



**Alpha One Foundation**

ANNUAL REPORT 2010



## Contents

1. Executive Summary .....	2
2. What is the Alpha One Foundation? .....	3
3. The Prevalence of Alpha-1 Antitrypsin Deficiency in Ireland .....	4
4. The National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme .....	9
5. Rare Alpha-1 Antitrypsin Mutations in the Irish Population .....	13
6. The National Alpha-1 Patient Registry .....	15
7. A Study of Health Related Quality of Life in Alpha-1 Individuals .....	18
8. Pioneering Research into Alpha-1 Antitrypsin Deficiency .....	22
9. Research Studies/Programmes .....	24
10. Conferences .....	34
11. What is Alpha-1? .....	36
12. What does it mean to be an Alpha-1 Carrier? .....	39
13. Recent Events .....	42
14. Patient Support Group .....	43
15. Acknowledgements .....	44

# 1. Executive Summary

**During the past twelve months** the Alpha One Foundation has continued to increase awareness of Alpha-1 Antitrypsin Deficiency. Our endeavours to raise public awareness are linked to the promotion of basic and clinical research into Alpha-1.

The National Screening Targeted Detection Programme receives samples from over 20 Hospitals, GP practices, and family members of known Alpha's. We have screened almost 5,000 individuals for Alpha-1. Upon diagnosis patients can now be fast tracked to our new dedicated Alpha-1 Clinic based in Beaumont Hospital.

The National Alpha-1 Registry continues to grow and expand and is now successfully capturing all the data necessary to monitor the health of our Alpha-1 patients and this will in turn lead to improved patient care and treatment. We earnestly encourage all our patients to sign up to the National Alpha-1 Patient Registry.

The Alpha One Foundation continues our active participation with the Medical Research Charities Group, (Rare Diseases Working Group, Registry Working Group), Irish Donor Network (IDN), Irish Platform for Patient Organisations, Science and Industry (IPPOSI) and the European Organisation for Rare Diseases (EURORDIS). We hosted an information stand and presented novel research at the Irish Thoracic Society (ITS) conference in November 2009 in Galway. Our research was also presented at the American Thoracic Society Meeting in New Orleans May 2010.

We are delighted to welcome Dr Kevin Molloy to the Foundation, he is continuing research into MZ (carrier) Alpha-1 patients. The study's aim is to clarify if MZ patients are at a greater risk of developing COPD, this research is in conjunction with Dr. Edward Silverman in Harvard University. This year the Alpha One Foundation also welcomed Dr. Ilaria Ferrarotti, Senior Scientist and responsible for the Italian Alpha-1 screening programme at the University of Pavia who visited our research laboratories for three months.

Our patient support group held a Charity Fashion Sale in Maynooth, Co. Kildare last February. This was a tremendous success. The group also successfully raised funds through the Flora Women's Mini Marathon. Funds raised were presented to the Foundation for a Sebia Hydrasys machine. This equipment is used in our laboratory to diagnose Alpha-1. Crea Crosbie and Orla Keane from our patient support group represented the Foundation at the Alfa Europe Annual meeting in London this July. Patients from Europe shared their experiences, views, and plans in relation to Alpha-1. This meeting proved to be a useful and enriching experience for all attendees and for the medical personnel.

The Alpha One Foundation held its annual Chopin Anniversary Recital in the Mansion House, last October. In attendance were the Polish and French Ambassadors. During Chopin's life he suffered from chronic respiratory illness and died at a young age, most likely due to Alpha-1. We felt it appropriate to celebrate Chopin's life and draw attention to respiratory research especially into Alpha-1 Antitrypsin Deficiency. We would like to thank all those who were involved.

This brief overview may give you some idea of the research, activities and the progress being carried out by the Alpha One Foundation. This work is collaborative and I wish to thank my team colleagues for their diligence and cooperation which made the past year such a success for the Foundation.

**Kitty O'Connor**  
Chief Executive Officer

## 2. What is the Alpha One Foundation?

**The Alpha One Foundation** was founded in 2001, and is based in the RCSI Clinical Research Centre at Beaumont Hospital. The Alpha One Foundation is dedicated to increasing diagnosis, raising awareness and improving the treatment of Alpha-1 Antitrypsin Deficiency (Alpha-1).

Alpha-1 is a common inherited disease but is grossly under-diagnosed, and affects over 200,000 carriers on the island of Ireland, with 2,000 of these having very severe deficiency. If undetected, Alpha-1 leads to severe lung and liver disease. Most patients present in their 40s and 50s with emphysema. A sub-group presents with liver disease in the first year and may require liver transplantation. Of the first cohort of Irish lung transplant recipients, 50% were Alpha-1 patients. Early diagnosis is vital for health and welfare of our patients, and this is our main objective. For example, the average Alpha-1 patient in the US sees 5 doctors over 7 years for a correct diagnosis.

The Foundation employs a dedicated senior laboratory scientist, a laboratory technician and a specialist Alpha-1 nurse. Professor McElvaney is the clinical director overseeing the activities of the Alpha One Foundation. Patient focused service is essential for the delivery of care of AATD patients. The Foundation focuses on patient services, medical services and diagnostic services to hospital laboratories in the area of Alpha-1 antitrypsin deficiency.

The Alpha One Foundation has affiliations with the Irish Donor Network, the Irish Asthma Society, and the Irish Platform for Patient Organisations Science and Industry (IPPOSI). We support our active Alpha-1 patient support group which promotes understanding and awareness of the condition among patients and their families.

### **FUNCTIONS OF THE ALPHA ONE FOUNDATION:**

#### **1. National Targeted Detection Programme**

The World Health Organisation (WHO)

recommends the following patient groups should be screened for Alpha-1:

- All COPD patients
- All non-responsive asthmatics
- All cryptogenic liver disease patients
- All first-degree relatives of known Alphas
- Reduced serum levels of AAT
- Panniculitis patients

There are over 1,000 Alpha-1 blood tests performed FREE by the Alpha One Foundation every year. Over 20 hospitals currently send us Alpha-1 samples. *This is the only national Alpha-1 screening programme in the world.*

#### **Diagnostic Services Provided:**

There are 3 main diagnostic services provided for the diagnosis of Alpha-1 Antitrypsin Deficiency;

- Phenotyping
- Genotyping
- Quantification of serum AAT

#### **Who is Eligible to Access these Services?**

Healthcare professionals who are involved in the treatment, care and management of the patient groups specified in the WHO guidelines for screening. These include;

- Hospital laboratories
- Respiratory physicians
- GPs
- Research nurses
- Physiotherapists
- Smoking cessation officers

## **2. Family Screening**

Family screening provides an opportunity for early diagnosis of Alpha-1 and therefore can potentially reduce the risk of developing lung disease. We provide a full family testing service, for relatives of known Alpha-1 patients. The US Alpha-1 Foundation provides us with free finger-prick test kits and this allows us to quickly diagnose family members, often before lung or liver disease has developed in these individuals. We then offer them rapid access to our outpatient facilities. Alpha-1 patients are also provided with a counselling service, an Alpha-1 patient support group and access to our website [www.alpha1.ie](http://www.alpha1.ie).

understanding to newly diagnosed and known Alphas and their families. The group are also actively involved in fundraising events such as Women's Mini Marathon, fashion sales etc.

