Alpha one

Alpha One Foundation

ANNUAL REPORT 2008

Contents

Executive Summary

During the past twelve months we made quite a lot of progress with our main programme – The Targeted Detection Programme - and are proud to have tested 2,600 persons for Alpha-1 Antitrypsin Deficiency.

The credit for this is due in no small way to the untiring efforts of Kitty O'Connor and Tomás Carroll. They have built up very good working relationships with the labs in various hospitals throughout the country. We have now signed agreements with the Research Ethics Committees of all relevant hospitals and institutions.

Once again we have been successful in our application for matched research funding form the MRC/HRB scheme this year. This grant is for a three year period. We have now three projects running under the scheme.

Last year the Patient Support group was represented by John Hannan, Josephine McGuirk and Orla Keane at two international Alpha-1 patient meetings. The first was the Third World Patient Congress in Rome in September 2007. The second was the Alfa Europe Annual meeting in Prague in May 2008. These were two very useful meetings in which patients from Europe and all over the world shared their experiences, views and plans. They proved to be a useful and enriching experience for all attendees and for the medical personnel. The Rome Congress was addressed by both Prof Gerry McElvaney and Kitty O'Connor. This group also successfully raised funds through the Women's Mini Marathon. We thank all who were involved.

As current President of Alfa Europe and CEO of this Foundation, I represented both organisations in various fora at home and the EU. Theses included: a council of Europe Transplant meeting in Dublin, a European Symposium on Rare Diseases in Dublin, a meeting of the EU Transplant Taskforce in Brussels, a meeting of Orphanet (Rare Diseases) in Paris.

We also continued our active participation in: Medical Research Charities Group, Irish Donor Network, Irish Platform for Patient Organisations Science and Industry and EURORDIS. We hosted an information stand and presented at the Irish Thoracic Society (ITS) conference in November 2007 in Dublin and will do so also at this year's ITS conference in Belfast 2008. We also attended at the American Thoracic Society Meeting in Toronto in May 2008, presenting some of our work which demonstrates a much higher incidence of Alpha-1 on the island of Ireland, with an estimated 3,000 individuals at risk of developing this severe disease.

Our Registry continues to grow and improve and is now successfully capturing all the data necessary for patient welfare and research. We earnestly encourage more and more patients to sign up to the Registry. This year in close cooperation with Ecom Ireland we have developed a new website which will keep you up to date with all developments in the world of Alpha-1.

This brief overview may give you some idea of the work being done and the progress being made by the Alpha One Foundation. This work is collaborative and I wish to thank my team colleagues: Prof Gerry McElvaney, Tomás Carroll, Kitty O'Connor and Olwen Floyd for their diligence and cooperation which made the past one such a success for the Foundation.

Larry Warren, CEO.

Introduction

Alpha-1 antitrypsin (AAT) deficiency is a genetic disease that has numerous clinical implications and primarily affects the lungs and liver.

It may also have contributed to the premature death of Frédéric Chopin in 1849. The first formal report of the disease occurred a little more than 40 years ago, when Laurell, while reviewing tests in his laboratory noticed the absence of the Alpha-1 band during the analysis of serum proteins from 5 patients. Subsequent investigations by Eriksson revealed that 3 of those patients presented with early emphysema, and another had a family history of pulmonary emphysema. Thus, the disease and some of its principal characteristics began to be recognized. Since then, significant advances in understanding and in the care of individuals with AAT deficiency have occurred. Novel diagnostic techniques have also been developed, allowing the performance of large-scale epidemiological surveys-even allowing the genetic and pathophysiological basis of the disease to be studied.

There are an estimated 3,000 Irish citizens that have AAT deficiency and a further 700,000 Irish citizens estimated to be carriers for the disease. To date 120 individuals with AAT deficiency have been identified in Ireland. Less than 5% of those predicted to have AATD have been diagnosed and up to 3% of individuals with chronic obstructive pulmonary disease (COPD) may be undiagnosed AATD patients. US studies have showed it takes an average of 5 doctors and 7 years from time of first symptoms to diagnosis.

The ATS/ERS (WHO) Guidelines state that the following categories should be tested:

- All COPD patients
- All non-responsive asthmatics (adults/ adolescents)
- All patients with cryptogenic cirrhosis/liver disease
- All first degree relatives of patients/carriers with AAT deficiency.

OUR AIMS ARE TO:

- identify all individuals with Alpha-1 antitrypsin deficiency in the Irish population
- diagnose all referred patients free of charge
- provide immediate counselling
- refer each patient to the Foundation for support, advice, and access to resources, appropriate therapies, and clinical trial enrolment

This year, the Foundation has expanded its efforts to engage and educate a broader and more diverse audience about its mission. By strengthening its name across all platforms and to spread awareness of the quest to test and treat Alpha-1 Antitrypsin Deficiency.

The Foundation has expanded its online presence, improved its web content and significantly increased media coverage. Overall, more people have learned about the way the Foundation carries out its mission, from therapeutic development to fund-raising and from delivering patient care to advocacy.

National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme

Alpha-1 antitrypsin (AAT) is an antiprotease produced chiefly by the liver. Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder characterised by low serum levels of AAT and is associated with lung and liver disease. In May 2004 a national targeted detection programme for Alpha-1 antitrypsin deficiency was established in Beaumont Hospital. Funded directly by the Department of Health, the programme provides free testing to patients with chronic obstructive pulmonary disease (COPD), non-responsive asthma, cryptogenic liver disease and to relatives of AATD patients. A range of methods are used to diagnose AATD including phenotyping by isoelectric focussing and genotyping by RT-PCR. Upon diagnosis the Alpha One Foundation also provides a range of ancillary services to patients including counselling, expert advice, information packs and leaflets, and opportunities to enrol in clinical trials and to join the Alpha-1 patient support group.

Alpha-1 antitrypsin (AAT) is a 52kDa glycosylated protein. Produced in the liver and secreted into the blood, AAT diffuses into the lungs where it functions as an antiprotease. Antiproteases regulate and inactivate protein-splitting enzymes such as neutrophil elastase, an enzyme capable of destroying alveolar wall connective tissue. AAT is the most abundant antiprotease in the lung and therefore plays a major role in maintaining a healthy, functioning lung. Alpha-1 antitrypsin deficiency (AATD) is a hereditary autosomal codominant disorder caused by mutations in the AAT gene located on chromosome 14. Genetic variants of the AAT gene are characterised by their electrophoretic mobilities as medium (M), slow (S) or very slow (Z). The most common variants associated with disease are the S (Glu264Val) and Z (Glu342Lys) mutations, caused by a single amino acid replacement of glutamic acid at positions 264 and 342 of the polypeptide respectively. Both mutations result in decreased levels of circulating AAT due to retention of the aberrantly folded protein in the liver, and classically result in liver disease in children, and early onset emphysema or occasionally liver disease in adults. Moreover, the small amount of AAT that reaches the lung in AATD patients is inactivated by cigarette smoke. Smoking is the single biggest risk factor for the development of emphysema in AATD patients, and individuals with AATD who smoke develop severe, early-onset emphysema. The most commonly observed genotypes are MM (normal), MS, MZ (heterozygotes), SZ (compound heterozygote) and SS or ZZ (homozygotes). It is unclear, as yet, whether the carrier status (MS or MZ) confers an increased risk of disease.

AATD is under-diagnosed with prolonged delays in diagnosis common. In addition, the majority of AATD individuals with emphysema are misdiagnosed as COPD patients. A recent US study showed it takes an average 5.6 years from the time symptoms first appear to accurate diagnosis. Increased awareness and understanding of AATD is therefore vital to prevent the continuing under-diagnosis of this condition. To this end, we have launched a national registry of AATD patients and a website (www.Alpha-1.ie) providing a resource for doctors, patients, and the general public. All patients diagnosed through our targeted detection programme are offered a variety of services including counselling, expert advice, information packs/leaflets, and opportunities to enrol in clinical trials and to join the Alpha-1 patient support group. Based on studies in other European countries it is estimated that 3,000 Irish citizens have AATD and up to 700,000 Irish citizens are carriers, yet only 120 individuals with AATD have been identified in Ireland to date. A research project recently undertaken in our laboratory screened 1,000 anonymised DNA samples provided by the Trinity College Biobank for the presence of the S and Z mutations. This investigation of a sample Irish population revealed a gene frequency of 0.05 for the S mutation and 0.02 for the Z mutation, which is higher than anticipated based on studies in other European populations.

WHO (World Health Organisation) guidelines advocate targeted detection programmes for AATD in patients with COPD, non-responsive asthma or cryptogenic liver disease. In May 2004, a national targeted detection programme for AATD was launched by the Alpha One Foundation in Beaumont Hospital. The programme employs

a full time clinical research nurse who attends respiratory outpatient clinics where patients are targeted for screening. AATD can be diagnosed from a venous sample drawn during a blood test, or alternatively a finger-prick test can be used to collect a dried blood spot (DBS) sample on specially treated filter paper for DNA isolation. When a venous sample is obtained serum can be isolated from blood and used in two different assays. The first assay measures circulating levels of AAT by radial immunodiffusion (RID). The second assay, performed on serum isolated from a venous sample, is 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 (Figure 5). The various phenotypes are identified by comparison with reference standards (for example MM, SZ, ZZ). Standard protein electrophoresis is not precise enough for an accurate analysis of the various forms of AAT so isoelectric focussing must be performed to correctly diagnose patients. It is also worth considering that determination of AAT levels alone is insufficient evidence of AAT deficiency. AAT is an acute-phase protein and consequently levels can sometimes be falsely elevated. Therefore, determination of the quantitative level of AAT must be combined with phenotypic or genotypic analysis. In addition, our laboratory is participating in a pilot UK NEQAS (National External Quality Assessment Service) Alpha-1 Antitrypsin Phenotyping scheme since July 2007. Every three months the scheme provides us with two serum samples for inclusion in our screening programme and so far we have achieved 100% compliance with NEQAS.

In the last year a DNA genotyping system has been developed which can detect the two mutations (S and Z) responsible for almost 98% of all cases of AATD. After a short questionnaire is filled out for each patient, a lancet is used to obtain a small blood sample which is collected on specially treated filter paper. DNA isolated from this paper is then used to genotype the patient by RT-PCR (Real-Time Polymerase Chain Reaction), using primers and probes specific to each mutation. The major advantage of implementing the genotyping method is that the ease of sample collection and storage has allowed for self-testing in the home, and the finger-prick kit test is particularly useful for family screening. Information brochures on AATD and a stamped addressed envelope are supplied with each kit and the completed kit can be sent directly to the diagnostic laboratory in the RCSI Education & Research Centre at Beaumont Hospital.

In summary, AATD is more prevalent in Ireland than previously thought, even allowing for the targeted, symptomatic population investigated in this programme. The advantages of early and accurate diagnosis of AATD are manifold and include (1) closer observation and management of affected individuals, especially regarding pulmonary and liver health, (2) family member testing, at least some of whom may have lung or liver complications, (3) aggressive smoking cessation efforts, which have been associated with lower rates of smoking among AAT-deficient individuals, and (4) consideration of occupational hazards and environment as exposures to some occupational dusts and vapours can accelerate pulmonary decline. Once identified, AATD patients have the opportunity to enrol in clinical trials currently taking place in Beaumont Hospital, such as the AAT augmentation therapy clinical trial for ZZ individuals, and the MZ family study which is attempting to fully clarify the

	Phenotype	AAT Level (g/L)	What Does It Mean?
NORMAL	**MM**	Normal range 1.0-2.2g/L	Does not carry any altered AAT genes and will not develop disease
CARRIER	**MZ/MS/MI**	0.616-1.51g/L	Mild to moderate AAT deficiency – carries an altered AAT gene and may develop disease
AAT DEFICIENCY	**ZZ/SZ/SS**	0.084-0.661g/L	Moderate to severe AAT deficiency – carries two altered AAT genes and will develop disease

Table 1: Explanation of the various AAT phenotypes and their clinical consequences

risk of COPD in MZ individuals. To conclude, the importance of an early diagnosis of AATD cannot be over-emphasised as the resulting appropriate medical follow-up and lifestyle changes can help prevent or at least postpone the development of AATD-related lung and liver disease.

