1 Antitrypsin Deficiency can be detected using this method.

The programme has been running for the three years. To date we have tested 2,000 individuals throughout Ireland. We use a combination of venous blood collection to determine phenotype and the dried blood spot method (involving a simple finger prick), to determine genotype (confined to Z and S alleles). It is important to remember that a reduced AAT level taken alone is insufficient evidence of AAT deficiency. This is because AAT levels can be falsely elevated due to the fact that AAT is an acute-phase protein. Therefore, determination of the quantitative level of AAT must be combined with phenotypic or genotypic analysis for a correct diagnosis of AATD.

The results of the tests are as follows: 1553 MM, 184 MZ, 43 ZZ, 26 SZ, 7 SS, 156 MS and 31 rarer phenotypes, these figures are from September 2007.

During the year we have visited and received support from the major teaching hospitals, who have all signed up to the programme. During the visits we presented the aims of the targeted detection programme to the respective respiratory teams which were very well received. We also presented at the Irish Thoracic Society, American Thoracic Society and European Respiratory Society international meetings.

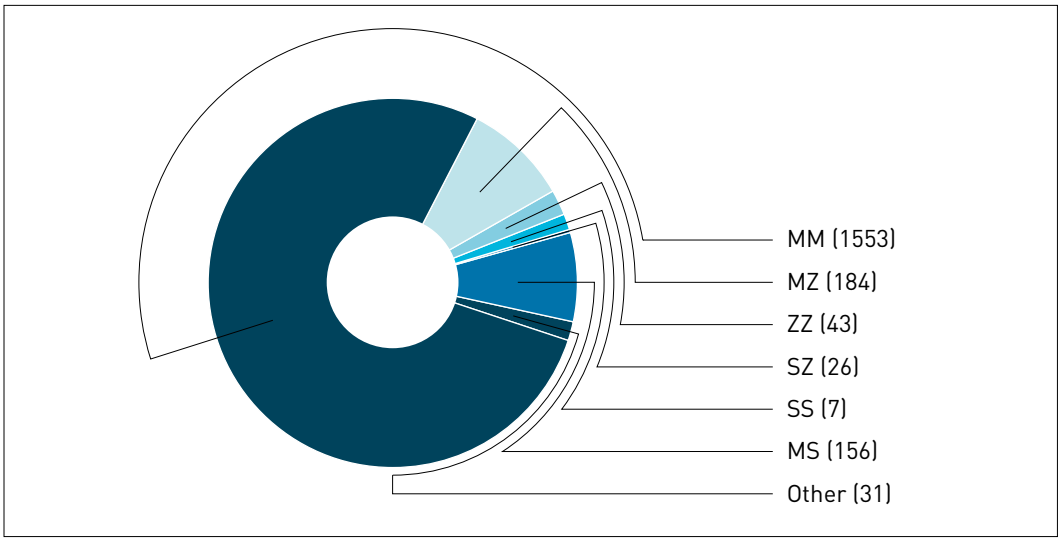

Fig. 4 — AAT Phenotypes detected in Irish Population (September 2007)

Phenotype	Numbers Identified
MM	1553
MZ	184
ZZ	43
SZ	26
SS	7
MS	156
Other	31
Total	*2000*

Geographical Location of Z Phenotypes Identified in Ireland

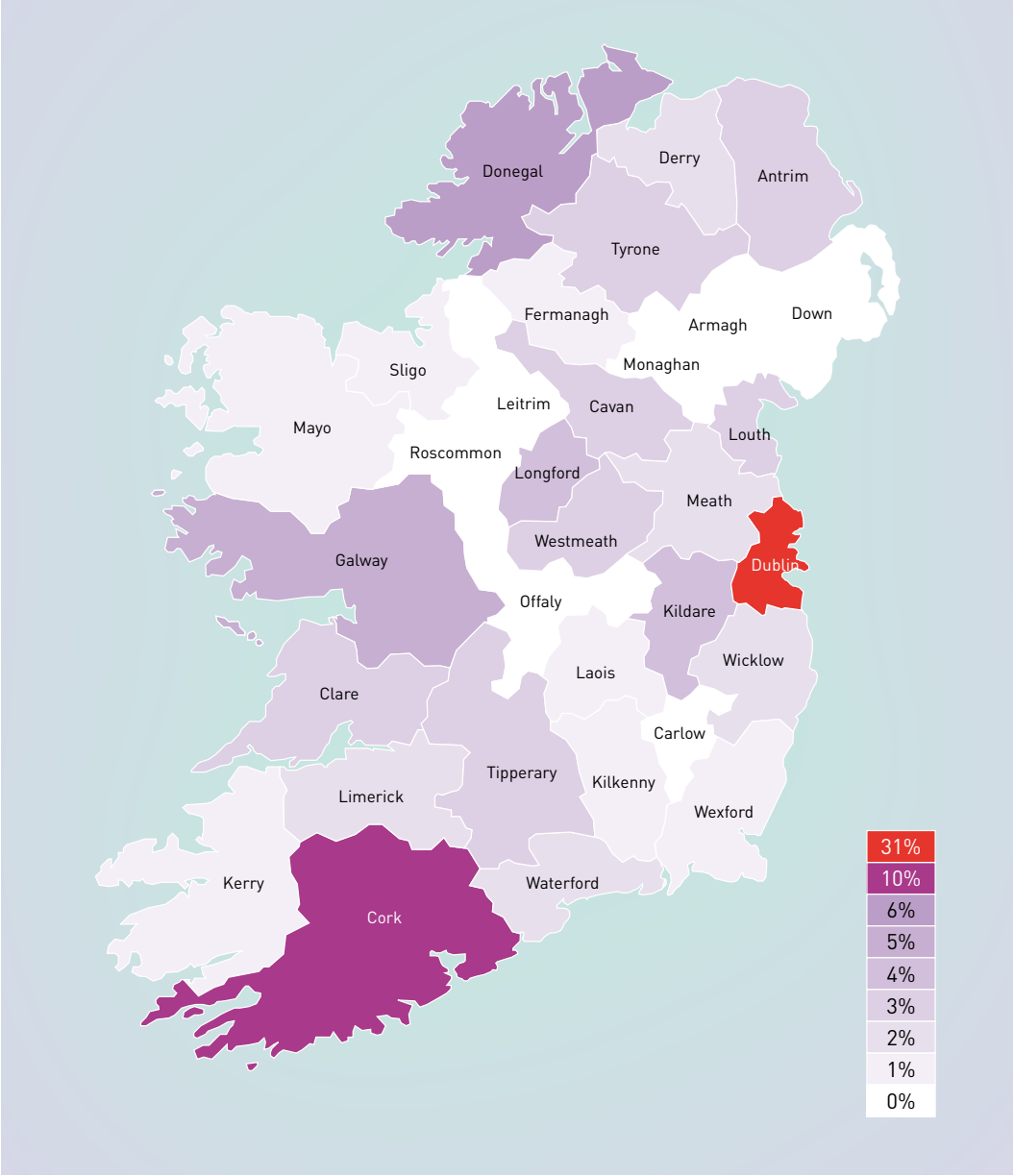

Fig. 5 — Location of Z Phenotypes Identified in Ireland

This map represents a geographical breakdown of the Z phenotypes (i.e. ZZ, SZ, MZ) in Ireland identified so far, compiled from data accumulated from our Screening Programme since 2004 and our Alpha-1 Registry. This will be updated on a yearly basis and as our screening centres increase throughout the country we hope to accumulate a complete representation of the frequency of Z and S genes in Ireland.