## **3. Evaluation of New Treatments**

New treatments for Irish Alpha-1 patients in clinical trials at the moment include intravenous replacement therapy, inhaled Alpha-1, and gene therapy treatment.

## **4. National Alpha-1 Referral Centre**

As the national referral centre for Alpha-1 we provide a rapid access Alpha-1 clinic for newly-diagnosed Alphas. The Alpha-1 patient is seen by a team involving doctors, nurses, and physiotherapists and international best practice standards of care are initiated.

## **5. National Advocacy Role**

The Alpha One Foundation played a lead role in supporting Minister Martin's successful ban on smoking in the workplace. We also played a role in the H1N1 vaccination programme and are frequent contributors to World COPD (chronic obstructive pulmonary disease) Day.

## **6. National Alpha-1 Patient Registry**

We host the national Alpha-1 patient registry which gives us essential clinical information about Alpha-1 patients and their disease progression. This active registry allows us to improve their care and treatment, and plan for the future services required by these patients.

## **7. Patient Support Group**

This group of patients provide support and

### 3. The Prevalence of Alpha-1 Antitrypsin Deficiency in Ireland

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#### INTRODUCTION

Alpha-1 antitrypsin (AAT) deficiency is a hereditary disorder first described in the early 1960s when emphysema was described in patients with low plasma levels of AAT protein (Laurell and Eriksson 1963). The condition is associated with a substantially increased risk for the development of pulmonary emphysema by the third or fourth decades of life and is also associated with risks for development of hepatic disease (Sveger 1976), cutaneous panniculitis (Edmonds, Hodge et al. 1991), bronchiectasis (King, Stone et al. 1996), vasculitis (Lewis, Kallenbach et al. 1985), Wegener's granulomatosis (Barnett, Sekosan et al. 1999), arterial aneurysm (Schievink, Puumala et al. 1996), lung cancer (Yang, Bamlet et al. 2005), and renal disease (Davis, Burke et al. 1992). AAT deficiency is characterised by misfolding of AAT protein and belongs to a class of genetic diseases termed conformational disorders (Greene, Miller et al. 2008). Other conformational disorders include cystic fibrosis, Parkinson's and Huntington's diseases, all associated with intracellular accumulation of misfolded proteins.

The SERPINA1 gene which produces the AAT protein is highly pleiomorphic, with over 100 variants identified to date (DeMeo and Silverman 2004). Mutations which confer an increased risk

of developing pulmonary emphysema and/or liver disease are those in which deficiency alleles are combined in homozygous or heterozygous states, yielding AAT serum levels below a putative protective threshold of 11µmol/L. The most common variants associated with disease are the Z (Glu342Lys) and S (Glu264Val) mutations, caused by a single amino acid replacement of glutamic acid at positions 342 and 264 of the polypeptide respectively (Greene, Miller et al. 2008). The class of AAT variants termed "null" mutations lead to a complete absence of AAT production and while extremely rare, confer a particularly high risk of emphysema (Fregonese, Stolk et al. 2008).

AATD is an under-diagnosed condition with most cases misdiagnosed as COPD or non-responsive asthma. As a result, long delays between presentation of first symptoms and correct diagnosis are commonplace (Stoller, Sandhaus et al. 2005). Guidelines issued by both the World Health Organisation and the American Thoracic Society/European Respiratory Society recommend the establishment of targeted screening programmes for the detection of patients with AATD (1997; 2003). In addition, while a large number of cohorts have been investigated, many of these studies were based on screening symptomatic patients, and performed on small groups of less than 500 individuals with an accompanying high risk of error.

Apart from a few notable exceptions, such as the Swedish neonatal screening study (Sveger 1976), the lack of large population based studies using random sampling means the true prevalence of AATD in most European countries remains unknown. To date no studies have been performed to investigate the prevalence of AATD in Ireland. To address the paucity of data relating to AATD in the Irish setting, we analysed 1,100 individuals taken at random from the general population for the Z and S mutations.

#### METHODS

##### Subjects

A total of 1,100 individuals were screened from the Trinity Biobank DNA collection at St. James's Hospital, Trinity College Dublin (TCD). The Trinity



Biobank is a national buccal swab DNA collection selected at random from the electoral register. Isocode paper kits were provided to the Economic and Social Research Institute (ESRI) random-sampling service together with an explanatory brochure and prepaid envelope addressed to the Biobank. The kits were posted to potential donors and returned anonymously to the Biobank.

**Genotyping**

Genotyping was performed using a LightCycler 480 System (Roche) with specific primers and probes (Metabion) for the S and Z mutations as described in a previous publication (Rodriguez, Jardi et al. 2002).

**Data elaboration and statistical analysis**

The prevalence and numbers of genotypes in the Irish population was calculated by applying the Hardy-Weinberg principle.

**RESULTS**

Frequency of S and Z alleles in a random sample of the Irish Population

In the Trinity Biobank collection, 113 MS heterozygotes, 46 MZ heterozygotes, 2 SS homozygotes, and 2 SZ compound heterozygotes were identified (Figure 3.1). This data yields a frequency of 0.0541 for the S allele and 0.0218 for the Z allele in the Irish population.

Assuming Hardy-Weinberg equilibrium and based on a population of 4.24 million inhabitants (Census of Ireland 2006, [www.cso.ie](http://www.cso.ie)) these allele frequencies yield 2,015 ZZ individuals, 10,001 SZ individuals and 12,409

SS individuals (Table 3.1). Thus, the estimated prevalence of severe AATD (ZZ homozygotes) in Ireland is 1/2104. In addition to ZZ AATD, the estimated prevalence of intermediate AATD (SZ compound heterozygote) is 1/424, with this phenotype also at increased risk of lung and liver disease, while the estimated prevalence of mild AATD (SS homozygote) is 1/341. Finally, in terms of carriers, the calculated S and Z allele frequencies yield 170,832 MZ heterozygotes and 423,947 MS heterozygotes. The estimated prevalence of carriers is 1/24 for MZ and 1/10 for MS. The high prevalence of MZ heterozygotes is assuming greater importance as studies are underway to delineate whether the MZ phenotype is an independent risk factor for developing COPD.