Further information can be obtained from:
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Email: catoconnor@rcsi.ie / Tel: 01 8093871

RESULTS OF THE ALPHA-1 ANTITRYPSIN DEFICIENCY TARGETED DETECTION PROGRAMME

The programme has been running for four years since beginning in May 2004. During this period we have tested 2,600 individuals throughout Ireland. We use a combination of venous blood collection to determine phenotype and 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 serum AAT must be combined with phenotypic or genotypic analysis for a correct diagnosis.

So far we have identified 70 severely deficient AATD patients and almost 600 moderately deficient individuals (carriers). In a targeted population of 2,600 individuals, over 25% possessed at least one abnormal AAT gene. The full results of the programme, with figures compiled up to September 1st 2008, are as follows:

ZZ	33
SZ	37
MZ	360
MS	228
SS	12
Rarer phenotypes identified, including IZ, IS, and MI	6

Table 2.

TARGETED SCREENING IN IRELAND TO DATE

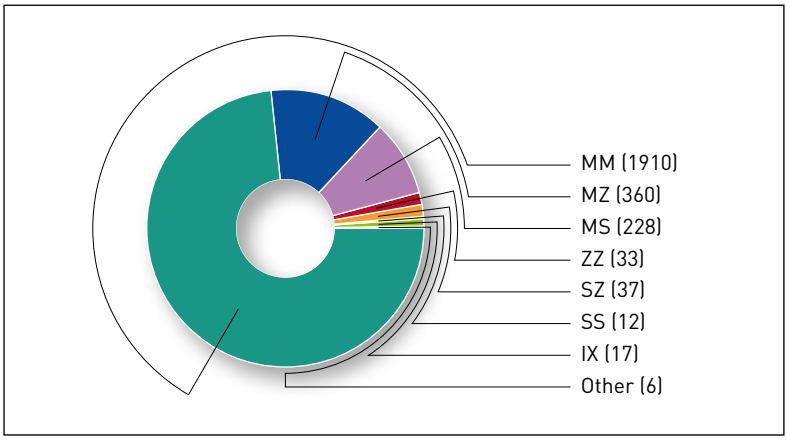


Figure 2: Screening in Ireland to date

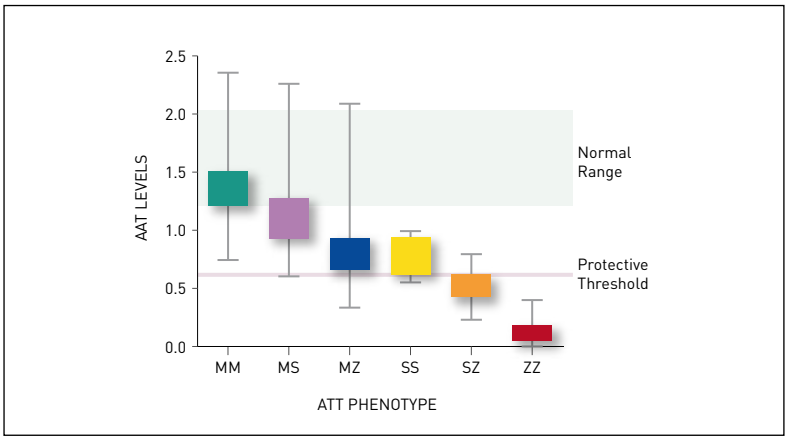


Figure 3: AAT Levels v Phenotype in Ireland

During the year we have presented to and met with various respiratory journal clubs, biochemistry and immunology laboratories throughout hospitals in Ireland. As a result we are now receiving samples for measuring Alpha-1 antitrypsin levels and for Alpha-1 antitrypsin phenotyping from various respiratory consultants and laboratories throughout Ireland. During our visits to the various Hospital labs it has come to our attention that many Biochemistry and Immunology laboratories do not have the resources, equipment or personnel to measure and/or phenotype for Alpha-1 antitrypsin. In many cases, the labs in question send these Alpha-1 requests to a

private company Claymon Laboratories Limited which charges €60 for AAT levels and €160 for AAT phenotyping. Alternatively, some other labs send their Alpha-1 requests to the Protein Reference Unit in the Northern General Hospital, Sheffield, England and this is also costly for individual labs. We provide AAT measurement and AAT phenotyping for **FREE** and we are aware that departmental budget control is of vital importance within hospitals. So, on top of our ancillary resources such as the opportunity to enrol in clinical trials and the Alpha-1 Patient Support Group, the fact that we provide AAT levels and phenotyping **FREE** of charge is an added financial incentive for various hospitals around the country to use our services.

PILOT ALPHA-1 ANTITRYPSIN DEFICIENCY SCREENING PROGRAMME TO DETERMINE THE PREVALENCE OF ALPHA-1 ANTITRYPSIN DEFICIENCY IN IRELAND

Funding Body: Talecris Biotherapeutics

We recently undertook a research project to identify the incidence of AATD in a representative sample of the general population of Ireland. This involved screening 1000 anonymised DNA samples for the presence of the S and Z mutations and was undertaken in collaboration with Dr Joe McPartlin of the Trinity College Biobank. The gene frequencies revealed for both the S and Z mutation were higher than anticipated based on studies in other European populations.

For the purpose of this study the randomised nationally-based buccal swab collection from the Trinity Biobank was investigated. Ethical approval for the collection was granted by the joint Hospital Ethics Committee of Tallaght and St James's Hospitals. The confidentiality of the participant's data and samples was respected and ensured by irreversible anonymisation. DNA was extracted from buccal swabs according to the Biobank standard protocols. AAT deficient individuals were identified through separate genotyping assays for the S and Z mutations using Real-Time PCR technology and melt curve analysis on a Roche Lightcycler.

A Z genotyping assay was performed on 1095 DNA samples provided by the Biobank. This revealed 46 MZ or carrier individuals in the population. The frequency of the Z gene in this population was, therefore, 0.022. An S assay, carried out on 960 biobank samples, revealed 98 MS carriers and 1 SS (homozygote) individual. The frequency of the S gene in this population was, therefore, 0.053. In total, between the two assays, 3 AAT deficient individuals were identified, constituting 2 SZ and 1 SS genotype. A total of 140 AATD carriers were detected, constituting 46 MZ and 98 MS individuals.

DEFICIENCY PHENOTYPE	NO. DETECTED/NO. SCREENED
MZ	46/1095
MS	98/960
SS	1/960
SZ	2/2055

Table 3. Number of Deficiency Phenotypes Detected in Population Screened

The percentage of deficiency alleles detected was higher than anticipated from studies in other populations. The allele frequencies for S and Z in Ireland were previously estimated at between 0.02-0.04 and 0.005-0.015 (Luisetti et al, Thorax 2004). The S variant, thought common to the Iberian Peninsula, was detected with unusually high frequency in the Irish population. Our pilot study shows S and Z alleles occur at frequencies of 0.053 and 0.022 respectively in the Irish population. As the random sample was from the 32 counties, and extrapolating from a population of 6 million on the island of Ireland this would suggest there are approximately 3,000 ZZ and 14,000 SZ AAT deficient individuals and over 700,000 MZ carriers on the island of Ireland. Compared to the gene frequencies in our targeted detection programme the Z mutation would appear to be much more clinically significant with a higher penetrance than S in the two populations we have evaluated.

The prospect that Alpha-1 antitrypsin deficiency is much more common in Ireland than previously

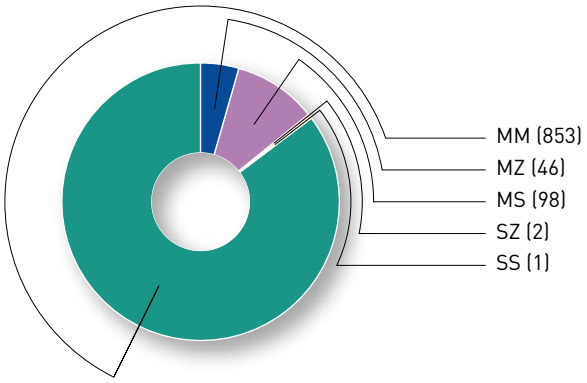
thought will help highlight the fact that all COPD, non-responsive asthma and cryptogenic liver disease patients should be tested for AATD. Increased awareness and understanding of AATD is vital to prevent the continuing under-diagnosis of this condition. Early diagnosis of AATD, with appropriate medical follow-up and lifestyle changes, can prevent, or at least postpone, AATD complications.

ADOPTION OF NEW SEBIA PHENOTYPING METHOD

In the last year we have acquired a new piece of equipment for the Targeted Detection Programme. This has allowed us to implement a more accurate method of phenotyping. We can now identify the different Alpha-1 antitrypsin phenotypes with an increased sensitivity of detection. The Sebia Hydragel 18 AAT Isofocusing kit is designed for the qualitative detection and identification of the different phenotypes of Alpha-1 antitrypsin (AAT) in the electrophoretic patterns of human sera. The procedure includes isoelectricfocusing on agarose gel, performed on the semi-automatic HYDRASYS system, followed by immunofixation with anti-Alpha-1 antitrypsin antiserum.

The assay is carried out in two stages. Firstly, isoelectrofocusing on agarose gel is used to fractionate the proteins in the serum samples. This is followed by immunofixation with enzyme (peroxidase)-labelled anti-Alpha-1 antitrypsin antiserum to identify the various phenotypes of Alpha-1 antitrypsin.

All in all, we have found this new phenotyping method to be highly specific, rapid and simple to perform. It represents a more accurate method of screening for Alpha-1 antitrypsin deficiency and improves the identification of not only the most common but also the various rare AAT phenotypes.



- 1,000 patients screened from TCD / St James's Hospital Biobank
- 46 MZ, 98 MS, 2 SZ, and 1 SS identified
- Contrasts with targeted population, showing high S (0.05) vs Z (0.02) incidence

Figure 4: Sample Irish Population

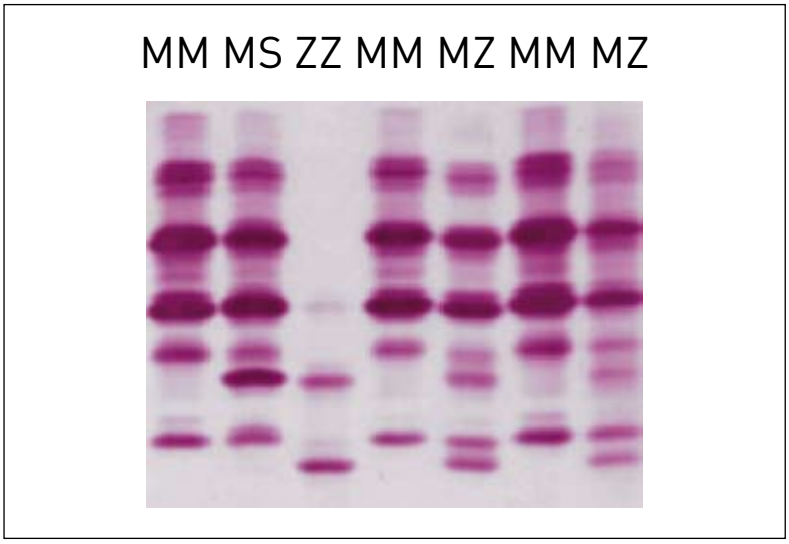


Figure 5. A typical isoelectric focussing gel for AAT phenotyping.