References

Rodriguez F, Jardi R, Costa X, Cotrina M, Galimany R, Vidal R, Miravitlles M. Rapid Screening for Alpha-1 Antitrypsin Deficiency in Patients with Chronic Obstructive Pulmonary Disease using Dried Blood Specimens; *Am J Respir Crit Care Med*; 2002 Sep 15; 166 (6); 814-7

Antitrypsin Report – Mouse, Minnie

REPORT DETAILS	
Patient ID:	XXXX
Name:	Minnie Mouse
Gender:	Female
Date Of Birth:	01/01/01
MRN:	XXXXX
Consultant:	Dr Stephen Lane (Peamount)
Hospital:	Peamount Hospital, Newcastle, Co.Dublin
Date Sample Taken:	01/01/01
Phenotype:	MM
Phenotype Level:	X.XX
Phenotype Percent:	XX%

All data reported by Dr. Tomas Carroll & Olwen Floyd

NOTE:- Under the terms of Disability Act 2005 part 4, section 2: it is ILLEGAL to disclose any information gained by genetic testing (such as this) for the purpose of insurance, assurance, pension, mortgage etc.

Alpha-1 Antitrypsin (AAT) Deficiency

AAT-deficient patients are at high risk of developing life-threatening lung and liver disease. The normal AAT protein is the M variant, synthesised in the liver and present in sufficient amounts to provide a protective anti-protease screen in the lung. The most common severely deficient variant is the Z protein, causing decreased circulating levels of AAT. The Z variant folds incorrectly and polymerises in the liver, preventing its release with concomitant reduced blood and lung levels. Z AAT accumulation can also cause liver disease. Patients with the ZZ phenotype typically have 5-15% of normal AAT levels, while MZ patients (1 normal, 1 deficient) have 50-80% of normal AAT levels. Another less severe deficient variant is the S protein. SS homozygous patients are predisposed to developing emphysema but not liver disease, with MS carriers (1 normal, 1 deficient) possessing almost normal AAT levels. Another possible phenotype is SZ (2 deficient AAT variants) with circulating AAT levels decreased to 25-40% of normal, and these patients have an increased risk of lung or liver disease. There are at least 20 other rare variants of the AAT protein, such as I, F, and null variants, which confer varying degrees of deficiency.

The most important thing to remember is that cigarette smoke is the single biggest risk factor for Alpha-1 patients in the development of emphysema.

	Phenotype	What Does It Mean?
Normal	MM	Does not have the disorder and does not carry any altered AAT genes
Carrier	MZ/MS	Mild to moderate AAT deficiency - may develop disease symptoms and does carry an altered AAT gene
AAT Deficiency	ZZ/SZ/SS	Moderate to severe AAT deficiency - will develop disease eventually and does carry two altered AAT genes

Registry

OBJECTIVES

- Objectives of the registry are to identify, collect, classify, record, store and analyse information relating to the prevalence, incidence and treatment of Alpha-1 Antitrypsin Deficiency in Ireland.
- Collect, classify record and store information in relation to existing and newly diagnosed Alpha-1 patients.
- Promote and facilitate the use of the data collected in approved research projects and in the planning and management of services.
- Provide advice, information and assistance in relation to any aspect of Alpha-1 to various Health Boards and other service providers and persons with Alpha-1.
- Provide data on long term prognosis for Alpha-1 in Ireland - including treatment outcomes, management regimes, quality of care, and international best practice.
- Provide data on long term prognosis for Alpha-1 patients in Ireland and compare this information with international data.
- Register all Alpha-1 patients whose usual residence is in Ireland and to record genotype of all Alpha-1 patients treated in Ireland.

The Alpha-1 Registry of Ireland has again increased its enrolment for 2007. An annual assessment section has recently been completed by our computer programmers and this has enabled us to complete both diagnosis and annual clinical data for each patient. This has advantages in terms of having the ability to analyse the data obtained. For the next few months we will concentrate on enrolment as much as possible with increasing emphasis on Consent forms. Full ascertainment will bring a number of benefits:

- We will have a much better idea of our population distribution.
- We will be able to perform statistical analysis on the 'age of diagnosis' to determine whether there are differences between sexes and /or genotype.
- We will be able to move towards producing in this country and compare them with other countries.

This year we can provide comprehensive reports of patients on the registry. These include gender, genotype, diagnosis details, complications suffered, pulmonary function test and GOLD guideline standards. These reports are available on an individual basis as well as group basis.

Regarding the accumulation of annual assessment records, we have a total of 31 annual assessments recorded on the Registry.

GENERAL DATA

Gender

The total number of male and females on the Alpha-1 Registry is fairly evenly split between male (55%) and female (45%) (Figure 6).

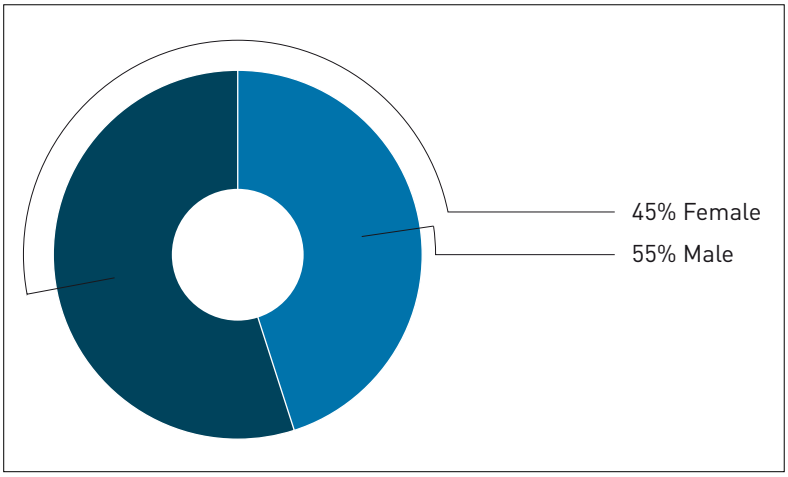

Fig. 6 — Proportion of male and female patients enrolled in Registry

During the past 12 months we have gathered additional genotype data as well as increased the total number of Alpha-1 patients on the database, with 54 patients in total. The new genotypes added are SZ and MZ (Carrier) (Figure 7). Additional patients and data are needed to generate statistical reports for these genotypes at present.