Genotype	Prevalence [% , 95% CI]	Nos. in Irish Population
MS	1/10 [10.00%, 9.70-10.30%]	423,947
MZ	1/24 [4.17%, 4.00-4.40%]	170,832
SS	1/341 [0.29%, 0.20-0.40%]	12,409
SZ	1/424 [0.24%, 0.23-0.25%]	10,001
ZZ	1/2,104 [0.05%, 0.04-0.06%]	2,015

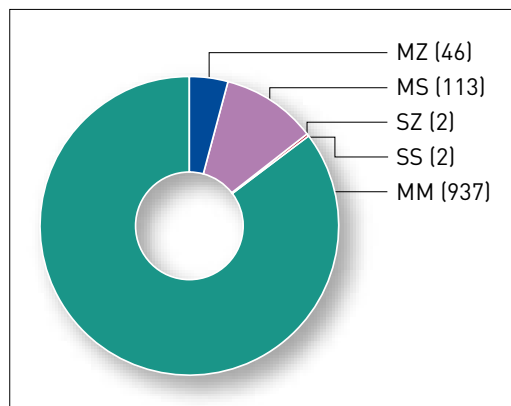
**TABLE 3.1:** Estimated AAT genotype prevalence and predicted numbers in Irish population of 4.24 million (Census 2006, [www.cso.ie](http://www.cso.ie)).

**DISCUSSION**

Our study describes for the first time the prevalence of AATD in a randomly selected sample of 1,100 individuals from the Irish population. In the general population the S mutation occurs at a frequency of 0.0541, while the Z mutation occurs at a frequency of 0.0218, meaning 10% of the Irish population carries a copy of the S allele, with 4.17% of the population carrying a copy of the Z allele. Combining the two, almost 15% of the Irish population possesses one copy of either S or Z mutation, yielding an overall carrier rate of approximately 1 in 7.

The S allele is associated with a mild plasma deficiency of AAT and S AAT protein is less

**FIGURE 3.1:** Analysis of AAT mutations in Ireland. 1,100 DNA samples in the Biobank collection were genotyped for the S and Z mutations.



polymerogenic than the Z form (Mahadeva, Chang et al. 1999), with the S allele only becoming clinically relevant when co-inherited with Z or other deficient alleles. For example, the SZ genotype is a significant risk factor for COPD (Holme and Stockley 2009) and liver disease (Sveger 1976), while the risk of COPD due to the MS genotype is not substantially elevated (Dahl, Hersh et al. 2005).

Throughout Europe the frequency of the S and Z mutations varies widely between countries, geographic regions, and ethnic groups. Approximately 6% of northern Europeans carry the S allele and 3-4% carry the Z allele. The highest frequency of the S allele is found in the Iberian Peninsula with a mean gene frequency of 0.0564, and decreases along a southwest to northeast axis, suggesting the mutation is likely to have arisen in the region. Placing our results in a European context, we observe that the frequency of 0.0541 for the S mutation in Ireland is among the highest in Europe, and similar to the Iberian Peninsula. The frequency of the Z variant is highest in northern and western European countries with a mean gene frequency of 0.014, and in contrast to S, the distribution of the Z allele gradually decreases along a north-west to south-east gradient (Luisetti and Seersholm 2004). Similar to the S allele, the frequency of 0.0218 for the Z allele in the Irish population is also among the highest in Europe. Moreover, it is worth considering the gene frequencies described in the Biobank cohort may be under-estimated as the genotyping methods employed can only identify S and Z alleles. Other more rare AAT mutations might also be present in this population.

The high prevalence of AATD in Ireland is not without precedent. Ireland has the highest incidence of cystic fibrosis (Devaney, Glennon et al. 2003) and haemochromatosis (Byrnes, Ryan et al. 2001) in Europe, as well as high frequencies of other genetic diseases (Mattiangeli, Ryan et al. 2006). This can be partly explained by the geographical isolation of an island on the fringes of Western Europe, with the genetic background of the population remaining largely undisturbed by the demographic movements that have prevailed on

mainland Europe. The Z allele is thought to have arisen from a single origin 66 generations or 2,000 years ago (Cox, Woo et al. 1985). The high frequency in southern Scandinavia suggests that the mutation arose in the Viking population and was distributed across northern Europe by Viking raiders between 800 and 1200 AD. The relatively high frequency of the Z allele in the Irish population may represent a Viking genetic footprint resulting from significant settlement in Ireland in the period from 800 to 1200 AD when large towns and urban centres were established by Viking settlers including modern Dublin, Limerick and Cork (Duffy 2000).

The relatively high frequency of the S mutation could suggest that the tribes who first settled on Irish shores may have migrated from the Iberian Peninsula. The S mutation is thought to have first arisen in the north of the Iberian Peninsula and subsequently spread throughout Europe during mass migration (Luisetti and Seersholm 2004). For example, one of the highest reported frequencies of the S allele in Europe is in the region of Galicia in north-western Spain (Carracedo and Concheiro 1983), and in general high S frequencies are found all along the western Atlantic seaboard (Luisetti and Seersholm 2004). Other genetic similarities have been described that suggest a shared ancestral heritage among the populations on the Atlantic façade of Europe, stretching from northern Iberia to western Scandinavia and dating back to the end of the last Ice Age (McEvoy, Richards et al. 2004).

Another intriguing reason for the high incidence of AATD in European populations that has been postulated is that the S and Z mutations confer a survival advantage on heterozygotes, of particular relevance in the pre-antibiotic era (Lomas 2006). Polymers of Z AAT protein have been found in lung lavage and shown to act as neutrophil chemoattractants (Mulgrew, Taggart et al. 2004), and an enhanced inflammatory response has been demonstrated in MZ heterozygotes (Malerba, Ricciardolo et al. 2006). The proposed hypothesis suggests the Z and S alleles favour the generation of polymers at sites of inflammation and these polymers help focus and amplify the host inflammatory



response to eradicate invading infectious organisms.

In summary, the findings of our study have significant consequences. We show that AATD is twice as prevalent as previously estimated in Ireland (Luisetti and Seersholm 2004), with over 2,000 ZZ and 10,000 SZ individuals at significantly increased risk of developing lung and liver disease. A further 170,000 MZ heterozygotes are estimated in the Irish population and this patient group may also be at risk of developing COPD, particularly in individuals who smoke (Hersh, Dahl et al. 2004). The continuing lack of awareness and under-diagnosis of this condition is alarming considering the high numbers of individuals at risk due to deficient SERPINA1 mutations. 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, (4) consideration of occupational hazards and environment as exposures to some occupational dusts and vapours can accelerate pulmonary decline, and (5) significant economic benefits arising from the reduced burden on health providers (Hogarth and Rachelefsky 2008).

Finally, it is clear from the data presented here that the statement "AATD is not a rare disease but a disease that is rarely diagnosed" is even more apt in the Irish setting (de Serres 2003). 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.