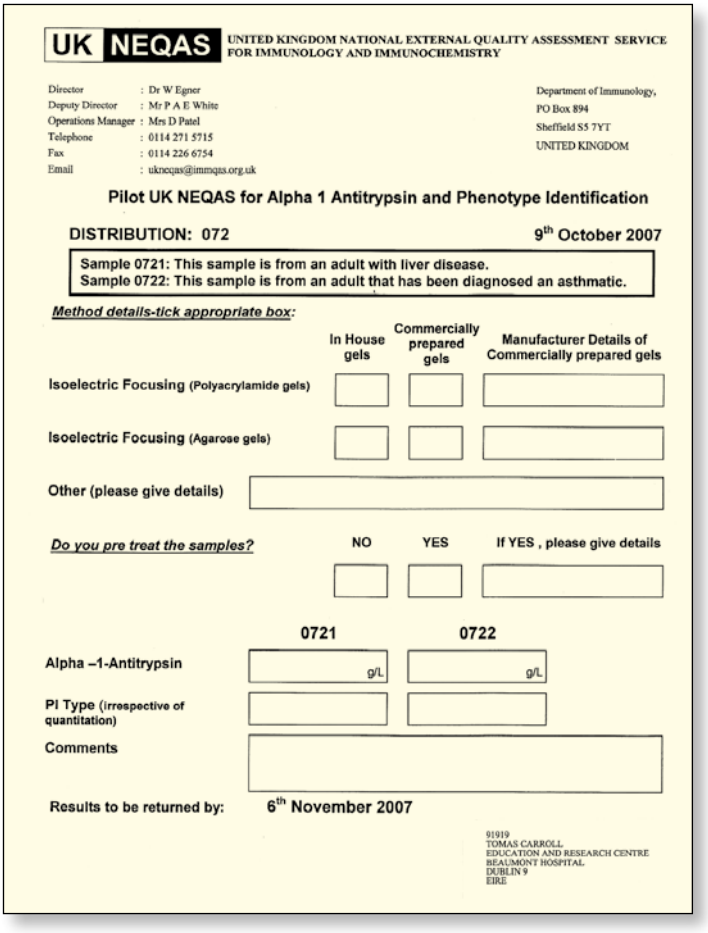
Quality Assurance and Accreditation

For over a year now we have been participating in a pilot scheme operated by the UK National External Quality Assessment Service (NEQAS).

Every three months the scheme provides us with two serum samples for inclusion in our screening programme. Each sample is accompanied with clinical details and is tested according to our routine methods. After each sample has been phenotyped and the Alpha-1 antitrypsin level measured by radial immunodiffusion, the results are returned to NEQAS for analysis. Since we began participating in the scheme we have achieved 100% compliance with NEQAS.

Accreditation is the formal recognition of a body's competence to conduct a specific activity such as medical laboratory testing.

This recognition is based on a specific series of International and European standards. In Ireland, laboratory accreditation is granted by the Irish National Accreditation Board (INAB). This is a voluntary scheme open to any laboratory performing testing and/or calibration. We are currently in the process of applying for accreditation for the Alpha-1 antitrypsin deficiency targeted detection programme from INAB. While this is an arduous and timely process we hope that once we are fully accredited with INAB we should be able to increase the number of screening centres to which we offer our services.



UK NEQAS UNITED KINGDOM NATIONAL EXTERNAL QUALITY ASSESSMENT SERVICE FOR IMMUNOLOGY AND IMMUNOCHEMISTRY

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UNITED KINGDOM

Pilot UK NEQAS for Alpha 1 Antitrypsin and Phenotype Identification

DISTRIBUTION: 072 **9th October 2007**

Sample 0721: This sample is from an adult with liver disease.
Sample 0722: This sample is from an adult that has been diagnosed an asthmatic.

Method details-tick appropriate box:

	In House gels	Commercially prepared gels	Manufacturer Details of Commercially prepared gels
Isoelectric Focusing (Polyacrylamide gels)			
Isoelectric Focusing (Agarose gels)			
Other (please give details)			

Do you pre treat the samples?	NO	YES	If YES , please give details

	0721	0722
Alpha –1-Antitrypsin	g/L	g/L
PI Type (irrespective of quantitation)		
Comments		

Results to be returned by: 6th November 2007

91919
TOMAS CARROLL
EDUCATION AND RESEARCH CENTRE
BEAUMONT HOSPITAL
DUBLIN 9
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National Alpha-1 Registry

In the past year we have started a National Alpha-1 Registry to track the health of people with AAT deficiency across Ireland.

The type of information collected includes height, weight, gender, genotype, pulmonary function test results, liver function tests, hospitalisations, and complications related to lung and liver manifestations of AATD. Information in the registry helps clinicians and researchers see new trends, design clinical trials to test new therapies and improve the delivery of care for people with AATD.

We hope that this patient registry will increase our understanding of AAT deficiency. Additionally, we are engaged in active, wide-ranging investigations to improve the diagnosis, monitoring, and treatment of the disease.

The main objectives of the patient registry are as follows:

1. To establish a database of patients and their clinical details
2. Promote basic and clinical research into AAT and coordinate this activity.
3. To collect, assess and disseminate information concerning all aspects of AAT.
4. To encourage and support awareness of AAT.

According to figures complied by the Alpha One Foundation's census we have a 50% enrolment rate of ZZ patient's in our National Registry; this provides us with an enrolment target and we project with additional personnel and services in this area we will to obtain a 80% enrolment rate in 2009-10. Our long-term aim is to have a dedicated person to the position of administrator for the registry which will increase our enrolment numbers and research outcomes.

GENDER DISTRIBUTION

The total number of male and female patients on the Alpha-1 Registry is fairly evenly split between the genders, male (56%) and female (44%) (Figure 7).

Our figures show that males represent 65% of ZZ on our registry and females 35%. SZ patients have a 64% representation by females and 35% males. There is an equal number of MZ male and female with 1 female SS and 1 male MS patient.

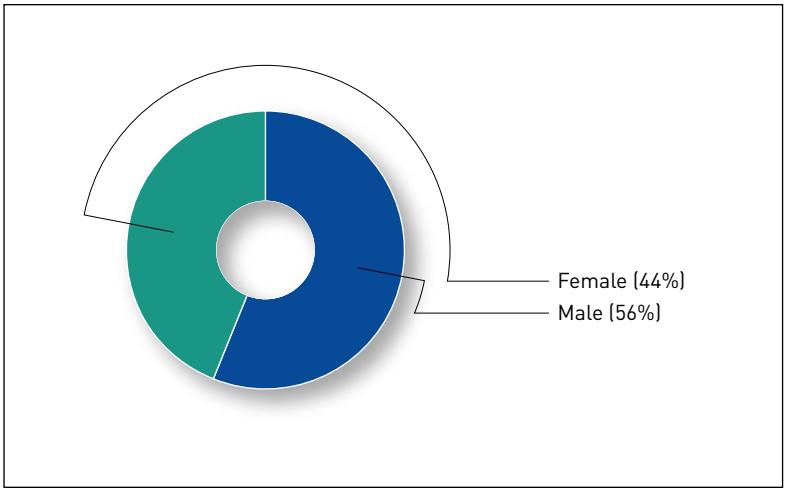

Figure 6: Proportion of male and female patients enrolled in registry

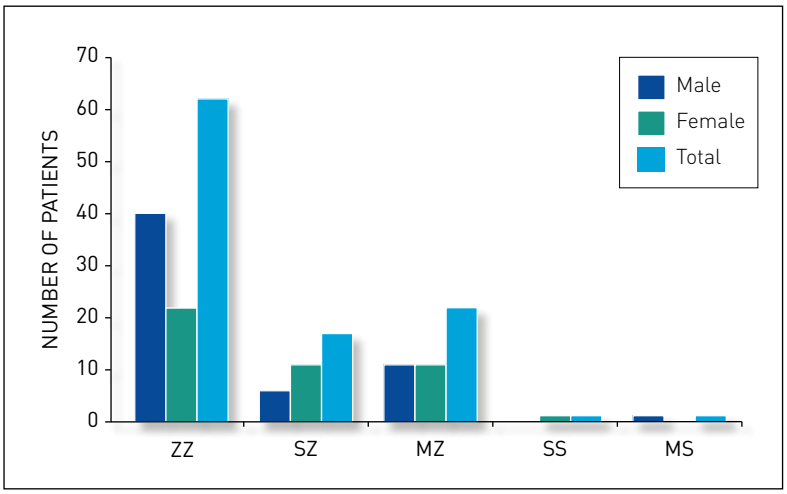

Figure 7: Gender distribution and genotype

INITIAL REASONS FOR ALPHA-1 ANTITRYPSIN TESTING

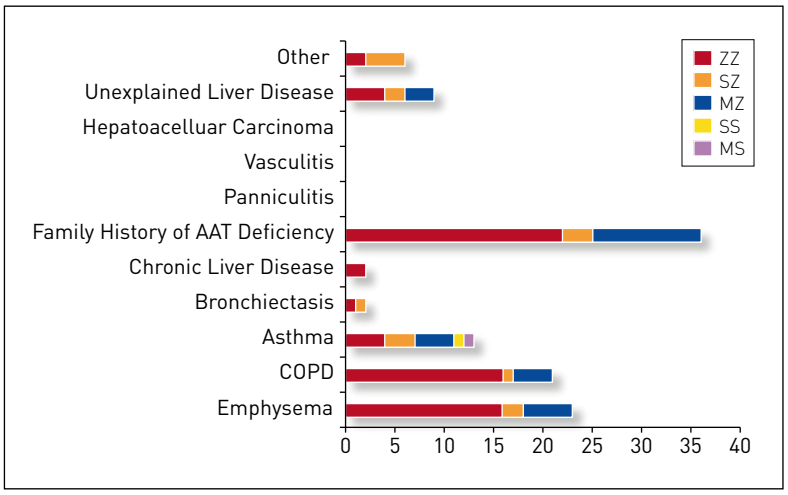

Figure 8: Initial Reasons for Alpha-1 Antitrypsin Testing

A person's symptoms at diagnosis are of vital importance for our research. This information gives us an indication of when and how Alpha-1 patients are being diagnosed, which is of significance to current screening practices in place in Ireland and further afield. From further research we can try to address issues such as delays in diagnosis, misdiagnosis, and the importance of family screening. Method of diagnosis has been divided into 11 symptoms for ZZ, SZ and MZ, SS and MS individuals.

From our data we have established that 24% of ZZ patients presented with emphysema and 25% COPD as the initial reason for diagnosis, 6% with asthma and 33% were diagnosed as part of family screening. Unexplained liver disease counted for 6% and 3% with chronic liver disease. The other 3% included CREST syndrome, cerebral haemorrhage and Sjogren's syndrome.

In the SZ patients emphysema and COPD accounted for 13% and 6% respectively. While a larger portion (19%) presented with asthma, again family screening had a large representation with 19% of patients being diagnosed through this method. 13% of SZ cases were diagnosed by screening unexplained liver disease patients.

In the MZ patient group, again emphysema and COPD accounted for 19% and 15% respectively while asthma was the reason for diagnosing 15%. Family screening is the most common reason for diagnosis with 40% being the largest of any other genotype.

In the SS and MS population, asthma was the only reason for testing.

PULMONARY FUNCTION TESTS

The spirometric changes resulting from AAT-deficiency related emphysema are the same described in smoking-related COPD: airflow obstruction, represented by a decrease in forced expiratory volume in one second/ forced vital capacity (FEV1/FVC) ratio and in FEV1; and normal or decreased FVC. Full pulmonary function testing reveals increased residual volume and greater total lung capacity, as well as decreased diffusing capacity of the lung for carbon monoxide. Due to air trapping, pulmonary volumes, as measured by plethysmography, are typically greater than those measured by gas-dilution methods.

In our ZZ population the average FEV1% predicted ranges from 40-60% in comparison to SZ and MZ where the majority ranges from 80-130% FEV1 % predicted.

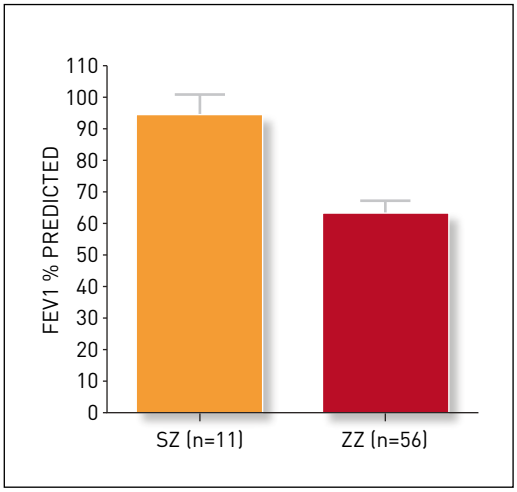

Figure 9: FEV1% of Registry AAT PAtients

BMI

Pulmonary function test (PFT) and height/weight from which you can calculate Body Mass Index, or BMI) are generally measured at the same time. The BMI is an indication of the relationship between height and weight of a person. The height and weight are generally taken every time pulmonary function test are performed, so the BMI can be calculated alongside the PFT data. BMI is an important figure to track over time, as it will give an indication of nutritional status. Average BMI ranges from 19.1 to 25.8 for women and 20.7 to 26.4 for men.