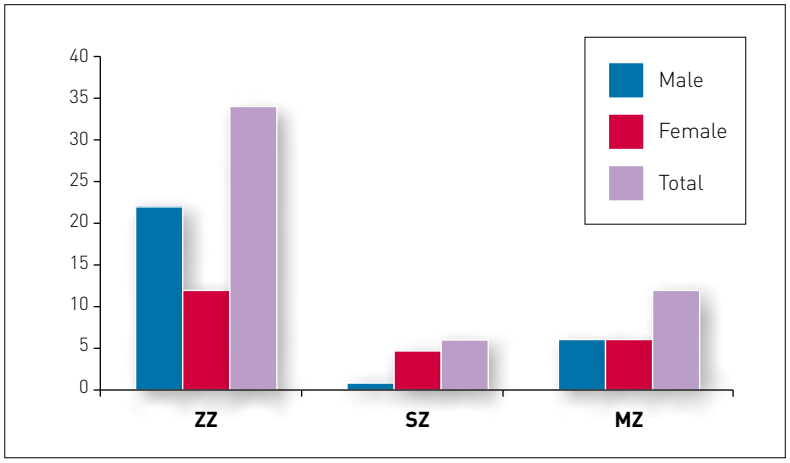

Fig. 7 — Gender Distribution and Genotype

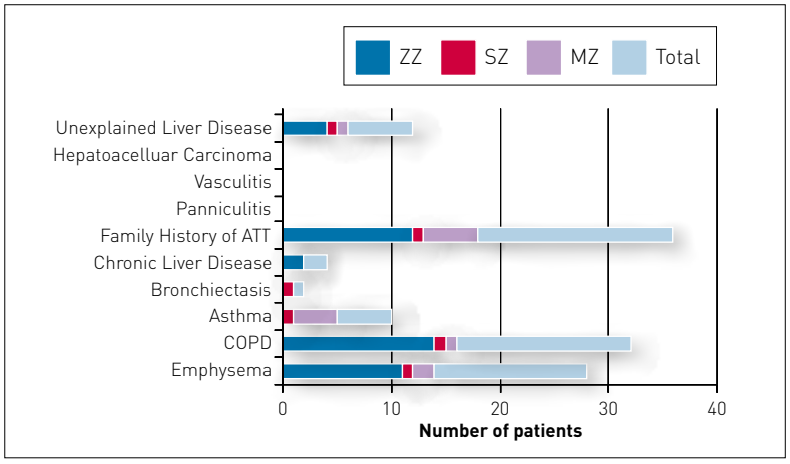

Fig. 8 — Method of Diagnosis and Genotype

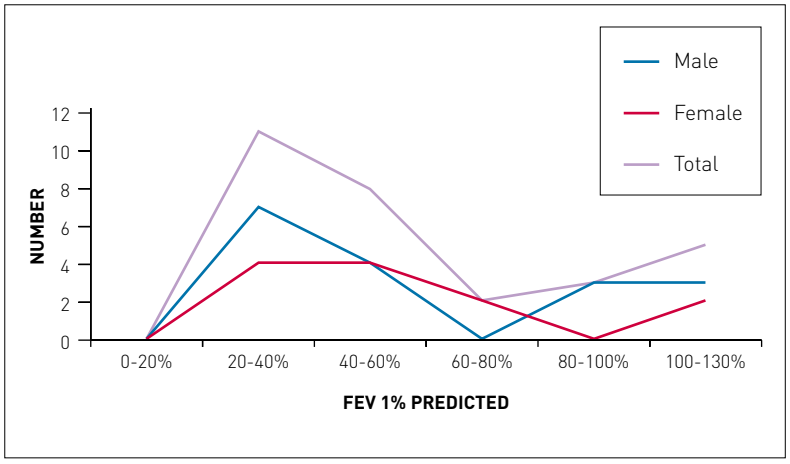

Fig. 9 — FEV 1% Predicted, male and female in ZZ

METHOD OF DIAGNOSIS

It is important to record a person's symptoms at diagnosis. We use a list of 11 symptoms and each person may have several symptoms. Our list has been divided into symptoms that brought a person to the attention of a physician (for example 'Asthma'). The most frequently recorded symptoms are family history, emphysema and COPD.

FEV1% PREDICTED AND FVC% PREDICTED

The pulmonary function tests (PFT) that are recorded in the Registry are 'Forced Expiratory Volume in 1 Second' and 'Forced Vital Capacity'. Both of these tests are indicators of the condition of lung functioning.

We have the capacity to record up to four PFT's in each Annual Assessment. As more data accumulates, we will be able to combine years of test results to show longitudinal movement for each Alpha-1 individual.

The FEV1 predicted and FVC predicted averages for ZZ patients are divided into male and female patients.

The PFT results show, that males start out with a higher number at <40% FEV1% predicted compared to females, and a higher number of males at >80% FEV1% predicted than females, with the peak total number at the 20-40% FEV1% predicted.

COMPLICATIONS

We have recorded complications that have occurred since the patient's last assessment. Among ZZ patients, respiratory exacerbation is the most common complication at 49%, compared with 2% Pneumothorax.

It is important to examine the complications that occur with less frequency as they can emerge in any patient and awareness of these is important. Data surrounding complications can reveal a lot of information about individuals with Alpha-1, the Alpha-1 centres and the overall trends in the country. It can be a barometer of well-being. It can be correlated to hospitalisations and used in pharmacological economic studies.

GOLD GUIDELINES

The Global Initiative for Obstructive Lung Disease, GOLD, has set out to raise clinical interest in the diagnosis and management of chronic obstructive pulmonary disease across the world with the aim "to improve prevention and treatment of this lung disease". The GOLD organisation is a committee of leaders in the field which is sponsored by 14 pharmaceutical companies with an interest in this area of medical practice. It was set up in 1997 with the collaboration of the National Heart, Lung, and Blood Institute, National Institutes of Health, USA, and the World Health Organization. We have found 44% of ZZ patients are Stage III according to the GOLD Guidelines, 19% are Stage II and 37% are Stage I (Figure 11).

SPIROMETRIC CLASSIFICATION OF COPD SEVERITY

Stage Characteristics:

I: Mild COPD • FEV1/FVC < 70%
• FEV1 ≤ 80% predicted

II: Moderate COPD • FEV1/FVC < 70%
• 50% ≤ FEV1 < 80% predicted

III: Severe COPD • FEV1/FVC < 70%
• 30% ≤ FEV1 < 50% predicted

IV: Very Severe COPD • FEV1/FVC < 70%
• FEV1 < 30% predicted or FEV1 < 50% predicted plus chronic respiratory failure

FEV1: forced expiratory volume in one second; FVC: forced vital capacity; respiratory failure: arterial partial pressure of oxygen (PaO2) less than 8.0 kPa (60 mm Hg) with or without arterial partial pressure of CO2 (PaCO2) greater than 6.7 kPa (50 mm Hg) while breathing air at sea level.