**NOTE:** This research forms part of a manuscript submitted for publication in September 2010.

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## 4. The National Targeted Detection Screening Programme

### TESTING

WHO (World Health Organisation) guidelines, ATS/ERS (American Thoracic Society and European Respiratory Society) 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, based in the RCSI Education and Research Centre at Beaumont Hospital. 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 for DNA isolation and analysis for AAT mutations.

Our principal diagnostic method using serum samples from suspected AATD individuals employs the Hydragel 18 AAT isofocusing kit (Sebia). This is designed for the qualitative detection and identification of the different phenotypes of alpha-1 antitrypsin (AAT) circulating in human blood (Figure 4.1A). The procedure involves isoelectric focusing on agarose gel, performed on the semi-automatic HYDRASYS system, followed by immunofixation with anti-AAT antiserum. The assay is carried out in two stages. Firstly, isoelectrofocusing on agarose gel is used to separate the proteins in serum samples from suspected AATD individuals. This is followed by immunofixation with enzyme-labelled anti-AAT antiserum to identify the various phenotypes of AAT. This method has been found to be highly specific, rapid and simple to perform (F. Zerimech *et al*, Clinical Chemistry and Laboratory Medicine 2008). It represents a more accurate method of screening for AATD and improves the identification of not only the most common phenotypes but also various rare AAT phenotypes. This year we are delighted to announce generous funding from the Alpha-1 Patient Support Group for the payment and maintenance of this equipment.

The DNA genotyping system has been developed to detect the two mutations (S and Z) responsible for over 95% of all cases of AATD (Figure 4.1B). After a short questionnaire is filled out for each patient, a lancet is used to obtain

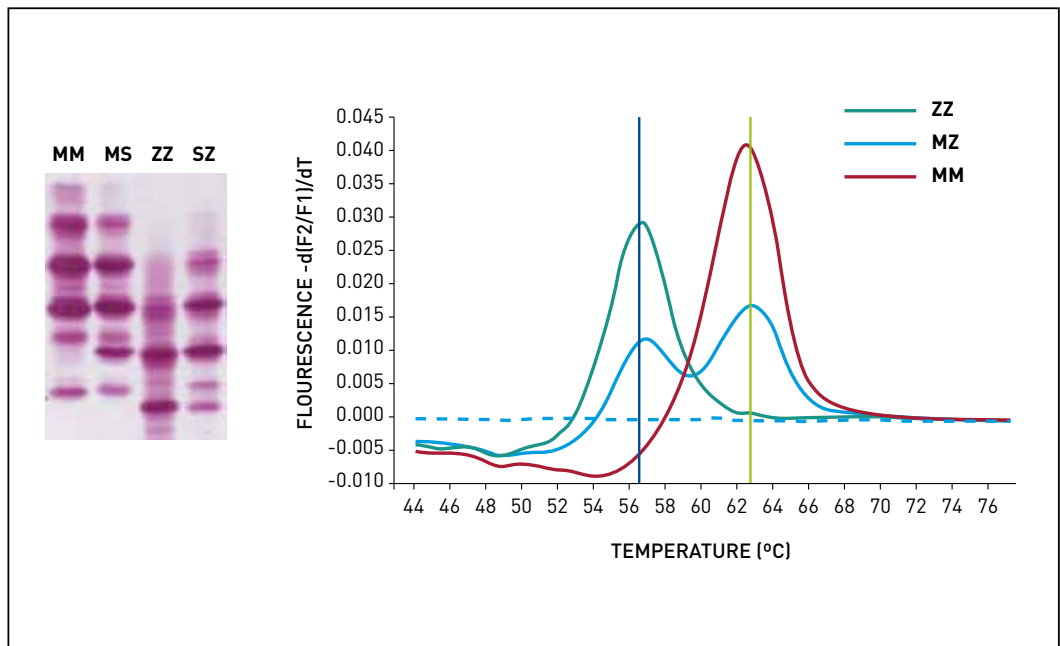
a small blood sample which is collected on specially treated filter paper. DNA isolated from this paper is then used to genotype the patient by RT-PCR (Real-Time Polymerase Chain Reaction), using primers and probes specific to each mutation. The major advantage of the genotyping method is that the ease of sample collection and storage has allowed for self-testing in the home, and the finger-prick kit test is particularly suited to family screening (see the DBS diagnostic algorithm in Figure 4.2).

As of September 2010 we are pleased to announce that all determination of AAT levels by the Alpha One Foundation is performed in collaboration with Dr. Bill Tormey, Consultant Chemical Pathologist and the Department of Chemical Pathology in Beaumont Hospital. The measurement of AAT levels is performed on the BN II nephelometer (Dade-Behring), a fully automated system for plasma protein determinations. The Department of Chemical Pathology has recently attained CPA accreditation so this means that all our AAT measurements are performed to the highest internationally accepted standards.