Our data suggests our ZZ, SZ and MZ patients a have a BMI within normal ranges.

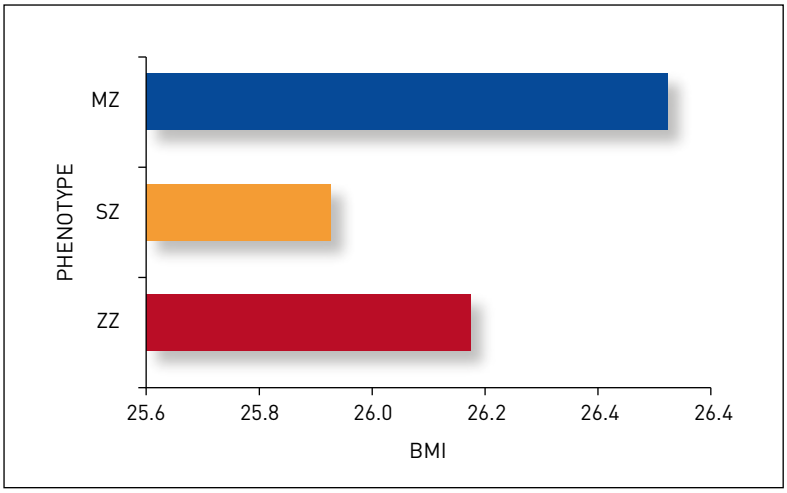

Figure 10: Average BMI (Kg/m2)

COMPLICATIONS

We record complications that occur in our patients at each annual assessment. Findings from the registry of complications in AAT deficient individuals of the ZZ phenotype, include respiratory exacerbation (46%), bronchopneumonia (9%), asthma (12%) and liver disorders (14%)(e.g., chronic hepatitis, cirrhosis, and hepatoma).

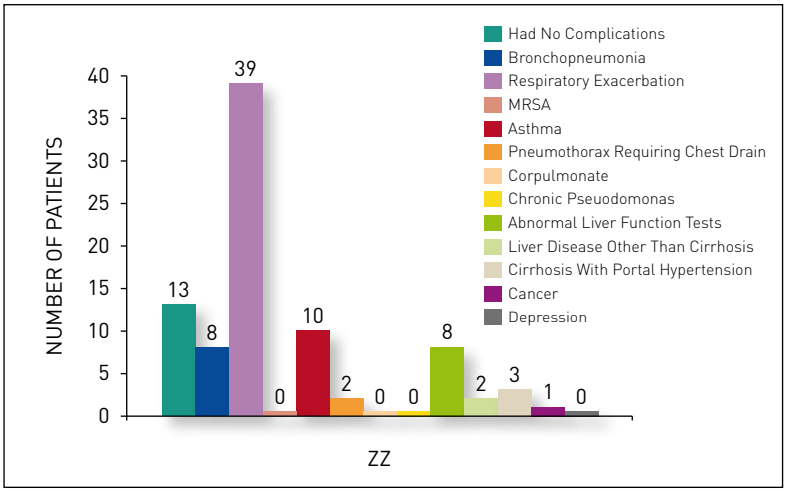

Figure 11: Complications

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 USA, National Institutes of Health USA, and the World Health Organisation.

From our patient registry we have found that 47% of ZZ patients are at stage III according to the GOLD Guidelines, 23% at stage II and 22% are at stage I.

From the graph, *Figure 12*, we can see that the majority of our ZZ patients are stage 3 (severe COPD) and a small minority are stage 4 (very severe COPD).

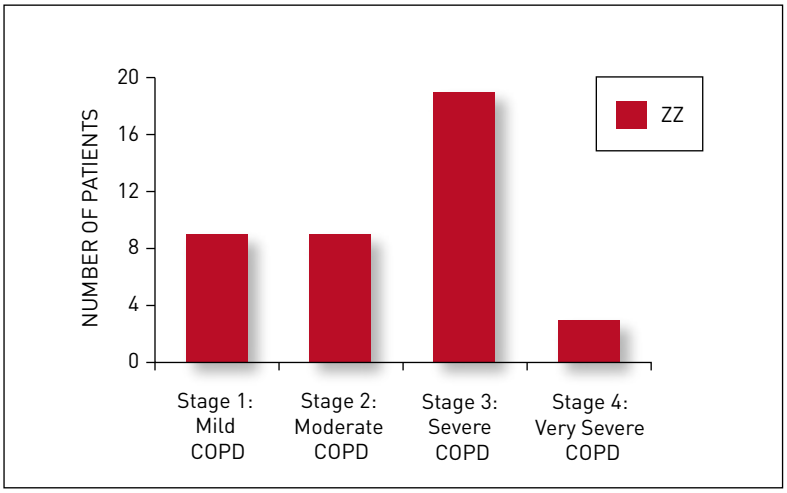

Figure 12: COPD Severity in ZZ Patients

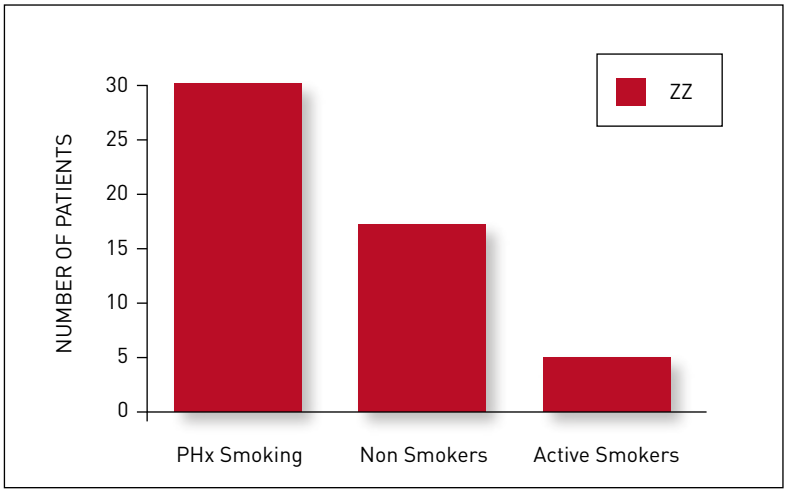

Figure 13: Smoking History of ZZ Patients

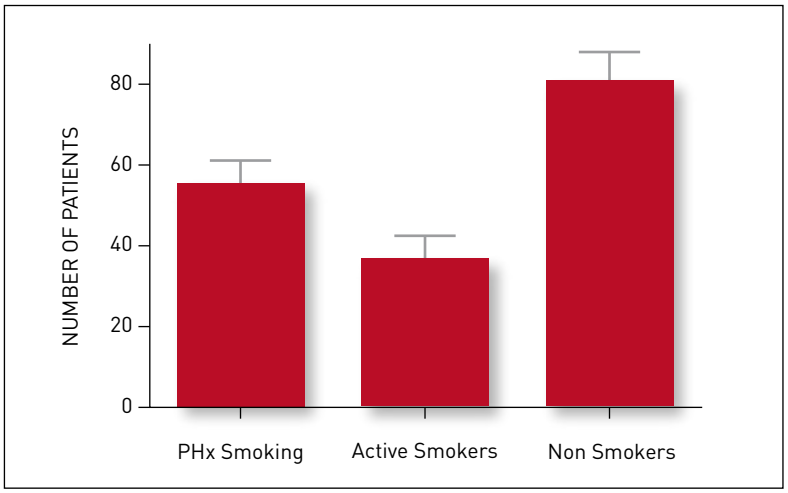

Figure 14: Smoking Status of ZZ Patients

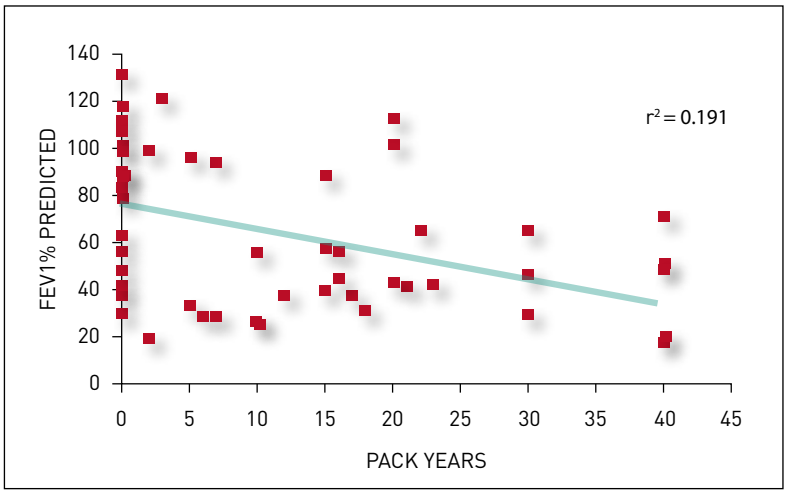

Figure 15: Effect of pack years on FEV1 of ZZ Patients

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.

SMOKING HISTORY

Pulmonary disease caused by AAT deficiency is clinically different from smoking-related COPD due to the fact that it has an earlier onset (fourth or fifth decade of life compared to sixth or seventh decade of life) and it is disproportionate to the tobacco intake.

Our data shows that the non-smoking ZZ population has a mean FEV1% predicted better in comparison to the ZZ population with a smoking history. This finding is also confirmed in our SZ population.

We have also shown pack years in comparison to FEV1% predicted for ZZ and SZ patients.

IRISH THORACIC SOCIETY ANNUAL MEETING, BELFAST 2008 - ABSTRACTS FOR PRESENTATION

THE INCIDENCE OF ALPHA-1 ANTITRYPSIN DEFICIENCY IN IRELAND

T. Carroll[3], O. Floyd[3], C. O'Connor[3], J. McPartlin[2], C. Taggart[1], R. Costello[3], S. J. O'Neill[3] and N. G. McElvaney[3]

1. ***School of Dentistry, Queens University, Belfast.***
2. ***Trinity Biobank, Institute of Molecular Medicine, Trinity Centre, St James's Hospital, Dublin.***
3. ***Department of Respiratory Research, RCSI Education and Research Centre, Beaumont Hospital, Dublin***

AAT deficiency (AATD) is a hereditary disorder, resulting from mutations in the SERPINA1 gene, and classically presents with early-onset emphysema and liver disease. The most common mutation causing AATD is the Z mutation, with the S mutation also associated with lung disease. AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. The World Health Organisation advocates screening COPD, poorly-controlled asthma, cryptogenic liver disease patients and first degree relatives of known AATD patients.

2,600 individuals with COPD, asthma, or cryptogenic liver disease were screened in the national targeted detection programme. 1,000 healthy individuals from the TCD Biobank were genotyped for S and Z alleles.

Targeted screening identified 33 ZZ, 37 SZ, 12 SS, 358 MZ, 228 MS, and 12 MI individuals, yielding gene frequencies of 0.055 and 0.09 for S and Z respectively. Biobank screening of 1,000 healthy individuals identified 98 MS, 46 MZ, 2 SZ and a single SS case, yielding gene frequencies of 0.053 and 0.022 for S and Z.

The allele frequencies for S and Z in Ireland were previously estimated at between 0.02-0.04 and 0.005-0.015.[1] Our pilot study shows S and Z alleles occur at higher frequencies, suggesting 3,000 ZZ individuals and over 700,000 carriers on the island of Ireland. The Z mutation is more clinically significant with a higher penetrance than S in the groups we have evaluated.

(1) Thorax. 2004; 59:164-169.

ALPHA-1 ANTITRYPSIN DEFICIENCY ZZ COPD COMPARED TO MM COPD

O. Floyd[2], T. Carroll[2], C. O'Connor[2], C. Taggart[1], R. Costello[2], S. J. O'Neill[2] and N. G. McElvaney[2]

1. ***School of Dentistry, Queens University, Belfast.***
2. ***Department of Respiratory Research, RCSI Education and Research Centre, Beaumont Hospital, Dublin.***

AAT deficiency (AATD) is a hereditary disorder, resulting from mutations in the SERPINA1 gene, classically presenting with early-onset emphysema and liver disease. The most common mutation associated with AAT deficiency is the Z mutation, with the S mutation also associated with lung disease. AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. World Health Organisation guidelines advocate screening patients with COPD, asthma, cryptogenic liver disease and first degree relatives of known AATD patients.