Improved treatments can be developed with the help of patient registries. Alpha-1 Antitrypsin Deficiency registries gather information on all aspects of a patient's condition. They act as storehouses for treatment and infection statistics. Detailed anaylsis of this information can yield significant findings about the most effective treatment for Alpha-1. It is through this analysis that better management of Alpha-1 may be achieved.

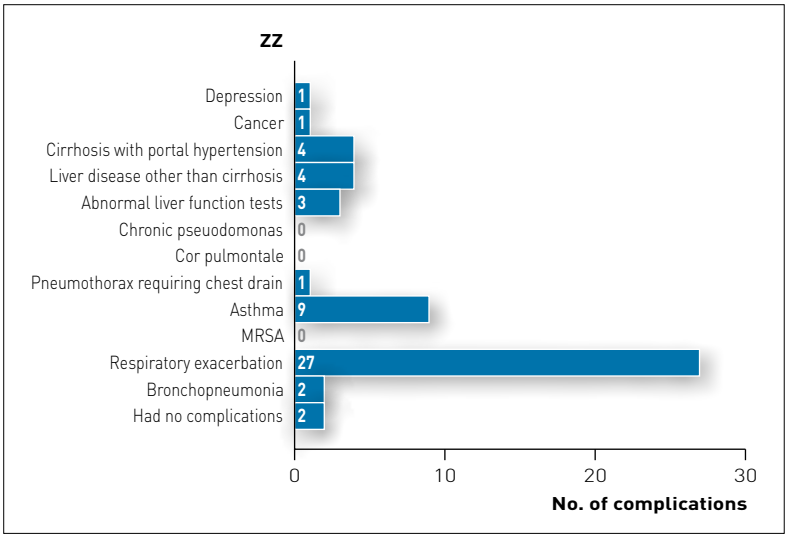

Fig. 10 — Complications in ZZ patients

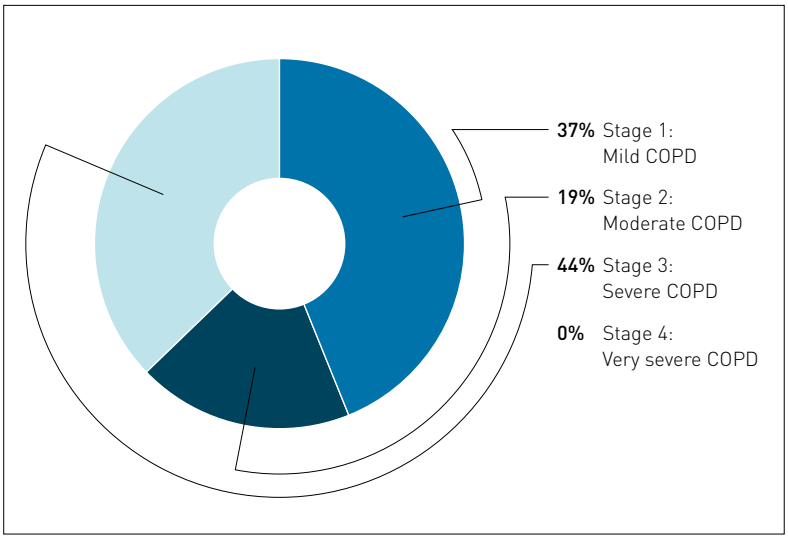

Fig. 11 — GOLD Guidelines Stages in ZZ patients

Research Programmes

We take a very broad view of research including: basic science, translational science, clinical trials, psychosocial studies, dietetics, establishing standards and detection programmes. In fact, a study of anything that might affect the health of Alpha-1 patients would be seen by us as health research.

Current research projects include:

1. Alpha-1 Augmentation Therapy Clinical trial
2. Pilot Alpha-1 Antitrypsin Deficiency Screening Programme in a normal population
3. Clarification of the Risk of COPD in PI MZ Individuals
4. Identification and characterisation of novel pro-inflammatory proteases that exacerbate lung disease associated with Z Alpha-1 antitrypsin deficiency
5. Elucidation of Unfolded Protein Response pathways activated in lung and liver disease associated with Z Alpha-1 antitrypsin deficiency
6. Elucidation of a Role for Foxa2 in Alpha-1 Antitrypsin Deficiency
7. Immune Cell Function in Alpha-1 Antitrypsin Deficiency

ALPHA-1 AUGMENTATION THERAPY CLINICAL TRIAL

This study is being conducted in Beaumont Hospital by Professor McElvaney and his team.

This is a placebo-controlled, double blind, multicentre phase III / IV study to compare the efficacy and safety of the drug Zemaira® in patients with Emphysema due to Alpha-1 proteinase inhibitor deficiency. The duration for each patient is two years.

We have recruited 11 patients so far and they are all at various stages in the trial. The trial involves having weekly intravenous infusions of Zemaira®, an Alpha-1 proteinase inhibitor or a placebo which is a dummy treatment that looks like the real thing but is not. As the study is double- blinded, neither the participating patients nor our study staff know which therapy has been assigned to them. There is an equal chance of receiving either treatment.

The infusions are given either in Beaumont Hospital or the patient's own home, taking on average 20 minutes. Every three months patients are required to attend Beaumont hospital so that routine tests can be carried out. These include:

- Monitoring of vital signs, i.e. blood pressure, weight etc
- Blood tests.
- Pulmonary Function tests.
- Physical Examination by physician.
- Cotinine test (urine test that detects nicotine) is required.

At certain visits a Quality of Life questionnaire and CT scan are performed. These help to investigate the effect of Zemaira® on the development of patients Emphysema.

The main inclusion criteria for all patients that enter onto the study are:

- Diagnosis of Alpha-1 antitrypsin deficiency.
- Non smokers or Ex-Smokers who have stopped at least six months prior to screening.
- Age range of 18 – 65 years of age, male and female.
- Emphysema with an FEV1 of 35-70% predicted range.

As with other Alpha-1 therapies, Zemaira® may not be appropriate for the following adults:

- Individuals with a known hypersensitivity and/or history of anaphylaxis or severe systemic reaction to A1-PI products or their components.
- Individuals with selective IgA deficiencies who have known antibodies against IgA

This is due to Zemaira® being derived from human plasma. All patients however will be assessed on an individual basis.

In previous clinical studies, Zemaira® has been shown to be generally well tolerated and provides patients with half or less the infusion time of other available Alpha-1 augmentation therapies available.