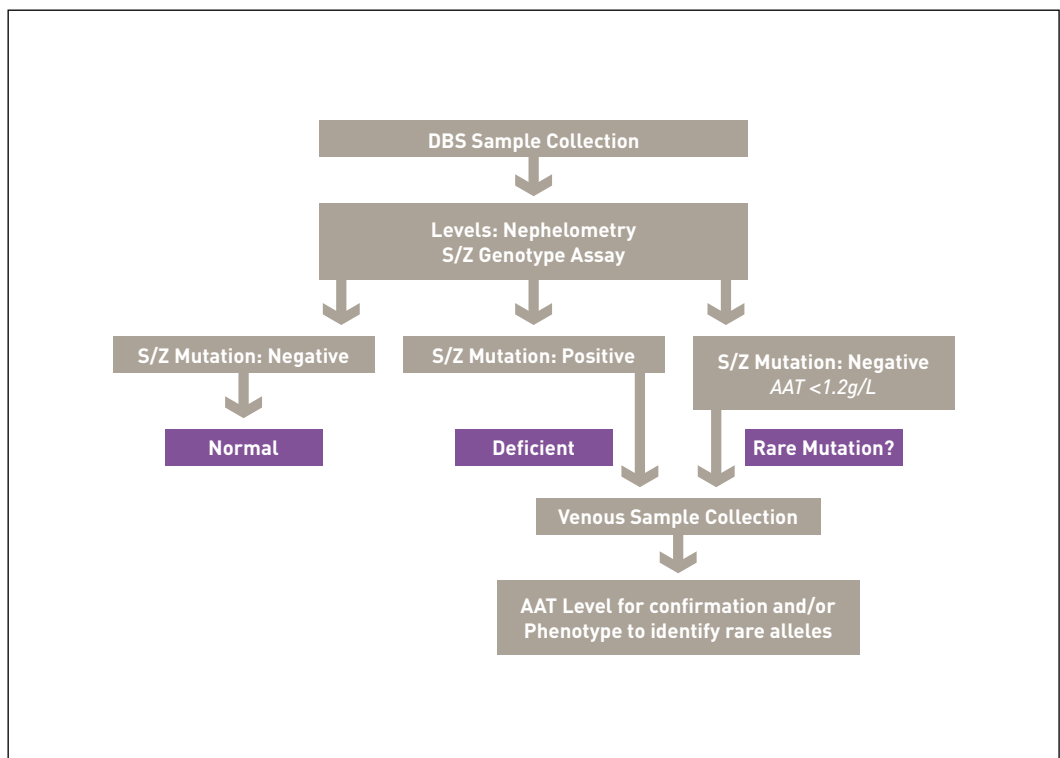
### RESULTS TO DATE

So far almost 5,000 individuals with COPD, asthma, liver disease or first-degree relatives of known AATD individuals have been screened in our national targeted detection programme since its inception in May 2004. A total of 72 ZZ (severe AATD) individuals have been identified but we have also detected 77 SZ, who are also at risk of developing lung and liver disease (Figure 4.1). In addition, a large number of other clinically significant phenotypes have been detected including 27 SS, 745 MZ, 465 MS, 24 MI, 8 IS, and 3 IZ phenotypes. The percentage of deficiency alleles (almost 25%) detected has been very high and the S variant, more prevalent in the Iberian Peninsula, has been detected with an unusually high frequency. Several rare AAT mutations were also identified in the Irish population, including I, F, V, X<sub>christchurch</sub>, Z<sub>bristol</sub>, and M<sub>malton</sub> and further analysis will reveal whether these phenotypes predispose individuals to lung or liver disease. The main outcome of this screening programme is that diagnosed

**FIGURE 4.1:** Methods employed for diagnosis of AAT mutations. A. Typical isoelectric focusing gel for AAT phenotype identification. B. Genotyping assay for the Z mutation.



**FIGURE 4.2:** Diagnostic algorithm used for genotyping DBS samples. It must be remembered that any diagnosis can only be confirmed by combining both AAT level and genotype.



individuals have the chance to receive proper treatment and management of their hitherto undiagnosed condition and are offered fast referrals to our dedicated Alpha-1 clinic in Beaumont Hospital.

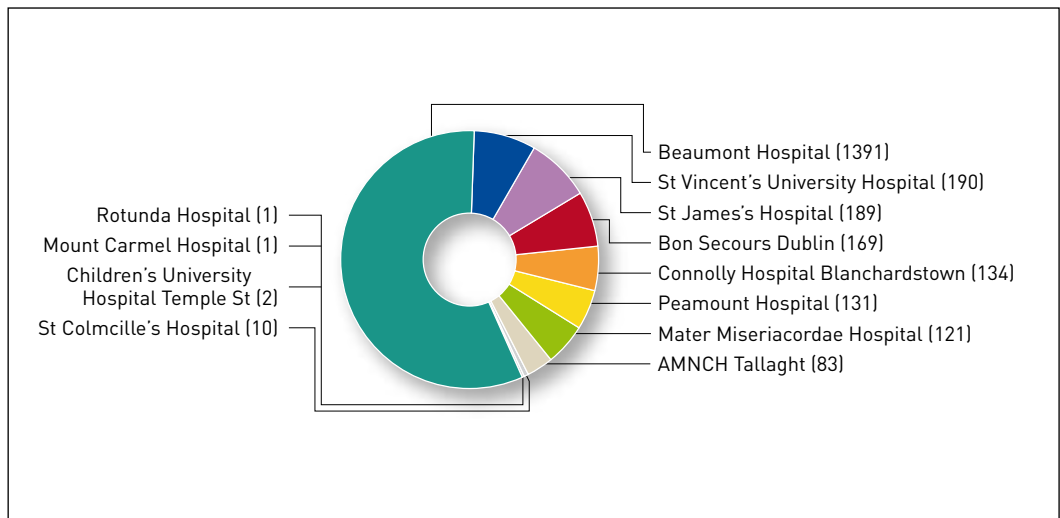
The current total of individuals screened in the Targeted Detection Screening Programme to date is 4291 from Hospitals nationwide, with a further 532 individuals tested as a result of family screening. Samples are received from the majority of the hospitals in Ireland as well as GP requests. In the Dublin area more than half of all samples are from Beaumont Hospital (58%) (Figure 4.3), but this is partly explained by Beaumont being the only referral centre in the first two years of the programme. Since then the targeted detection screening programme has been extended nationally.

The targeted detection screening programme has received 1869 samples nationally (excluding Dublin), with 31% of samples were received from Cork University Hospital (CUH), our largest referral centre (Figure 4.4).

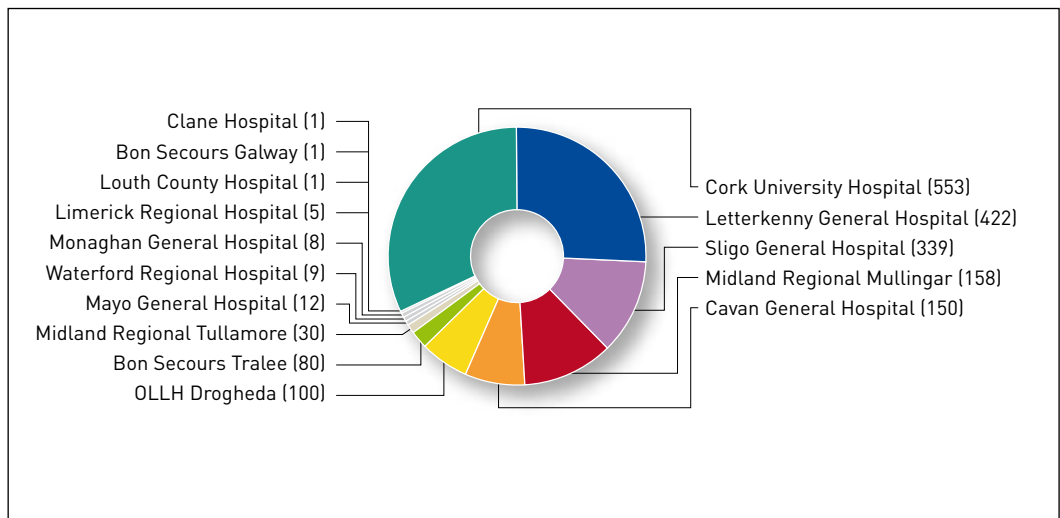
<b>Dublin</b>	
Beaumont Hospital	1391
St Vincent's University Hospital	190
St James's Hospital	189
Bon Secours Dublin	169
Connolly Hospital Blanchardstown	134
Peamount Hospital	131
Mater Misericordiae Hospital	121
AMNCH Tallaght	83
St Colmcille's Hospital	10
Children's University Hospital Temple St	2
Mount Carmel Hospital	1
Rotunda Hospital	1
<b>Total</b>	<b>2422</b>