ZZ AATD patients on the National Alpha-1 Registry (n=61, 49.3+/-1.3 years, 39 male, 22 female) were compared to a cohort of MM COPD patients (n=100, 60.4+/-1.3 years, 40 male, 60 female).

Mean AAT levels in the ZZ group were 0.127+/-0.013g/L compared to 1.393+/-0.03g/L in the MM COPD cohort. The mean FEV1 for all ZZ patients was 63.0+/-4.2% compared to 62.8+/-2.6% for MM COPD patients. However, when ZZ cases identified by family screening were removed, the mean FEV1 of the ZZ cohort was lower than the MM group (55+/-4.8%, p=0.005, compared to MM group). When MM and ZZ groups were stratified by smoking status, ZZ smokers had mean FEV1 of 51.0+/-4.4% compared to 82.6+/-6.7% for never smokers, while MM smokers had mean FEV1 of 60.9+/-3.7% compared to 65.6+/-3.5%

for never smokers. These findings underline the clinical significance of the ZZ phenotype and smoking in the development of COPD.

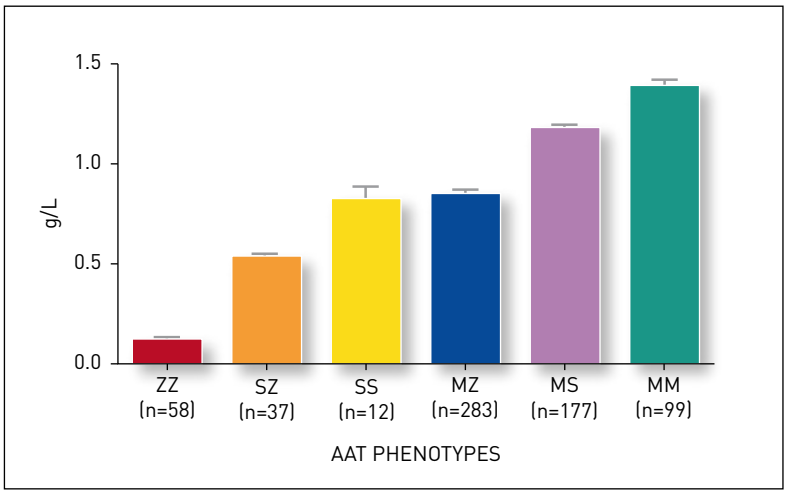

Figure 16: AAT levels of various phenotypes

CHARACTERISTICS OF AN IRISH REGISTRY OF ALPHA-1 ANTITRYPSIN DEFICIENCY PATIENTS WITH ZZ AND SZ PHENOTYPES

I. Hennessy, C. O'Connor, T. Carroll, O. Floyd, P. Rowland, P. Branagan, S. Chotirmall, and N. G. McElvaney

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

Alpha-1 antitrypsin (AAT) is produced by hepatocytes, and is the most important antiprotease in the lung. AAT deficiency (AATD) is a hereditary disorder resulting from mutations in the AAT gene, presenting with emphysema in adults and liver disease in childhood. WHO guidelines advocate a targeted strategy in screening COPD, non-responsive asthma, and cryptogenic liver disease patients and also relatives of known AATD patients.

The most common phenotype associated with disease is ZZ followed by SZ. A chart review of AATD patients on the National Alpha-1 Registry was performed on ZZ (n=61) and SZ (n=12) patients.

The mean age at diagnosis for ZZ patients was 43.6 +/- 2.0 years for males and 42.2 +/- 2.6 years for females. We demonstrate that ZZ individuals identified as a result of family screening have significantly increased FEV1 (78.5 +/- 6.9%, 47.3+/-2.4 years) when compared to ZZ patients identified by targeted symptomatic screening (55.0 +/- 4.8%, 52.0+/-1.3, p=0.0062). ZZ and SZ patients who smoked had significantly decreased lung function compared to non-smoking ZZ and SZ and that a positive correlation between pack years and FEV1 exists.

Our results emphasize the need for increased awareness and early detection of asymptomatic AATD. Identification of patients from a targeted detection programme should include aggressive family screening and allow the initiation of preventative measures before significant lung disease has occurred.

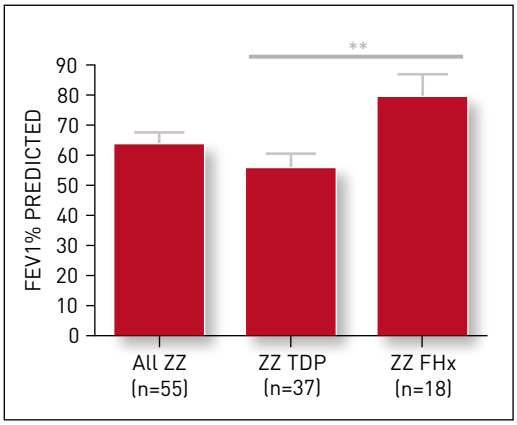

Figure 17: FEV1 % of ZZ Patients on National Registry

CHARACTERISTICS OF AN IRISH POPULATION WITH MZ PHENOTYPE ALPHA-1 ANTITRYPSIN DEFICIENCY

F. AlRoumi, T. Carroll, V. B. Morris, C. O'Connor, O. Floyd, N. G. McElvaney.

Department of Respiratory Research, RCSI Education and Research Centre, Beaumont Hospital, Dublin.

Alpha-1 antitrypsin (AAT) is a serum glycoprotein that inhibits proteases, and is produced mainly

by hepatocytes. It is particularly important in dampening the action of neutrophil elastase, which can damage the lungs. AAT deficiency results from both a qualitative and a quantitative deficiency of the protein which predisposes to the development of emphysema, chronic bronchitis, bronchiectasis, and liver disease.

We investigated 95 patients registered on the Irish Alpha-1 database as AAT deficiency MZ phenotype. The information gathered from the database was supplemented with chart reviews for clinical information and pulmonary function tests.

MZ patients had a mean FEV1% predicted of 81.9 +/- 3.3%. We note that there is a negative correlation between cigarette pack years and FEV1% predicted (r2=0.18). The serum level of AAT does not necessarily correlate negatively with FEV1 (r2=0.02). Nearly 60% of MZ patients were detected by family screening of known AAT deficient patients.

It remains uncertain whether MZ patients are predisposed to AAT deficiency sequelae when compared to the general population. We aim to settle this uncertainty by comprehensively describing the characteristics of this cohort of patients. This may represent a change in the way MZ patients are managed and could implicate earlier preventative measures to decrease the likelihood of developing emphysema.

CLINICAL TRIAL

The Alpha One Foundation has coordinated several clinical trials and research studies for Alpha-1 patients throughout the island of Ireland and these are discussed in more detail in our 'Research Programme' section. The graph, *Figure 18*, gives an indication of patients on the patient registry involved in research studies conducted by the Alpha One Foundation.

TRANSPLANT STATUS

Lung and liver transplantation is unfortunately a reality for a small proportion of our patients. The transplant status of Alpha-1 patients gives us an indication of the progression and severity of AATD, and also helps us assess the implications of early diagnosis of this disease.

Over the past year our patient registry shows the number of ZZ patients on the active transplant list is the same as the number of patients who have already received transplants (lung and liver). In addition, we have a significant number of ZZ patients currently being assessed for transplant *(Figure 19)*.

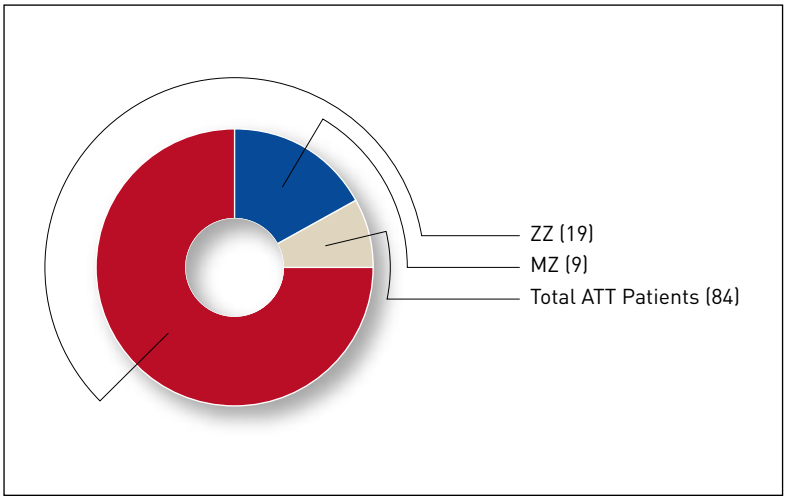

Figure 18: Number of AAT Patients Involved in Research

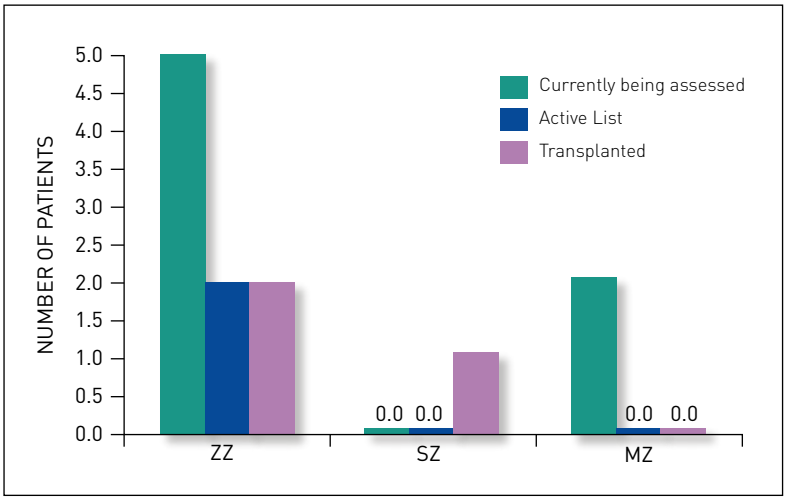

Figure 19: Transplant Status

Research Programmes

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-blinded, multicentre phase III / IV study to compare the efficacy and safety of the drug Zemaira® in patients with Emphysema due to Alpha-1 antitrypsin deficiency. The duration for each patient is 2 years.

We have recruited 16 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 antitrypsin product or a placebo (which is a mock treatment that looks like the real thing but has none of the activity). As the study is double-blinded, neither the participating patients nor our study staff knows which therapy has been assigned to them. There is an equal chance of receiving either treatment. As of March 2008 we have had 6 patients graduate onto the extension phase of the study. This is where each patient receives Zemaira® for up to another two years.

The infusions are given either in Beaumont Hospital or in the patient's own home and take 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 and progression of emphysema within the patients.

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

- Diagnosis of Alpha-1 antitrypsin deficiency (ZZ phenotype).
- Non smokers or Ex-Smokers who have stopped at least 6 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 Alpha-1 antitrypsin 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.

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

Máire Stack
Research Nurse, Study Co-Ordinator
Tel: 01 8093864 / 01 8093876

CLARIFICATION OF THE RISK OF COPD IN ALPHA-1 ANTITRYPSIN (MZ) INDIVIDUALS

Funding Body: Alpha-1 Foundation (USA)
Project Description: This clinical research study, to clarify the risk of COPD in MZ individuals, commenced in July 2007 and is supervised by Professor Gerry McElvaney, Department of Medicine RCSI, Smurfit Building, Beaumont Hospital, Dublin 9, Ireland.

The purpose of this study is to obtain information about individuals (and their family members) that are carriers of Alpha-1 antitrypsin (AAT). Acquisition of an abnormal Alpha-1 gene from each parent leads to severe deficiency in Alpha-1 protein levels which may result in serious lung disease in adults and/or liver disease in infants, children and adults. If an individual inherits an abnormal Alpha-1 gene from only one parent, they are a carrier and may be predisposed to developing lung disease.

The main objective of this study is to determine whether carriers of Alpha-1 antitrypsin deficiency are at an increased risk of developing lung disease. We aim to identify subtle changes in lung function especially in close family members that may allow earlier intervention and treatment. We also aim to investigate whether there are any environmental factors that interact with the abnormal Alpha-1 gene that predisposes some but not others to serious lung disease. If identified correctly, such environmental factors may then be avoided thus preventing the development of serious lung disease in carriers of Alpha-1 antitrypsin deficiency.