If you would like any further information on Zemaira® or you are interested in taking part in the trial, Please feel free to contact either:

Charlotte Hall
Research Nurse, Study Co-Ordinator
Tel: 01 8093864
charlottehall@rcsi.ie
Or
Máire Stack
Research Nurse, Study Co-Ordinator
Tel: 01 8093876
mstack@rcsi.ie

CLARIFICATION OF THE RISK OF COPD IN PI MZ INDIVIDUALS

We invite PI MZ individuals and their family members to take part in this new study, conducted by Professor Gerry McElvaney, Department of Medicine, Beaumont Hospital. We will be testing for PI MZ COPD risk specifically within families that have an identified PI MZ COPD individual.

One of the major areas of interest in Alpha-1 Antitrypsin (AAT) Deficiency research is whether individuals with the PI MZ genotype are more susceptible to develop chronic obstructive pulmonary disease (COPD). Although many studies of the PI MZ type and COPD risk have been performed, the results of these previous studies have been largely inconsistent. Resolving the ongoing controversy about COPD risk in PI MZ subjects will require a large and carefully studied population. Studying families of PI MZ and PI MM individuals can be a very powerful way to determine if PI MZ is really a risk factor for COPD.

We will enroll 400 parents and siblings of 100 PI MZ COPD individuals and perform spirometry testing, phlebotomy (for PI typing and future genetic modifier studies), and a questionnaire. Approximately 100 PI MZ individuals with diagnosed COPD will be invited to perform a screening visit in Beaumont Hospital or in their home.

The first study visit will be used to determine whether the PI MZ individual meet the following inclusion and exclusion criteria:

1) Age >30
2) GOLD stage 3 or 4 COPD (post-bronchodilator FEV1 <50% predicted; ratio <0.7)
3) No other lung diseases that would affect pulmonary function testing
4) Confirmed PI MZ phenotype

The potential PI MZ COPD individual will perform spirometry, complete family history and respiratory questionnaires, and phlebotomy to confirm their PI type and provide DNA samples.

Exclusion criteria for relatives are:

1) Lung diseases e.g. interstitial lung diseases (patients with asthma will be included)
2) PI types other than PI MM or PI MZ
3) Not full biological siblings of the PI MZ COPD proband.

The success of our study critically depends upon obtaining as many relatives as possible to participate in the research study. There is no obligation whatsoever to participate in this research study; whether individuals participate will have no influence on their regular treatment or medical care in any way. While the results of this study would not be expected to benefit them or their family directly, the knowledge gained might eventually benefit others at risk of COPD.

Our goal is to include as many siblings and parents as possible and we encourage at least four family members of each PI MZ individual to participate in this research. We will determine whether the PI MZ type is associated with COPD and whether cigarette smoking confers increased COPD risk in PI MZ individuals.

If any patients fulfill the above criteria, please contact:

Dr Valerie Morris
Alpha One Suite
Smurfit Building
Beaumont Hospital
Dublin 9
Email: vbmorris@rcsi.ie
Tel. 085 7255506.

PILOT ALPHA-1 ANTITRYPSIN DEFICIENCY SCREENING PROGRAMME:

Funding body: Talecris Biotherapeutics

Project Description: Alpha-1 antitrypsin deficiency (AATD) is an under-diagnosed condition. It is estimated that there are approximately 1,200 individuals with Alpha-1 antitrypsin deficiency in Ireland but as yet less than 5% have been identified. The World Health Organization recommends screening of individuals with chronic obstructive pulmonary disease, non-responsive asthma and cryptogenic liver disease for Alpha-1 antitrypsin deficiency.

The objective of the project is to screen for Alpha-1 antitrypsin deficiency in a random sample of the general population. This will allow us to more precisely determine the incidence of this disease in Ireland. To date in Ireland the only screening for AATD is a targeted detection programme, with specific patient populations tested for the condition.

The sample population in this study is from the Biobank in Trinity College Dublin, which has recently completed a national randomised buccal swab collection (1000 samples), based on the electoral register. The DNA has been collected and stored on Isocode (FTA Elute) paper, and is suitable for survey-type studies, investigating gene frequencies, and profiling the distribution of genotypic markers.

Duration: 1 year

IDENTIFICATION AND CHARACTERISATION OF NOVEL PRO-INFLAMMATORY PROTEASES THAT EXACERBATE LUNG DISEASE ASSOCIATED WITH Z ALPHA-1 ANTITRYPSIN DEFICIENCY

Funding Body: Health Research Board (HRB)/ Medical Research Charities Group (MRCG)

Project Description: Alpha-1 antitrypsin (AAT) is a serine anti-protease produced chiefly in the liver. AAT deficiency is an autosomal recessive disease that results in AAT being misfolded and thus accumulates within hepatocytes and colangiocytes. This disease results in early onset emphysema and liver disease and is thought to affect approximately 1,200 Irish people. Emphysema associated with AAT deficiency is characterized by a pro-inflammatory phenotype and an imbalance in the protease:antiprotease ratio in the lung. Augmentation therapy with recombinant or plasma-purified AAT can restore the protease: antiprotease balance in the AAT deficient lung however it does not reverse the proinflammatory events. Therefore understanding the molecular mechanisms that regulate inflammation in the lung in AAT deficiency is a priority. The role of Neutrophil Elastase (NE) in pulmonary inflammation is a key research focus of this laboratory. To date our studies have identified one intracellular signaling pathway regulated by NE leading to interleukin-8 (IL-8) production and investigated ways to inhibit this pathway in airway epithelial cells. We have also identified Toll-like receptor 4 and the epidermal growth factor receptor (EGFR) as targets of NE. EGFR is a key receptor involved in promoting inflammation within the lung environment. The classical and well-documented pathway of EGFR activation via NE in alveolar epithelial cells involves the epithelial-derived protease TACE. TACE in turns cleaves the ligand TGF alpha enabling it to bind to EGFR and thus increase the pro-inflammatory response. The aim of this study is to identify other key proteases involved in NE transactivation of EGFR on the bronchial epithelium using 16HBE14o- cells and AAT endobronchial biopsy samples. Our initial studies clearly show that

other proteases besides TACE can activate EGFR in response to NE treatment. It is our aim to identify these proteases, elucidate their role in the pro-inflammatory response regulated by EGFR and investigate their interaction with important immune cells.