<b>Nationwide</b>	
Cork University Hospital	553
Letterkenny General Hospital	422
Sligo General Hospital	339
Midland Regional Mullingar	158
Cavan General Hospital	150
OLLH Drogheda	100
Bon Secours Tralee	80
Midland Regional Tullamore	30
Mayo General Hospital	12
Waterford Regional Hospital	9
Monaghan General Hospital	8
Limerick Regional Hospital	5
Clane Hospital	1
Bon Secours Galway	1
Louth County Hospital	1
<b>Total</b>	<b>1869</b>

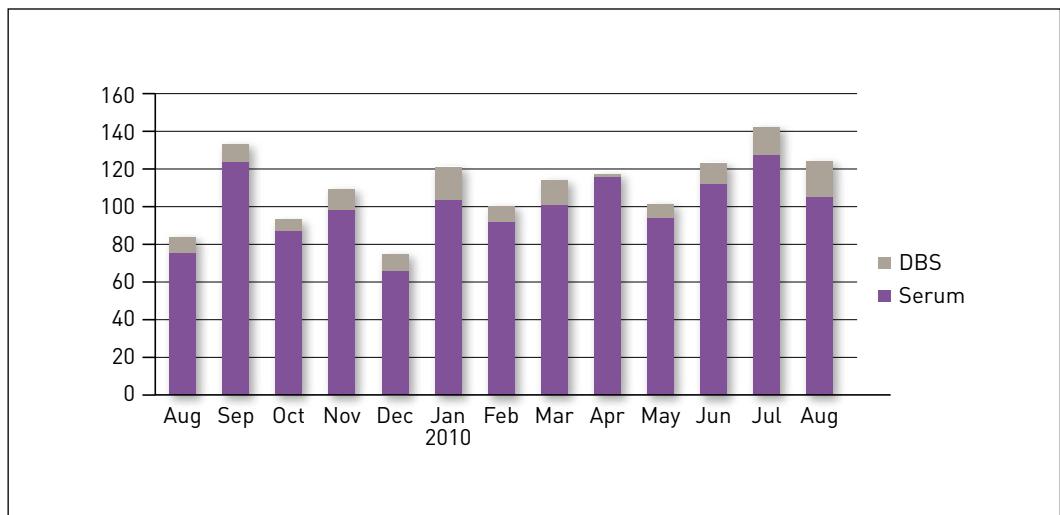
**FIGURE 4.3:** Samples received from Dublin Hospitals



**FIGURE 4.4:** Samples received from National Hospitals (excluding Dublin)



**FIGURE 4.5:** Samples Received from August 2009-August 2010





**FIGURE 4.6:** Sample of a typical Alpha-1 Antitrypsin Deficiency Report

<b>Alpha-1 Antitrypsin Deficiency Report - 0722</b>													
<b>Report Details</b>													
<b>Patient ID:</b>	2085												
<b>Name:</b>	0722,												
<b>Gender:</b>	Female												
<b>Date Of Birth:</b>	Unknown												
<b>MRN:</b>	Not Available												
<b>Laboratory Number:</b>													
<b>Consultant:</b>	Dr UK NEQAS												
<b>Hospital:</b>													
<b>Date Sample Taken:</b>	09/10/2007												
<b>Phenotype:</b>	SZ												
<b>Phenotype Level:</b>	g/L (Normal range 1.0 - 2.2g/L)												
<b>Phenotype Percent:</b>	%												
<b>All data reported by Dr. Tomás Carroll</b>													
<b>NOTE:- Under the terms of Disability Act 2005 part 4, section 2: it is ILLEGAL to disclose any information gained by genetic testing (such as this) for the purpose of insurance, assurance, pension, mortgage etc.</b>													
<b>Alpha-1 Antitrypsin (AAT) Deficiency</b>													
<p>AAT-deficient patients are at high risk of developing life-threatening lung and liver disease. The normal AAT protein is the M variant, synthesised in the liver and present in sufficient amounts to provide a protective anti-protease screen in the lung. The most common severely deficient variant is the Z protein, causing decreased circulating levels of AAT. The Z variant folds incorrectly and polymerises in the liver, preventing its release with concomitant reduced blood and lung levels. Z AAT accumulation can also cause liver disease. Patients with the ZZ phenotype typically have 5-15% of normal AAT levels, while MZ patients (1 normal, 1 deficient) have 50-80% of normal AAT levels. Another less severe deficient variant is the S protein. SS homozygous patients are predisposed to developing emphysema but not liver disease, with MS carriers (1 normal, 1 deficient) possessing almost normal AAT levels. Another possible phenotype is SZ (2 deficient AAT variants) with circulating AAT levels decreased to 25-40% of normal, and these patients have an increased risk of lung or liver disease. There are at least 20 other rare variants of the AAT protein, such as I, F, and null variants, which confer varying degrees of deficiency.</p> <p>The most important thing to remember is that cigarette smoke is the single biggest risk factor for Alpha-1 patients in the development of emphysema.</p>													
	<table border="1"> <thead> <tr> <th></th> <th><b>Phenotype</b></th> <th><b>What Does It Mean?</b></th> </tr> </thead> <tbody> <tr> <td><b>Normal</b></td> <td>MM</td> <td>Does not have the disorder and does not carry any altered AAT genes</td> </tr> <tr> <td><b>Carrier</b></td> <td>MZ/MS</td> <td>Mild to moderate AAT deficiency - may develop disease symptoms and does carry an altered AAT gene</td> </tr> <tr> <td><b>AAT Deficiency</b></td> <td>ZZ/SZ/SS</td> <td>Moderate to severe AAT deficiency - will develop disease eventually and does carry two altered AAT genes</td> </tr> </tbody> </table>		<b>Phenotype</b>	<b>What Does It Mean?</b>	<b>Normal</b>	MM	Does not have the disorder and does not carry any altered AAT genes	<b>Carrier</b>	MZ/MS	Mild to moderate AAT deficiency - may develop disease symptoms and does carry an altered AAT gene	<b>AAT Deficiency</b>	ZZ/SZ/SS	Moderate to severe AAT deficiency - will develop disease eventually and does carry two altered AAT genes
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Alpha One Foundation, RCSI Building, Beaumont Hospital, Dublin 9 Tel +353 1 8093871 Fax +353 1 8093809 Email: alpha1@rcsi.ie Web: www.alpha1.ie Participant in the UK NEQAS pilot scheme for AAT phenotyping													

## 5. Rare Alpha-1 Antitrypsin Mutations in the Irish Population

**The two most common mutations** associated with AATD are the Z and S mutations. Together, these two clinically significant variants are responsible for over 95% of all cases of lung and liver disease in Alpha-1 individuals.