Our aim is to enroll 400 parents and siblings of 100 Alpha-1 antitrypsin carriers (PIMZ) with diagnosed GOLD Stage 3 or 4 COPD into this study. The inclusion criteria for PIMZ carriers are as follows:

- Age >30
- GOLD Stage 3 or 4 COPD (post-bronchodilator FEV1 <50% predicted; FEV1/FVC ratio 0.7)
- Confirmed PIMZ genotype
- No other lung diseases that would affect pulmonary function testing (PFT)

The exclusion criteria for relatives of the above PIMZ carriers are as follows:

- Any interstitial lung diseases
- PI types other than PIMM or PIMZ
- Non-biological siblings of the PIMZ COPD proband

Each individual will perform a lung function test (using a portable spirometer), complete a detailed questionnaire (respiratory and liver questions, family history, smoking history etc) and provide blood samples to confirm their carrier status and allow DNA extraction.

In the last year, we have recruited 125 individuals into the study from 25 families. Our preliminary results have shown approximately equal numbers of MZ carriers and MM individuals within each family. Our goal is to include as many siblings and parents from each family to participate in this ground-breaking clinical research study. We will determine whether the PI MZ carrier status is associated with an increased risk of COPD and whether cigarette smoking confers an increased risk of COPD in carriers of Alpha-1 antitrypsin deficiency.

If there are patients that fulfill the above criteria and are interested in partaking in this clinical research study, please contact:

Dr Valerie Morris
Respiratory Research Laboratory
Smurfit Building
Building Hospital,
Dublin 9.

Tel: +353-1-8093861 or 085-7255506
vbmorris@Hotmail.com

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

Poster presentations

DA Bergin, Greene CM, Taggart CC, O'Neill SJ and McElvaney NG. Activation of the EGFR by Human Neutrophil Elastase. Royal College of Surgeons in Ireland Research Day, RCSI St Stephen's Green, Dublin, April 2006.

DA Bergin, CM Greene, CC Taggart, EE Sterchi, SJ O'Neill, NG McElvaney. Activation of the epidermal growth factor receptor by Human neutrophil elastase in Alpha-1 antitrypsin

deficiency. Annual meeting of the Irish Thoracic Society, Limerick, Ireland, November 2006.

DA Bergin, CM Greene, CC Taggart, EE Sterchi, SJ O'Neill, NG McElvaney. The Involvement of a Zinc Endopeptidase, Meprin, in NE-Induced Inflammation in Airway Epithelial Cells. American Thoracic Society, San Francisco, USA, May 2007.

Oral presentations
Bergin, D. Activation of the epidermal growth factor receptor by Human Neutrophil Elastase in Alpha-1 Antitrypsin Deficiency. Invited Speaker at the Metzincin Metalloproteases In Health and Disease, Ascona, Switzerland October 2006.

Abstracts
American Thoracic Society, Toronto, May 2008.

ORAL PRESENTATIONS WITHIN THE MINI SYMPOSIUM SESSION, D96 - PHYSIOLOGY AND MOLECULAR PATHOLOGY OF ALPHA-1 ANTITRYPSIN AND RELATED SERPINS, WEDNESDAY, 21ST MAY 2008.

TITLE: THE ANTIPROTEASE ALPHA-1-ANTITRYPSIN INHIBITS N-FORMYL-METHIONYL-LEUCYL-PHENYLALANINE (FMLP) INDUCED NADPH OXIDASE ACTIVITY OF HUMAN NEUTROPHILS.

Authors: Bergin DA, Reeves EP, O'Neill SJ and McElvaney NG. Respiratory Research, Dept of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland

Rationale: The anti inflammatory effects of AAT are not only related to modulation of serine proteases activity. It is now recoginised that AAT can regulate disease process including apopotosis, reactive oxygen species production/ toxicity, LPS induced inflammation, anti viral effects and cell mediated cytokine/chemokine release. Exposure of neutrophils to a vatiety of stimuli activates a membrane bound NADPH-oxidase to catalyse the generation of superoxide anion radical (O2-). It has been shown that O2- production initiated by concanavalin A is inhibited by AAT, an effect distinct from O2- scavenging [1]. Work carried out within our own laboratory further strengthens this latter observation as only three of nine methionines present within AAT are accessable to oxidation [2]. In the present study we further examined the anti inflammatory activities of AAT, and investigated whether NADPH oxidase activation via the G-protein coupled fMLP receptor is inhibited by AAT.

Methodology: Production of O2- by 2x105 neutrophils was measured by determining the superoxide dismutase (SOD)-inhibitable reduction of cytochrome c.

Results: The rate of O2- production elicited by fMLP (10-6M) was significantly inhibited in the presence of AAT (1μM). In addition inhibition of O2- production was dose dependent and almost completely inhibited by 7.7 μM AAT.

Conclusion: To summarize, this data further supports the theory that the anti-inflammatory effects of AAT are not only related to the modulation of its serine protease activity.

[1] Bucurenci N. et al., FEBS. Vol. 300, pp. 21-24.
[2] Taggart C. J. et al., Biol. Chem. Vol. 275, pp. 27258-27265.

Funding: Alpha One Foundation Ireland, US Alpha-1 Foundation, Medical Research Charities Group, Health Research Board.

TITLE: ALPHA-1 ANTITRYPSIN AND THE PHAGOCYTIC NEUTROPHIL REVISITED

Authors: Bergin DA, Reeves EP, O'Neill SJ and McElvaney NG. Respiratory Research, Dept of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland

Introduction: It has previously been demonstrated thatÐ AAT is synthesised by circulating neutrophils [1] and is packaged along with elastase within the primary granules of these cells [2]. Thus there remains a paradox as to why an enzyme and cognate inhibitor would simultaneously compartmentalize, potentially impeding protease antimicrobial activity. Here

we set out to determine and understand the relationship between AAT and the phagocytic neutrophil.

Methods: This study utilised unprimed neutrophils isolated from blood of healthy donors. The localisation of AAT in neutrophils was evaluated using sub cellular fractionation by sucrose gradient ultra centrifugation, by western blotting and confocal immunofluorescence. We evaluated AAT shedding from neutrophils primed by TNFÐ, IL-8 and LPS by immunoblotting and FACS analysis. Furthermore we employed a biochemical approach to evaluate the mechanism of interaction between AAT and the neutrophil membrane.

Results: Our results indicate that despite what previous literature has stated, AAT is localised on the outer surface of the membrane and within secretory vesicles of neutrophils. We show upon addition of priming agents, TNFÐ, IL-8 and LPS, AAT is shed from the cell. We also demonstrate the linkage mechanism between AAT and the neutrophil membrane.

Conclusion: This study has established the localisation of AAT within the neutrophil. Similarly to L-selectin, AAT shedding occurs upon priming of the cell. Furthermore we have evaluated the mechanism for AAT linkage to the membrane. This study aims to understand the pivotal role which AAT plays in neutrophil biology with particular emphasis on AAT deficiency.

[1] RM du Bois et al. 1991. Blood 77:2724.
[2] Mason DY et al. 1991. Am J Pathol 139:623.

Funding: Alpha One Foundation Ireland, US Alpha-1 Foundation, Medical Research Charities Group, Health Research Board.

1) For this project a Travel Grant was awarded from the ATS to Dr David Bergin. Travel Grant ATS 2008, Genentech/Novartis Award: $500.
2) This project has also been accepted for funding from the Health Research Board (HRB)/ Medical Research Charities Group (MRCG), 3 years duration from January 2009.

DEFINING THE ROLE OF SELENOPROTEIN S IN Z ALPHA-1 ANTITRYPSIN DEFICIENCY

Funding body: US Alpha One Foundation.
Duration - 1 year.

Description: Z Alpha-1 antitrypsin deficiency (ZAATD) is a genetic disorder that can affect either the lungs or liver. The liver disease arises as a result of the accumulation of a misfolded protein (ZAAT) within a compartment inside the liver cells that causes a stress on these cells and impairs their normal function. It also prevents ZAAT being released from these cells into the circulation. The majority of ZAAT in the body is made in the liver from where it travels to the lung and carries out a protective role. Thus lung disease occurs because there is too little ZAAT in the lung. However ZAAT can also be expressed by cells in the lung, and the misfolded form may affect the function of cells in the lung and further contribute to the lung disease.

This project investigates the role of a protein called Selenoprotein S1 (SEPS1) as a potential factor that can prevent the accumulation of misfolded ZAAT within liver or lung cells. SEPS1 is known to help stressed cells by enhancing their ability to cope with the burden of misfolded proteins such as ZAAT. In order for SEPS1 to function correctly it requires the essential trace mineral selenium. Here we investigate if adding selenium to cells can improve the way SEPS1 functions. We know of three responses that are affected in liver and/or lung cells due to ZAAT accumulation – the ER overload response, the unfolded protein response and apoptosis. This project tests the effect of selenium and different SEPS1 levels on each of these three responses. The data generated will provide important new information regarding how lung and liver disease occurs in ZAATD and may point towards potential new treatments for the disorder.

Presentations:

1. Irish Thoracic Society, Davenport Hotel, Dublin, Ireland, 9th-10th November 2007.
2. Beaumont Hospital Sheppard Prize, Dublin, Ireland, 19th February 2008.
3. American Thoracic Society Toronto, Wednesday 21st May 2008, Oral presentation.

Abstracts

"Defining the role of selenoprotein S in Z variant Alpha-1 antitrypsin deficiency." Irish Thoracic Society 2007 and abstract published in Irish Journal of Medical Science November 2007.

"Defining the role of selenoprotein S in Z variant Alpha-1 antitrypsin deficiency." American Thoracic Society 2008 and abstract published in Proceedings of the ATS May 2008.

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

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

CONFERENCE ABSTRACTS

American Thoracic Society Annual Conference 2008

Title: Evidence for the Activation of the Unfolded Protein Response (UPR) in Monocytes from Alpha-1 Antitrypsin Deficient Individuals

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, Ireland.

Rationale: Alpha-1 antitrypsin (AAT) is a serine protease inhibitor synthesised mainly 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. AAT is also synthesised in monocytes, and locally produced within the lung by alveolar macrophages and epithelial cells. 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 monocytes from AATD patients.

Methods: Monocytes were isolated from healthy individuals and from AATD ZZ patients. Immunoblotting and quantitative RT-PCR were used to investigate UPR activation.

Results: We show that elements of the UPR, such as the chaperones glucose-regulated protein 78 and 94 (grp78 and grp94), the activated transcription factors 3 and 4 (ATF3 and ATF4) and C/EBP-homologous protein (CHOP), can be activated in monocytes by treatment with Thapsigargin, a known ER stress inducer. To further evaluate UPR activation in vivo we show the transcription factor X-box binding protein-1 (XBP-1) is spliced and activated in ZZ monocytes, and not in monocytes from normal (MM) individuals. In addition, we demonstrate the phosphorylation of eukaryotic initiation factor 2Ð (eIF2Ð) in ZZ monocytes, responsible for shutting down global protein synthesis, the first step in the UPR. Finally, we show that the chaperones grp78 and grp94, and components of the ER-associated degradation pathway (ERAD) are induced in ZZ monocytes, and not in MM monocytes.

Conclusions: Monocytes produce AAT, representing another source of misfolded protein. We have shown for the first time that the unfolded protein response is activated in monocytes from AATD patients, probably by misfolded Z AAT. This UPR activation may impair monocyte function and contribute to the pro-inflammatory milieu of the AATD lung.

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

American Thoracic Society Annual Conference 2008

Title: Prevalence of Alpha-1 Antitrypsin Deficiency in Ireland

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

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

Rationale: AAT deficiency is a hereditary autosomal codominant disorder, resulting from mutations in the AAT gene, and classically presents with emphysema and liver disease. 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 carrier status 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.

Methods: 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 was used to identify AAT variants.

Results: 2,000 individuals with COPD or asthma attending respiratory outpatient clinics were screened in a national targeted detection programme. A further 1,000 healthy individuals in the general population were also screened for S and Z alleles in a pilot study. The targeted programme identified 43 ZZ, 28 SZ, 7 SS, 195 MZ, 158 MS, and 6 MI individuals, as well as several other rarer phenotypes. The pilot screen of 1,000 healthy individuals identified 98 MS, 46 MZ, 2 SZ, and a single SS case.