Duration: 3 years from January 2007

ELUCIDATION OF UNFOLDED PROTEIN RESPONSE PATHWAYS ACTIVATED IN LUNG AND LIVER DISEASE ASSOCIATED WITH Z ALPHA-1 ANTITRYPSIN DEFICIENCY

Awarding Body: Health Research Board (HRB)/ Medical Research Charities Group (MRCG)

Project Description: Current treatments for AAT deficiency are based on augmentation therapy with recombinant or plasma-purified AAT and focus almost completely on treating the pulmonary emphysema associated with the disorder. However, the long term efficacy of augmentation therapy has yet to be established, and represents a very expensive therapeutic option. Lung or liver transplantation provides the only effective means of intervention for AAT deficient patients with end-stage lung and liver disease. Unfortunately, while transplantation has been shown to successfully achieve AAT serum conversion, its usefulness as a treatment is confounded by a lack of suitable donors, concomitant immunosuppressive therapy, and high mortality rates. For these reasons it is critically important to develop less invasive therapeutic strategies for the treatment of the lung and liver disease associated with AATD. Conformational diseases are associated with rogue protein accumulation in tissues and cellular compartments. AAT deficiency is one such genetic disease characterised by the accumulation of incorrectly folded AAT in liver cells. The accumulation of mutant Z AAT protein within the cell switches on several protective mechanisms, including the unfolded protein response (UPR). With prolonged activation the UPR becomes harmful to the cell, causes inflammation and ultimately cell death. This is responsible for the cirrhosis observed in the AATD-associated liver disease, but may also be partly responsible for the emphysema seen in AATD-associated lung disease. This may explain why augmentation therapy has yet to be proven clinically effective, and why AAT deficient patients who have received liver transplants still exhibit local inflammation in the lung. We have previously identified intracellular events involved in the molecular pathogenesis of AATD-induced liver disease using an *in vitro* model system of Z AAT accumulation in liver cells. We will demonstrate that abnormalities in Z AAT-induced liver disease can also be present in Z AAT-induced lung disease. We aim to highlight the crucial involvement of the UPR in Z AAT-associated lung and liver disease. We will show that Z AAT activates the UPR, knocking off protein synthesis, turning on a plethora of UPR-related genes, and activating machinery which degrades the misfolded Z AAT. We will evaluate the role of UPR mediators *in vivo* in lung and liver biopsies from individuals with AATD compared to healthy normal individuals. This discovery-driven project proposal will lead to a greater understanding of AATD, generate several hypotheses, and allow us to identify novel therapeutic avenues for the treatment of AATD-related lung and liver disease.

Duration: 3 years from January 2007

ELUCIDATION OF A ROLE FOR FOXA2 IN ALPHA-1 ANTITRYPSIN DEFICIENCY

Awarding Body: Alpha-1 Foundation (USA)

Project Description: Alpha-1 Antitrypsin (AAT) is synthesised in the liver and travels to the lung forming a protective screen against harmful proteases, bacteria and cigarette smoke. AAT deficiency (AATD) is a hereditary disorder associated with lung and liver disease. The transcription factor forkhead box a2 (Foxa2) has been shown to regulate the expression of genes that are critical to lung morphogenesis, host defence, and function (Wan *et al.*, 2004). Foxa2 also regulates gene expression in the liver, and is essential for the maintenance of glucose and lipid homeostasis (Puigserver and Rodgers, 2006). Work from Jeffrey Whitsett's

group in Cincinnati has demonstrated airspace enlargement, goblet cell hyperplasia, increased mucin production, and inflammation in the lungs of Foxa2 knockout mice (Wan *et al.*, 2004). These symptoms are characteristics of asthma, chronic obstructive pulmonary disease (COPD) and AATD, suggesting a role for Foxa2 in AATD. Preliminary findings from our group demonstrate that Foxa2 expression is decreased *in vitro* in Z AAT-transfected bronchial epithelial cells. Threonine phosphorylation by specific kinases inactivates Foxa2. Intracellular accumulation of misfolded proteins such as Z AAT induces ER stress response pathways, including the unfolded protein response (UPR). The UPR pathway comprises several kinases, and a putative Foxa2 kinase may be located here. This project will investigate the potential role of Foxa2 in AATD-related lung and liver disease. We will examine whether Foxa2 over-expression can reverse the harmful effects of Z AAT-induced inflammation *in vitro* in both lung and liver cell lines. The effects of various inflammatory agonists on Foxa2 expression will be investigated. We will also determine by immunohistochemistry whether Foxa2 expression is decreased *in vivo* by comparing non-AATD to AATD individuals. The elucidation of the molecular mechanisms associated with Z AAT-related lung and liver disease will lead to a further understanding of AATD, and accelerate the development of targeted therapeutic strategies.

Duration: 12 months from July 2007

IMMUNE CELL FUNCTION IN ALPHA-1 ANTITRYPSIN DEFICIENCY

Awarding Body: Talecris Biotherapeutics

Project Description:
Alpha-1 antitrypsin deficiency (AATD) is a hereditary disorder characterised by lung and liver manifestations. The most common form of AAT deficiency occurs due to the Z mutation, which encodes a glutamine to lysine substitution at position 342 of the AAT protein. This mutation causes the protein to fold aberrantly and accumulate in the endoplasmic reticulum (ER) of hepatocytes. The liver disease is believed to be associated with intracellular accumulation of AAT in the ER leading to ER stress responses whilst the lung disease is due to decreased levels of the AAT antiprotease in the airways, thereby facilitating proteolytic damage. In addition to hepatocytes, AAT is also expressed by other cell types including monocytes and neutrophils. We aim to demonstrate that ER accumulation of Z AAT in monocytes and neutrophils impacts on specific phenotypes and functions of these immune cells, contributing to the overall inflammatory disease process.

Duration: 12 months from September 2007

International Conferences:

American Thoracic Society 2007 Abstract

ALPHA-1 ANTITRYPSIN DEFICIENCY IN IRELAND: NATIONAL TARGETED DETECTION PROGRAMME

Author: T. Carroll, O. Floyd, C. O'Connor, C. Taggart, R. Costello, S. J. O'Neill and N. G. McElvaney

Institution: Department of Respiratory Research, RCSI Building, Beaumont Hospital, Dublin 9, Ireland.

Rationale: Alpha-1 antitrypsin (AAT), a serine protease inhibitor, is a glycoprotein synthesised chiefly in the liver and is the most important antiprotease in the lung. AAT deficiency is a hereditary autosomal codominant disorder, resulting from mutations in the AAT gene, and classically presents with emphysema in young- to middle-aged adults and liver disease in childhood and occasionally in adulthood. The most common phenotype presenting with clinical evidence of AAT deficiency is the Z phenotype, resulting in decreased levels of circulating AAT due to retention of the aberrantly folded protein in the liver. It is unclear whether the MZ phenotype confers increased risk for disease. Demographic studies indicate that AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. World Health Organisation guidelines advocate targeted detection programmes of patients with COPD and asthma.

Results: 1400 individuals with COPD or asthma attending respiratory outpatient clinics in Ireland were screened. A combination of serum AAT measurement by radial immunodiffusion (RID) or nephelometry, phenotyping by isoelectric focussing (IEF), and genotyping of DNA isolated from dried blood spot samples by PCR identified 24 ZZ, 113 MZ, 115 MS, 20 SZ, 1 SS patient, as well as 6 other rarer alleles.