It is therefore not surprising that the majority of tests used to diagnose AATD are designed to detect the Z and S mutations. However, there are over 100 other rare mutations found in the AAT gene and a number of these rare mutations have been discovered in the Irish population (Table 5.1). It is precisely because these mutations are so rare that difficulties arise in their correct identification and subsequent diagnosis of suspected AATD individuals.

Mutation	Molecular Basis	Cases	Cellular Effect	Disease Association
I	Arg39Cys	35	Intracellular accumulation	Lung & Liver
F	Arg223Cys	1	Reduced antiprotease activity	Lung
V	Gly148Asn	1	Reduced antiprotease level	Lung
M <sub>MALTON</sub>	Phe51 or 52	1	Intracellular accumulation & polymerisation	Lung & Liver
X <sub>CHRISTCHURCH</sub>	Glu363Lys	1	Reduced antiprotease level	Lung
Z <sub>BRISTOL</sub>	Thr85Met	1	Intracellular accumulation & defective glycosylation	Lung & liver

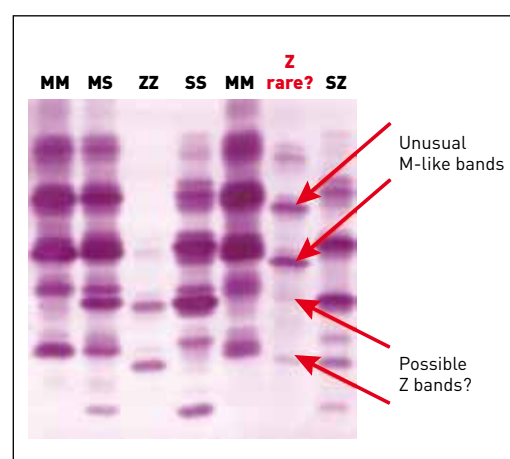
**TABLE 5.1:** AAT mutations identified to date in the Irish national AATD targeted detection programme.

### M<sub>MALTON</sub>: A DIAGNOSTIC JOURNEY TO A RARE AAT MUTATION

Ireland's position on the edge of Western Europe means our genetic background has remained undisturbed for centuries, and in terms of genetic diseases we have the highest incidence of cystic fibrosis and of haemochromatosis in Europe. Therefore, there may be a high possibility of novel and rare AAT mutations in the Irish population. Here, we present an unusual case from the past year.

A male in his 6th decade presented with a history of recurrent respiratory infections and very low AAT level (0.21 g/L). The individual was a lifelong non-smoker but had reduced lung function (FEV<sub>1</sub> = 44%). However, he had a lifelong occupational exposure to lung irritants. An AAT level of 0.21 g/L is normally suggestive of the ZZ phenotype so a number of assays were carried out for confirmation.

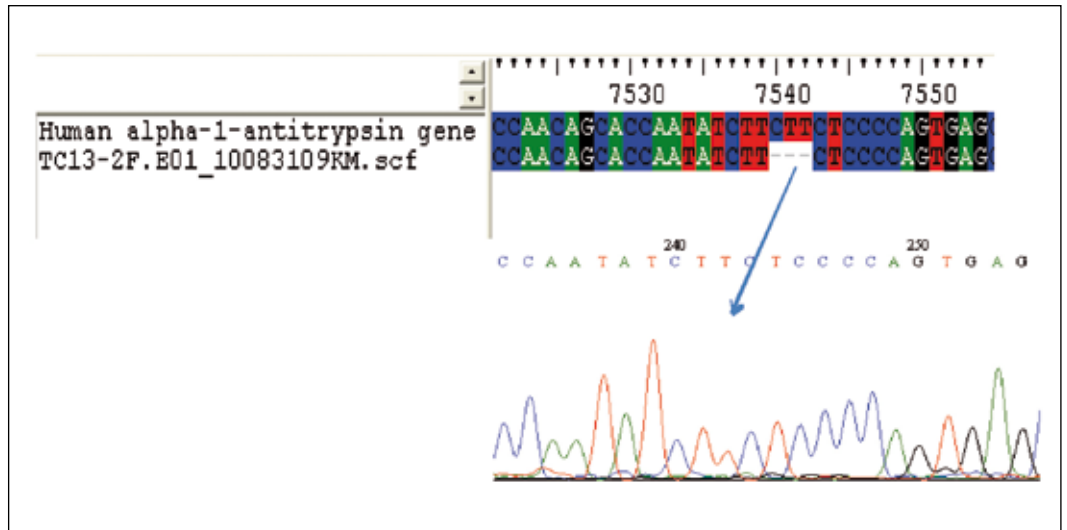
We performed isoelectric focusing (IEF) on serum from the individual and an unusual pattern was observed (Figure 5.1). The presence of a Z mutation was suspected, but another rare mutation was also present.



**FIGURE 5.1:** Unusual Mmalton phenotype detected in our IEF assay (Sebia Laboratories).

To confirm the presence of a rare mutation, DNA from the individual was analysed by sequencing the complete AAT gene. This complex procedure was kindly performed in the University of Pavia in Italy by Dr. Ilaria Ferrarotti and Prof. Maurizio Luisetti, and is the leading European laboratory in the identification of rare and novel AAT mutations. The individual was found to possess two copies (i.e. homozygous) of the very rare

**FIGURE 5.2:** DNA sequence analysis showing the deletion of 3 base pairs and confirming the presence of the  $M_{malton}$  mutation (University of Pavia).



$M_{malton}$  mutation (Figure 5.2). This mutation is caused by the deletion of three base pairs in the DNA sequence, and can be seen in the schematic where the normal AAT DNA sequence is aligned with DNA from the suspected individual.

The  $M_{malton}$  mutation causes the AAT protein to accumulate within liver cells, and this accumulation leads to the accompanying plasma deficiency. This in turn leads to a high risk of both lung and liver disease. In this regard,  $M_{malton}$  is very similar to the more common Z mutation. Interestingly,  $M_{malton}$  is found in high frequencies on the island of Sardinia in the Mediterranean.

This unusual case highlights the importance of a comprehensive diagnostic work up of all patients with low AAT levels. A low AAT level (< 1.2 g/L) indicates the presence of a mutation in the AAT gene and therefore should be phenotyped to identify the mutation(s). However, the rarer mutations besides the common Z and S often require the more comprehensive genetic analysis described above. In addition, these rare mutations can be missed or incorrectly identified as M using some of the more readily available commercial genotyping assays which only detect Z and S. On account of our collaboration with the University of Pavia, the Alpha One Foundation is in a strong position to diagnose any rare or novel AAT mutations in the Irish population.

## 6. National Alpha-1 Antitrypsin Deficiency Patient Registry

**The Alpha-1 registry** is a confidential database that collects information on patients with alpha-1 deficiency and alpha-1 carriers.