Conclusions: The percentage of deficiency alleles detected in the targeted population was higher than anticipated from studies in other populations. The S variant, thought common to the Iberian Peninsula, was detected with unusually high frequency in both targeted and the general population. Several other rarer phenotypes were also detected. Further analysis will reveal whether these phenotypes predispose individuals to lung disease.

Acknowledgements: Alpha One Foundation Ireland, Alpha-1 Foundation U.S., Department of Health and Children, the Royal College or Surgeons in Ireland and Talecris Biotherapeutics.

Association of Clinical Biochemists in Ireland (ACBI) annual conference 2008

Title: Prevalence of Alpha-1 Antitrypsin Deficiency in Ireland

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

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

Rationale: AAT deficiency is a hereditary autosomal codominant disorder, resulting from mutations in the AAT gene, and classically presents with emphysema and liver disease. 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 carrier status 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.

Methods: 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 was used to identify AAT variants.

Results: 2,500 individuals with COPD or asthma attending respiratory outpatient clinics were screened in a national targeted detection programme. A further 1,000 healthy individuals in the general population were also screened

for S and Z alleles in a pilot study. The targeted programme identified 66 ZZ, 41 SZ, 12 SS, 350 MZ, 222 MS, and 12 MI individuals, as well as several other rarer phenotypes. The pilot screen of 1,000 healthy individuals identified 98 MS, 46 MZ, 2 SZ individuals, and a single SS case.

Conclusions: The percentage of deficiency alleles detected in the targeted population was higher than anticipated from studies in other populations. The S variant, thought common to the Iberian Peninsula, was detected with unusually high frequency in both targeted and the general population. Several other rarer phenotypes were also detected. Further analysis will reveal whether these phenotypes predispose individuals to lung disease.

Acknowledgements: Alpha One Foundation Ireland, Alpha-1 Foundation U.S., Department of Health and Children, the Royal College or Surgeons in Ireland, and Talecris Biotherapeutics.

Members of our Targeted Detection Programme also participated in an international study which determined the first international (WHO) Alpha-1 antitrypsin standard, with the abstract below.

International collaborative study to establish the 1st international (WHO) standard for Alpha-1-antitrypsin

Author: C. Thelwell[1], P. Rigsby[2] and C. Longstaff[1]

Institution: (1) Biotherapeutics Group, Haemostasis Section and (2) Biostatistics Division, National Institute for Biological Standards and Control, South Mimms, Herts EN6 3QG, UK

Summary: An international collaborative study was organised to establish the 1st International Standard (IS) for Alpha-1-Antitrypsin (AAT) as agreed at the Alpha-1 Foundation Workshop, April 2005, Cincinnati, USA. The study involved 15 laboratories from 10 different countries. Laboratories were provided with detailed methods and critical reagents and were asked to measure the potency of four candidate standards (A, 05/150; B, 05/152; C, 05/162 and D, 05/172). In addition laboratories with relevant experience were invited to carry out extended characterisation of the materials, including total protein and antigen content for which an additional reference preparation was provided. Analysis of the data indicated that any of the candidates would be suitable based on potency determination; however candidate C had a slight advantage based on filling data. It is therefore proposed that candidate C (05/162) be adopted as the 1st International Standard for Alpha-1-Antitrypsin with a potency of 243 nmoles (12.4 mg) active AAT per ampoule.

Peer-reviewed Publications relating to Alpha-1 antitrypsin deficiency

Geraghty P, Rogan MP, Greene CM, Brantly ML, O'Neill SJ, Taggart CC, McElvaney NG. Alpha-1-antitrypsin aerosolised augmentation abrogates neutrophil elastase-induced expression of cathepsin B and matrix metalloprotease 2 in vivo and in vitro.
Thorax. 2008 Jul;63(7):621-6. Epub 2008 Feb 4.

Greene CM, Miller SD, Carroll T, McLean C, O'Mahony M, Lawless MW, O'Neill SJ, Taggart CC, McElvaney NG.
Alpha-1 antitrypsin deficiency: a conformational disease associated with lung and liver manifestations.
J Inherit Metab Dis. 2008 Feb;31(1):21-34. Epub 2008 Jan 16. Review.

Demeo DL, Campbell EJ, Barker AF, Brantly ML, Eden E, McElvaney NG, Rennard SI, Sandhaus RA, Stocks JM, Stoller JK, Strange C, Turino G, Silverman EK.
IL10 polymorphisms are associated with airflow obstruction in severe Alpha1-antitrypsin deficiency.
Am J Respir Cell Mol Biol. 2008 Jan;38(1):114-20. Epub 2007 Aug 9.

Mulgrew AT, Taggart CC, McElvaney NG. Alpha-1-antitrypsin deficiency: current concepts.
Lung. 2007 Jul-Aug;185(4):191-201. Epub 2007 Jun 12. Review.

Miller SD, Greene CM, McLean C, Lawless MW, Taggart CC, O'Neill SJ, McElvaney NG. Tauroursodeoxycholic acid inhibits apoptosis induced by Z Alpha-1 antitrypsin via inhibition of Bad.
Hepatology. 2007 Aug;46(2):496-503.

Demeo DL, Sandhaus RA, Barker AF, Brantly ML, Eden E, McElvaney NG, Rennard S, Burchard E, Stocks JM, Stoller JK, Strange C, Turino GM, Campbell EJ, Silverman EK. Determinants of airflow obstruction in severe Alpha-1-antitrypsin deficiency.
Thorax. 2007 Sep;62(9):806-13. Epub 2007 Mar 27.

Patient Support Group Meetings

Since April 2008 the Patient Support Group has organised five meetings, all members receive minutes and agenda of these meetings.

These were held in the Alpha One Foundation's office located in RCSI Building, Beaumont Hospital. This venue can be changed at any time when organised by the group.

Items discussed range from awareness to feedback of delegates to international patient meetings e.g. Prague May 2008.

Attendance has been low recently but we hope to improve this with an awareness campaign in October 2008. We would encourage patients to keep in contact with other patients and we hope to develop a newsletter updating members of news events and patient stories.

FUNDRAISING

Women's Mini Marathon June 2008

Fundraising from the Women's Mini Marathon in June 2008, being presented to the Alpha One Foundation from Anna Cassidy and her family.

Staff from the RCSI, Beaumont Hospital and Alpha One Foundation participating in the Women's Mini Marathon this year.

Web Expansion

The Foundation's newly redesigned Web site has nearly 70 percent more content. We hope this will result in 30 percent increase in the number of visitors.

Ecom Ireland which has developed and provides on-going service to our screening programme and Registry, also hosts our new website. We hope to provide the best possible user experience and accurately track web usage for patients, clinicians, researchers and general public. The site is also designed to encourage clinical trials participation and volunteer activities and on-line donations.

Conferences

ROME CONFERENCE

Delegates from 21 countries world wide gathered with fellow patients, carers, medical experts and pharmaceutical company representatives in the Eternal City for the 3rd Alpha 1 Patients Congress, hosted by the Italian Alpha-1 Association, on the weekend of 28th-30th September, 2007.

The Alpha One Foundation had two representatives present at the Congress, myself and John Hannan.

The Conference began on the evening of the 28th, when the assembled delegates and guests were welcomed by Larry Warren, our own CEO and President of Alfa Europe and Nuccia Gatta, President of the Italian Alpha-1 Association and Vice President of Alfa Europe. At this stage Larry gave a warm welcome and thanks to the Medical Professionals, who were attending the Conference, for their continued care and support, also to the Pharmaceutical Companies for their support

Larry Warren reported that the Alfa Europe Federation was now a legally recognised and registered organisation. It will be an organisation of many languages and cultures, and it is hoped will prove to be a voice in the EU that will increase awareness of Alpha-1 and promote more, much needed research into the illness.

To open the Conference all the Delegates we asked to give a brief presentation to show how their individual patient groups were working and how they were moving forward.

21 countries were represented, including for the first time the Czech Republic, who have only recently set up an AATD centre. In addition to European countries there were delegates from the USA, Puerto Rico, Argentina, Australia and New Zealand. Some countries have had patient groups for a number of years while others have only recently been formed. Patient groups were proactive in many aspects of their organizations, such as the setting up of support networks for patients, regional groups, supporting research programmes, advocating for clinical trials, working closely with the medical professionals, and producing leaflets and newsletters explaining about Alpha-1.

Saturday began at 9am, with a presentation from John Walsh, President of the Alpha One Foundation in the USA, entitled "Patients Empowerment". This talk proved to be very motivating. John first said how encouraging it was to see so many countries sending delegates to the conference. He said that patient groups give Alpha-1 patients

- Inspiration through collective and shared experience
- Courage to do things
- Empowerment to take action, through frustration and desperation
- Self confidence and power
- Alphas serving Alphas

Patients groups involve not only patients but loved ones, wider groups, communities, medical professionals, scientists, and also challenge patients to get up and become involved, and can encourage healthcare industries to aid research into therapies.

Continuing with his presentation, John said how he had picked up common themes from all the delegates

- Lack of awareness, low detection rates and diagnosis
- Lack of resources
- Limitation on access to therapies in some countries
- Start up Charities
- Limited or no support for patients
- Transplant issues
- The more aware the better the cure
- Quest for a cure(s) to conquer or eradicate the illness

The one message that came through is that there is under diagnosis, mis-diagnosis, and a lack of awareness and understanding, amongst the medical profession and the general public, of Alpha-1. It is patient groups who can change this, by making sure hospital consultants, GPs, nurses, family and friends know about the illness and the effect it has on people's lives, not just sufferers, but their carers and families.

Josephine McGuirk,
October 2007

ABSTRACTS FROM ROME CONFERENCE 2007

ALPHA1-ANTITRYPSIN DEFICIENCY: WHERE WE ARE, WHERE WE ARE GOING TO

Maurizio Luisetti

Center for Diagnosis of Inherited Alpha1-antitrypsin Deficiency Laboratory of Biochemistry and Genetics Institute of Respiratory Disease University of Pavia Fondazione IRCCS Policlinico San Matteo Pavia, Italy

At the beginning of the 1960s, two scientists in Sweden, Laurell & Eriksson, associated missing bands on paper electrophoresis in sera with subjects suffering from pulmonary emphysema and chronic liver disease. This was the first report of a disorder since then referred to as "Alpha1-antitrypsin Deficiency" (AATD). Now, 44 years after the discovery, AATD is in its middle age, and an astonishing amount of data have accumulated during four decades. We have currently a satisfactory view of the AATD epidemiology, at least in western countries; we have gained a good level of knwoledge about the pathogenesis of lung and liver disease. We have developed suitable methods for laboratory identification of protein and molecular abnormalities linked to AATD. A number of national registries for AATD have been established, as well as national patient support groups, and their federations work together for a better awareness of the disorder. Replacement therapy is available in a growing number of countries, and several hundreds of AATD subjects with lung disease are currently treated. But a number of relevant questions are still unanswered, and we hope we are able to offer satisfactory answers to these question in the next future. Basic questions, such as the definition of the exact ratio between asymptomatic individuals affected by AATD and individuals affected by AATD which develop lung/liver disease (and, more importantly, what differentiates the latter from the former), and clinical questions, such as the development of more effective "replacement" therapies, are among the questions AATD patients ask the scientific community. This is a commitment for the future.

PATHOGENESIS OF LIVER INJURY IN ALPHA-1-ANTITRYPSIN DEFICIENCY

F. Callea

Department of Pathology Children's Hospital Bambino Gesù Rome, Italy

Liver pathology is a major manifestation of Alpha-1-antitrypsin deficiency (AAT), restricted to the AAT mutations causing misfolding of the protein and accumulation in the Endoplasmic Reticulum (ER) of hepatocytes. So far three such mutations have been identified, Z, Mmalton, Siiyama, the Z variant being the most frequent. The spectrum of the associated liver pathology comprises neonatal cholestasis, chronic hepatitis, cirrhosis and HCC.

The mechanism of the liver damage is not fully understood. There are two major lines of thinking: 1) the storage is toxic per se, 2) additional factors are required for the development of the liver damage. The main argument favouring the second opinion is mainly based upon the observation that only a minority of AAT deficient individuals develop liver disease.