Conclusions: The percentage of deficiency alleles was higher than anticipated from studies in other populations. The S variant, common to the Iberian Peninsula, was detected with unusually high frequency. Several other rarer phenotypes were also detected. Further analysis will reveal whether these phenotypes predispose individuals to lung disease. Increased awareness and understanding of AAT deficiency will prevent the continuing under-diagnosis of this condition.

Acknowledgements: Alpha One Foundation Ireland, U.S. Alpha One Foundation, Department of Health and Children, and the Royal College or Surgeons in Ireland.

American Thoracic Society 2007 Abstract

Z ALPHA-1 ANTITRYPSIN INDUCES THE UNFOLDED PROTEIN RESPONSE (UPR), AN ER STRESS PATHWAY, IN BRONCHIAL EPITHELIAL CELLS

Author: T. Carroll, C. Greene, C. Taggart, S. J. O'Neill and N. G. McElvaney

Institution: Department of Respiratory Research, RCSI Education and Research Centre, Beaumont Hospital, Dublin 9

Rationale: Alpha-1 antitrypsin (AAT) is a serine protease inhibitor synthesised in the liver and functions as the most important antiprotease in the lung. AAT deficiency (AATD) is a hereditary disorder resulting from mutations in the AAT gene and presents with emphysema in young adults and liver disease in childhood. The most common phenotype presenting with clinical evidence of AATD is the Z phenotype, with decreased levels of circulating AAT due to retention of the aberrantly folded protein in the endoplasmic reticulum (ER) of hepatocytes. Having shown previously that ER stress pathways are activated in the liver we sought to elucidate whether misfolded Z AAT can induce ER stress pathways, specifically the UPR, in bronchial epithelial cells.

Results: We demonstrate that Z AAT induces the phosphorylation of eukaryotic initiation factor 2Ð (eIF2Ð) in 16HBE14o- cells, responsible for shutting down global protein synthesis, the first step in the UPR. Z AAT activates in vitro the chaperones glucose-regulated protein 78 and 94 (grp78 and grp94), and the transcription factors X-box binding protein-1 (XBP-1) and activated transcription factor 6 (ATF6), elements of the transcriptional program of the UPR. Finally, we show that Z AAT induces the chaperone p97/valosin-containing protein (p97/VCP) and C/EBP-homologous protein (CHOP), components of ERAD.

Conclusions: Airway epithelial cells produce AAT locally in the lung, representing another source of misfolded protein. We have shown that misfolded Z AAT induces ER stress pathways, specifically the UPR, in bronchial epithelial cells. As transplantation is the only current treatment for end stage AATD lung and liver disease, the inhibition and/or induction of disparate elements of the UPR may represent viable therapeutic modalities.

Acknowledgements: Alpha One Foundation Ireland, Royal College of Surgeons of Ireland, and Department of Health and Children AAT Targeted Detection Programme.

Alpha One Strategic Partnership

Since the establishment of the Alpha One in 2001, we have recognized the importance of establishing strategic partnerships with government bodies, international organisations, voluntary health groups and Collaborating Committees.

COLLABORATING COMMITTEES

Irish Asthma Society

Alpha-1 Foundation (US)

Alpha-1 Association (Italy)

Alpha-1 Association (Spain)

Plasma and Protein Therapeutics Association

EURORDIS: European Platform for Rare Diseases

RCSI Education and Research Centre Beaumont Hospital

EMEA: European Medicines Agency

ITS: Irish Thoracic Society

ANAIL: Irish Respiratory Nurses Association

European Alpha-1 Detection Consortium

ORGANISATIONAL MEMBERSHIPS

AIR: Alpha-1 International Registry

ALFA Europe

Medical Research Charities Group (MRCG)

Alpha One International Network

Irish Donor Network

Pro Health lobby of the Smoking Ban

IPOSSI: Irish Platform for Patients Organisations, Science and Industry

Awareness Campaign

Since the establishment of our patient support groups last year, we have developed a dynamic network of members throughout the country.

These members undertook an awareness campaign in June 2007. John Hannan, an Alpha-1 patient undertook a Horse Ride from Mizen Head to Malin Head in combination with G.P., Allied Health professional and patient meetings en route. John gained well deserved publicity from local radio stations and local newspapers. Our main aim of the awareness campaign is early detection of Alpha-1 by a FREE simple dried blood spot test, with follow up support and treatment from the Alpha One Foundation.

Meeting were held in Cork and Galway which were attended by GPs, respiratory nurses, physiotherapists, laboratory staff and respiratory technicians. Dr Charles Bredin, Respiratory Consultant, University College Hospital Cork and Dr Anton O' Regan, Respiratory Consultant, and University College Hospital Galway presented at these regional meetings.

Our patient meeting in Galway was a great opportunity for patients from the west of Ireland who previously were unable to attend meetings in Dublin to meet other patients from their local area and establish a network of their own. Speakers contributing to our information day were from local based services including Rebecca Levy from Galway Advocacy Service, and Bernie Dowling, Carer's Association, Galway.

On completion of the Horse Ride in Malin Head, we received overwhelming support from the local people and family members of Alpha-1 patient in the Inishowen area. A presentation and heartfelt acknowledgement was given to John Hannan and his support team on this long and arduous journey during the month of June. This celebration of events was held in Malin with support from local government and council officials, friends, family and members of the Alpha One Foundation.

Flora's Women's Mini Marathon, 4 June 2007

John Hannan, Mizen to Malin Head Horse Ride June 2007

The following are highlights from the awareness campaign:

WOMEN'S MINI MARATHON

The annual Women's Mini Marathon has again shown us the commitment and support of our patients their families, friends, relatives and staff. This year over 30 women participated in the Flora's Mini Marathon on June 4th raising over €4,000 for the Alpha One foundation. We very much appreciate the great efforts you made, not only in participating in the event but also in getting sponsors and in collecting the sponsor money. We realise that getting money from people is not an easy task. This significant sum will be put towards Alpha-1 education and research. Once again thank you and congratulations on a magnificent success.

MARATHON HORSE RIDE FROM MIZEN HEAD TO MALIN HEAD

A 57-year-old patient suffering from Alpha-1 will ride the length of Ireland from Mizen Head in the South to Malin Head in the North. Johnny Hannon wants to draw attention to his condition and make people, medical and lay, aware of it.

Asked why he was undertaking such an arduous task in his condition, Johnny said:

"When I was diagnosed with both lung and liver Alpha-1 in 2003 my specialist said that I had a condition that was not well known. So there must be thousands of people out there with it and who know nothing.

To have this condition is life changing. I had to change my whole work and lifestyle because of it.

More people, especially doctors in hospitals and GPs need to know more about it. They should have notices on their boards telling people about it. They should test all their liver and lung patients for it.

It may seem crazy to ride a horse the length of Ireland but I made up my mind that I just have to do something to bring Alpha-1 to the attention of doctors and patients, particularly those who have liver or lung problems.

Much more needs to be done to detect and help people with Alpha-1. The test is very simple - just like a cholesterol test and can be done FREE by the Alpha One Foundation in Beaumont Hospital."

The ride from Mizen Head in Cork to Malin Head in Donegal will pass through Goleen, Durrus, Macroom, Mallow, Charleville, Patrickswell, Limerick, Ennis, Gort, Oranmore, Galway, Tuam, Claremorris, Charlestown, Tobercurry, Sligo, Bundoran, Ballybofey, Letterkenny, Buncrana, Carndanagh to Malin Head.

Alpha-1 Antitrypsin Deficiency (Alpha-1) is one of the most common hereditary disorders. It affects both lungs and liver and can cause emphysema and cirrhosis of the liver. These can cause life threatening conditions in both adults and children. It is estimated that 1,200 people have Alpha-1 which is either undiagnosed or misdiagnosed as something else e.g. COPD or Asthma.

Awareness meetings will be held in Cork (Moran's Silver Springs Hotel) on Thursday 7th June at 8pm and in Galway (Marriott Courtyard) on Wednesday 13th June 8pm. All GPs in Cork and Galway along with nurses and physiotherapists are invited to the meeting.

On 13th June in the Marriott Courtyard, Galway there will be an information meeting for patients, their families and other interested people from 2pm Entrance to the meetings is free and is being organised by the Alpha One Foundation.

Lead speakers at the meetings will include, Prof Gerry McElvaney (Beaumont), Dr Cathal Bredin (CUH, Cork) and Dr Anton O' Regan (UCH, Galway).

For further information contact: Kitty O'Connor at Beaumont Hospital 01 809 3871, alpha1@rcsi.ie, catoconnor@rcsi.ie or Larry Warren (larryw@connect.ie).

Alpha One Foundation supports the Irish Donor Network — Always carry a Donor Card

MIZEN TO MALIN HEAD HORSE RIDE- CREATING AWARENESS FOR A GENETIC CONDITION ALPHA-1 ANTITRYPSIN DEFICIENCY.

On June 4th I set off on horse back from Mizen Head on the first leg of a journey of 428 miles/ 689 kilometres.

The sun was slitting the stones as I left Mizen Head accompanied by my friend Lorraine. We planned to ride an average of 20 miles /32 kilometres per day. Four and a half hours later we arrived in Durris which is just 32km from Mizen. Over the next few weeks we continued to average 32km per day.

We were accompanied on the trip by a back up team of six people. As we made our way we had a jeep travelling in front of us and a lorry behind with two to three spare horses. We also had a floating car which travelled ahead locating lay-byes etc. where we could pull in to eat or change horses. Each horse covered 16km per day which meant using four horses a day. We used a total of 18 horses for the duration of the 21-day ride.

Our route took us from Mizen head to Millstreet onward through Limerick, Ennis, Galway, Claremorris, Sligo, Letterkenny, Donegal, Malin and finally Malin Head.

Along the route we received lots of media publicity both on radio and in newspapers. We secured interviews on eleven radio stations and received coverage in 17 newspapers including some of the national daily papers. During the Ride two conferences were arranged in Cork and in Galway. Prof Gerry McElvaney came to address the conference in Cork together with Dr Charles Bredin of CUH. In Galway Dr Anton O'Regan spoke along with other relevant speakers. The attendance at some of the conferences was a little disappointing, increased support from general practitioners would have been greatly welcomed.

On arrival at Malin Head we received an overwhelming reception from friends, Beaumont Hospital Medical Staff and fellow patients. The reception was held in The Malin Head Hotel that night which was attended by a number of Donegal Lord Mayors and local representatives.

The whole event was for me, an Alpha-1 patient a very fulfilling, hard at times and very emotional event. Fellow Alpha-1's who helped me along the way included Anna Cassidy, Donegal, Stephen Crumlish, Donegal and Josephine McGuirk, Meath. The trip was a huge success and has resulted in an increased awareness among the general public and an increase in the number of queries to Kitty O'Connor, in Alpha One Foundation, alpha1@rcsi.ie / 01- 809 3871.

Finally I want to thank everyone who helped me on the way and all those who sent good wishes and gave their support.

A letter from the Alpha-1 Patient Support Group

Alpha one PATIENT SUPPORT GROUP

Dear Friends

The Alpha-1 Patient Support group was established in October 2006. It remains in its infancy but has achieved a multitude in its short life.

The aim of the support group is to involve patients in the development of the Alpha One Foundation, new advances in treatment and most importantly to supply information and support to patients and families affected by Alpha-1.

The patient group consists of monthly to two-monthly meetings in Beaumont this usually coincides with medical appointments for convenience. Larry Warren and Kitty O'Connor usually attend to give updates on events and further development of the Foundation.

As many patients are unable to attend telephone and email are used to keep them informed of proceedings.

In June 2007 we organised an Awareness campaign for the Cork, Galway and Donegal areas of Ireland to highlight this condition to the general public and encourage general practitioners and allied health professionals to screen for Alpha-1 with a Free simple Dried Blood spot test. This also gave us an opportunity to meet with other Alpha-1 patients and families to form friendships and alliances.

Patient information days are held during the year, these are organised through the Foundation. This is to update families on research trials, aspects of the disease that may affect them, services available, allied health professionals advice and an opportunity for patients to meet and express their experiences and views.

Our annual meeting in the Marino Institute in Drumcondra will be held in October this year and we strongly encourage patients to attend this annual event. For further details please contact Kitty O'Connor on 01-809 3871 or email alpha1@rcsi.ie.

To contact our support group please phone 087 230 4369. Our aim this year is to commence local patient only meetings around the country, the first being in the Sligo /Donegal area in September /October.

We have found an increased awareness among ourselves and propose to continue the support needed by all patients with Alpha-1 since establishing the group. We encourage all families, patients and person's affected by Alpha-1 to contact us.

Yours Sincerely

John Hannan, Josephine McGuirk

Acknowledgements

We would like to thank Alpha-1 Foundation in the US; Maurizio Luisetti MD, Center for Diagnosis of Inherited Alpha1-Antitrypsin Deficiency, Laboratory of Biochemistry and Genetics Institute for Respiratory Disease, University of Pavia, Italy; Marc Miravitlles, Servei de Pneumologia (ICPCT), Hospital Clínic Villarroel, Barcelona, for protocols and technical information; Dr Joseph McPartlin, Trinity Biobank, Institute of molecular Medicine, St James's Hospital, Dublin; ECOM – Ireland continues to consult, programme, handle queries and develop the website and database to the highest international standards. We would also like to thank the Department of Health and Children and Health Service Executive for their continued financial support.

There are an estimated 1,200 individuals with Alpha-1 Antitrypsin Deficiency (AATD) in Ireland. Less than five per cent of these have been identified.

Alpha One Suite, RCSI Building, Beaumont Hospital, Dublin 9, Ireland. Tel: +353 1 809 3871 Web: www.alpha1.ie