I would like to emphasize the point that the accumulation of the mutant AAT represents per se the elementary lesion of the disease and that is an unavoidable process. In this context I will be reviewing the patho-morphogenesis of the storage phenomenon and the main morphological alterations with special regard to new electron microscopic findings.

The epidemiological and clinical relevance of the morphological alterations are discussed also in view of the new experimental data on the pathways for degradation of the mutant proteins that accumulate in the ER.

PATHOGENESIS OF LUNG DISEASE

David A. Lomas

University of Cambridge, Cambridge, UK

Alpha1-Antitrypsin is produced from the liver and bathes all the tissues of the body. Its role is to protect against tissue damage from

enzymes released from neutrophils. The genetic deficiency of Alpha-1-antitrypsin that results from the Z mutation causes the protein to form polymers that are retained within the cells of the liver. This retention of protein causes liver disease. The resulting lack of plasma Alpha-1-antitrypsin (10-15% of normal levels) leaves the lungs exposed to attack by neutrophil enzymes and so results in emphysema.

We have recently shown that some of the Alpha-1-antitrypsin that enters the lung can change shape and form chains of polymers. These polymers are unable to function as inhibitors of neutrophil enzymes and so exacerbate the tissues damage. Moreover the polymers are themselves inflammatory and cause further attraction of neutrophils into the lungs. This in turn accelerates the inflammation and emphysema. All these factors are exacerbated by smoking.

My laboratory has defined the pathway by which polymers form and is currently developing strategies to block the polymerisation of Z Alpha-1-antitrypsin. Such a strategy will prevent the accumulation of Z Alpha-1-antitrypsin within hepatocytes thereby treating the associated liver disease. The release of Alpha-1-antitrypsin from the liver will increase the circulating levels of Alpha-1-antitrypsin and hence provide protection for the lungs against emphysema.

THE NEED OF STANDARDIZATION OF DIAGNOSIS

Ilaria Ferrarotti

Fondazione IRCCS Policlinico S. Matteo Pavia, Italy

AATD is a largely under-recognized condition and one of the most common severe hereditary disorders in the world.

To improve the diagnostic yield and to address the discrepancy between expected and diagnosed AATD cases, the recently published ATS/ERS Statement (2003) recommends diagnostic testing for all symptomatic adults with emphysema, COPD or asthma with incomplete reversible airflow obstruction, individuals with unexplained liver disease, asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (cigarette smoking, occupational exposure) and adults with necrotizing panniculitis.

The laboratory diagnosis of AATD has evolved from the initial description of the condition in 1963 based on paper electrophoresis – agar-gel electrophoresis – immunoelectrophoresis to the currently used methodology, that is a combination of serum AAT level determination, isoelectric focusing (IEF), genotyping, and sequencing. The availability of matrices such as the dried blood spot have facilitated the implementation of laboratory analyses for Alpha-1-antitrypsin deficiency, but have also challenged laboratories to develop more reliable and reproducible techniques starting from dried blood.

THE IMPORTANCE OF SCREENING

Luciano Corda

Centro Riferimento Deficit Alfa1-Antitripsina Medicina Respiratoria/Prima Medicina, Spedali Civili, Brescia Cattedra di Malattie dell'Apparato Respiratorio, Università di Brescia, Italy

Screening is performed to identify the presence of a disease or a risk factor for a disease, typically among asymptomatic persons. In this way, a disease, or risk factors for a disease, can be detected early, allowing either early treatment or prevention.

Screening tests are widely used by clinicians as part of the periodic health examination, as well as by public health officials. Examples of screening tests are as varied as blood tests to detect lead poisoning in young children, mammography to detect breast cancer and questionnaires to identify persons with alcohol or other drug problems. Even if Alpha-1-antitrypsin deficiency (AATD) is considered a rare disease, it is probably the most common widespread genetic abnormality.

AATD is rarely diagnosed, both because of poor awareness by health workers and lack of really implemented screening programs. An

underrecognition of AATD diagnosis persists in spite of an effort to enhance clinicians' awareness. From the clinical point of view the under-recognition might be explained by the facts that most of clinical phenotypes associated with AATD are not exclusive to this condition and that the abnormal genes have an incomplete penetrance (the relationship between genotype and clinical phenotype is not strong).

The availability of Alpha-1-antitrypsin replacement therapy for individuals with pulmonary emphysema associated with AATD encouraged the scientific community to establish and reinforce AATD screening.

Screening for AATD has been recommended by the World Health Organization (WHO) in 1997. The American Thoracic Society (ATS) together with the European Respiratory Society (ERS) in 2003 advice population screening when three main conditions occur: 1) high prevalence of AATD (more than 1/1500), 2) high prevalence of smokers or of people exposed to toxic inhalants 3) availability of an adequate genetic "counselling". A population screening might be very innovative making use of new genetic techniques. An adequate screening program and an early diagnosis of AATD may permit to apply primary (i.e. smoking cessation for lung disease or alcohol abstention for liver disorders) or secondary (i.e. treatment of related lung diseases) prevention measures.

At the moment there are evidences of neonatal screening, patient oriented detection (case-finding) and so called "targeted screening". Recently, the Italian Association of "Alphas" coordinated a screening for AATD in the entire population of a small village in a high risk area.

ONGOING RESEARCH AND DEVELOPMENT

Robert Stockley

University Hospital, Birmingham, UK

From the first recognition of Alpha-1 antitrypsin deficiency in 1963 the research field became very active exploring the deficiency as a cause of all COPD. In the early 1980s augmentation therapy was introduced and with the understanding that this was "a cure" research activity waned.

However in recent years a more inquisitive approach to the condition has emerged. Why do some AATD patients remain well? How does the lung disease present? How does it develop? How should patients be treated? What is the effect of chest infections? Can we prevent deterioration? Can we repair the damage?

These and many other questions challenge us for the next few years. Of importance clinical trials of old or new treatments will emerge and be delivered through the extensive collaboration of researchers and the developments of the AIR and AOF registries. However it is critical that these are co-ordinated and not replicated merely to appease regulatory bodies.

Close collaboration between medical registries, pharmaceutical companies and patient groups will be essential if we are to assess future therapies adequately.

THIRD ALFA EUROPE ALLIANCE MEETING; PRAGUE 9TH – 10TH MAY 2008

Agenda:

1. ***ONGOING RESEARCHES INTO ALPHA-1-ANTIRYPSIN DEFICIENCY***
   Dr Thomas Köhnlein MD,
   Hannover Medical School
2. ***EPIDEMIOLOGY AND DETECTION PROGRAM IN POLAND***
   Dr Pawel Kuca MD,
   National TB and Lung Diseases Research Institute
3. ***SITUATION OF ALPHA-1 PATIENTS IN THE CZECH REPUBLIC***
   MUDr Jan Chlumský, Ph.D,
   Department of Pneumonology, Thomayer Teaching Hospital, Prague
4. ***REPLACEMENT THERAPY AND EUROPE***
   Klaus Schäfer, ***Talecris Biotherapeutics GmbH in Frankfurt***

5. MEETING OF THE DELEGATES

Topics

1. Lung rehabilitation
2. Access to therapies
3. Access to insurance, mortgage, pension
4. Genetic discrimination within Europe

All delegates gathered at the Hotel Don Giovanni on the afternoon of Friday 9th May.. We were welcomed to Prague by the Federation President, Larry Warren and Silke Horakava from the Czech Alpha Group who were our hosts for the Conference.

The AGM, initially consisted of voting and legal formalities, Larry Warren presented his President's Report; in this he outlined events of the Conference in Rome last year.

- The alliance of the Alfa Europe Federation & the European Alpha 1 Foundation, has given the Federation a more stable footing and financial backing to run the Organisation and develop
- The Federation membership is growing, with France and the Czech Republic joining this year and Poland, UK, Scotland and Portugal soon to be joining
- It is important that The Federation is recognised by the EU Commission as a patient support partner, in order that Alpha Europe becomes recognised in the EU, this will eventually be for the benefit of all Alpha patients and their families
- Larry Warren reported that the Federation already
  * Is a member of the Plasma and Protein Users group, which meets 4 times a year to look at the supply of produce etc
  * Has been accepted as a member of EURORDIS, the European Alliance for patients with rare diseases
  * Has applied for membership of EPPOSI, which is an alliance of patient groups, science and industry
  * Intends to build a working relationship with AIR, the Alpha 1 International Registry.
  * The above will ensure that the Federation is part of the planning for therapies, registries or any other matters that will affect Alpha sufferers
- This year France has the EU Presidency and they have already given notice to the Federation that the French Government wishes to raise the profile of rare diseases (of which Alpha 1 is one) in the EU and look at how they are tackled and will hopefully draw up directives and recommendations to the Parliament and Commission, these will then be passed down to National Governments
- In order to raise the awareness of Alpha 1 within the EU, delegates were asked to take back to their various groups and associations a request that Alpha patients right to their local MEPs telling them about Alpha 1 as an illness, how it affects them personally and their families lives and the problems they encounter with receiving treatments, therapies and reimbursements. Here in the UK we are ahead of the game with this, but I would still urge you if you haven't written to your MEP please do so.

Nuccia Gatta, who has been Vice President and last year hosted the Rome Conference, explained how Italy along with Holland and Germany, have been doing the PAAIR (a collaboration between Patient Associations – PA- and the Alpha-1 International Registry – AIR), this will give the EU an example of how individuals involved in a rare disease can organise themselves to improve the diagnosis, care and treatment of a disease and the impact this has on patient care.

We next went on to the Treasurers report, given by Juergen Schultz, explained that the Federation now had finance coming in from the Foundation, this apart from the 50euros membership fee paid by each member country, is, at present, the Federation's main source of income. Conferences are funded by the Foundation, although the World Conference in Rome was funded by outside organisation.

After discussion of the reports, the meeting moved on to electing officers

- President – Larry Warren
- Vice President – Sandrine Lefrancois (nominated from the floor, Nuccia Gatta stood down)
- Treasurer- Juergen Schultz
- Auditor – Elke Sadtler-Lison

Orla Keane
May 2008

RARE DISEASE NEWS IN EUROPE

The Alpha One Foundation is a member of Transplant and Rare Disease Programme and we were represented at this meeting by Larry Warren, CEO.

The 'Eurordis Summer School' for capacity building for patient advocates took place from June 15 -19 in Barcelona, Spain. The event, which was the first of its kind, attracted 34 participants from 16 European countries, representing 21 rare diseases. The aim was to further the understanding of patient representatives on their role and potential input in the regulatory process of drug development and clinical trials in Europe. The programme was especially interesting for those patient advocates who are currently involved or are thinking about getting involved in the EU decision making process in the EU regulatory agency, the EMEA, as members of scientific committees and working parties. To date, Eurordis' patient representatives involved in drug development had not had the opportunity to meet together and interact with each other, regulators, academic partners and industry and to learn from their experience. The Eurordis Summer School offered this unique opportunity within a 4-day seminar, which was deemed very interactive by everyone who attended.

EURORDIS MEMBERSHIP MEETING: EMPOWERING PATIENTS' REPRESENTATIVES TOWARDS MULTIDISCIPLINARY AND COMPREHENSIVE CARE

This year's Eurordis Annual Membership Meeting, which took place on the 16 and 17 of May in the welcoming city of Copenhagen, attracted 155 participants from 24 countries. During this two-day conference, participants shaped the tools to advocate for Centres of Expertise and European Reference Networks, especially by debating and working on a Declaration of Principles and a Charter. These documents could help ensure that patient's input is included in national plans for rare diseases and Centres of Expertise. On their way out, the people who attended felt truly empowered to continue fighting in favour of patient-centred care for rare diseases at home and at European level.

Acknowledgements

We would like to thank the following:

- 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
- Helen Moore and Pat O'Brien in the Beaumont Hospital Biochemistry Department for their continued support
- Dr Joseph McPartlin, Trinity Biobank, Institute of molecular Medicine, St James's Hospital, Dublin
- ECOM – Ireland continues to consult, programme, build queries and develop our website and database to top international standards

We would also like to thank the Department of Health and Children and Health Service Executive for their continued financial support.

The Foundation's research demonstrates higher incidence of Alpha-1 on the island of Ireland than previously thought, with an estimated 3,000 individuals at risk of developing this severe disease compared to 1,600 individuals.

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