It gathers information regarding patient's alpha-1 level and genotype along with an individual's general health and how alpha-1 is affecting their livelihood. When patients attend the Alpha-1 clinic their permission is sought to allow their details to be entered into the Alpha-1 registry. Patients from 32 counties in Ireland have been included on the registry. This year we hope to increase patient numbers from other hospitals throughout the country.

In order to be included in the registry a patient must give their written consent which is collected on a consent form with the patient retaining a copy. Patients are provided with an information leaflet about the registry and can always withdraw their consent at a later date. This registry is a very important tool for clinical research, improving the care and treatment of Alpha-1 patients, and increasing the awareness of Alpha-1.

### WHO WILL PARTICIPATE IN THE ALPHA-1 REGISTRY?

All patients with Alpha-1 Antitrypsin Deficiency and Alpha-1 carriers treated in Ireland can participate in the registry. The more individuals that participate, the greater the quality of information available from the registry.

### WHAT IS THE REGISTRY?

The Alpha-1 registry is a confidential database, involving individuals diagnosed with Alpha-1 Antitrypsin Deficiency (Alpha-1) and individuals identified as Alpha-1 carriers. The registry was established in 2007 by the Alpha One Foundation.

### WHAT IS THE FUNCTION OF THE REGISTRY?

The registry's function is to improve our understanding of Alpha-1, promote the development and improvement of treatments, enhance patient care and management.

### WHO HAS ACCESS TO INFORMATION OF THE ALPHA-1 REGISTRY?

The registry database is based in the Alpha One Foundation in Beaumont Hospital. The database is encrypted so it cannot be accessed by anyone unless they have an encryption key. The database is also password protected. Staff of the Alpha One Foundation have individual passwords and are the only ones who can access the database.

### WHAT HAPPENS IF YOU GIVE YOUR CONSENT TO BE INCLUDED ON THE PATIENT REGISTRY?

Once you have given your consent a member of the Alpha One Foundation will have permission to look at your medical chart and transfer all relevant information to the secure registry. Not all information can be gathered from a patient's chart so certain questions may be asked by the consent taker when you give consent. These questions cover patient's family history for example if the patients close family members have been tested and if Alpha-1 has affected your job.

### WHAT HAPPENS IF YOU DON'T GIVE CONSENT?

Participation in the registry is voluntary. If you prefer not to be on the registry there is no penalty or change to your care.

### CAN I WITHDRAW FROM THE REGISTRY?

Any individual who is enrolled on the registry has the right to withdraw from the registry at any time. Any information on the individual captured on the registry will be removed.

For any further question or queries relating to the Alpha-1 Registry please contact:

**Geraldine O'Brien**, Research Scientist,  
Alpha-1 Suite, Beaumont Hospital, Dublin 9.  
Telephone: (01) 809 3871 Email: [alpha1@rcsi.ie](mailto:alpha1@rcsi.ie)

**Principle Investigator:** Prof Noel McElvaney,  
Department of Respiratory Medicine,  
Beaumont Hospital, Dublin 9. Tel: (01) 809 3764

### DEMOGRAPHIC CHARACTERISTICS

There are currently 164 patients on the Alpha-1 Patient Registry comprising ZZ, SZ, MZ, SS and MS genotypes. The largest patient group on the registry is the ZZ genotype of which 61%, (n = 49) are male and 39% (n = 31) are female. The mean age at diagnosis for ZZ patients was 44.87 +/- 1.99 years for males and 42.4 +/- 2.44 years for females (Table 6.1).

**TABLE 6.1: Demographic characteristics of ZZ patients on the Alpha-1 Registry**

	No	%
<b>Gender</b>		
Male	49	61.25
Female	31	38.75
<b>Initial Diagnosis</b>		
Symptomatic	47	58.75
Family	23	28.75
Other	10	12.5
<b>Smoking Status</b>		
Never	20	27.4
Ex-smoker	47	64.4
Current	6	8.2

### REASON FOR DIAGNOSIS

The initial reason for diagnosis of ZZ AATD individuals was analysed from the registry. Pulmonary symptoms 59%, and family screening 29% were the predominant reasons for diagnosis of ZZ AATD. Other reasons for diagnosis include liver disease, elevated liver function tests and panniculitis (Figure 6.1).

### HIGH RESOLUTION COMPUTERISED TOMOGRAPHY

High Resolution Computerised Tomography (HRCT) of thorax is performed on ZZ AATD individuals to analyse the structure of their lungs and possible lung disease. HRCT data was available on 71 ZZ AATD individuals. The predominant HRCT findings were 44% emphysema, 11% bronchiectasis and 4% fibrosis (Figure 6.2). 25% of ZZ AATD individuals had normal HRCT, of these 50% were symptomatic while the remaining were diagnosed via family screening (Figure 6.3). The diagnosis of symptomatic ZZ AATD individuals with normal HRCT were primarily due to low AAT levels, elevated LFTs, asthma and panniculitis. Family

screened ZZ AATD individuals show a reduced level of lung damage compared to symptomatic patients ZZ AATD patients (Figure 6.3).

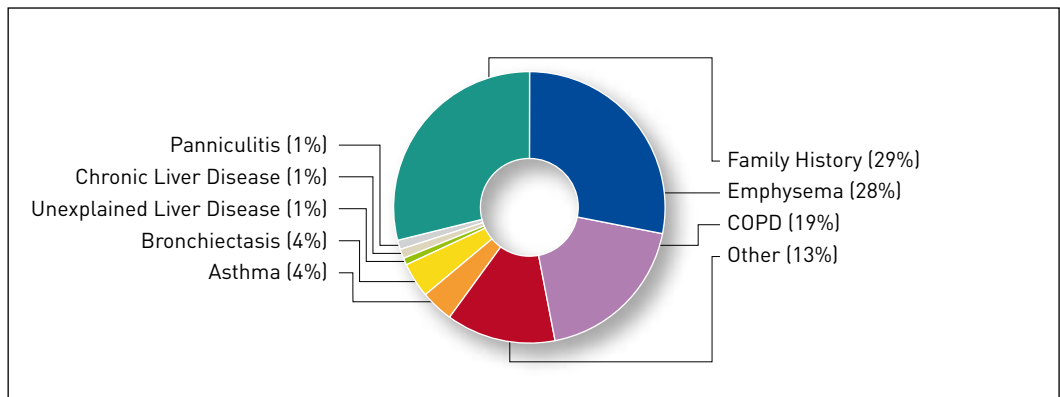
### PULMONARY FUNCTION TESTS

A Pulmonary Function Test (PFT) is a formal test carried out to measure lung function and is conducted on all patients. 'Forced Expiratory Volume in one second per litre' (FEV<sub>1</sub>) in terms of % predicted is one measurement which provides an indication of the condition of the lung.

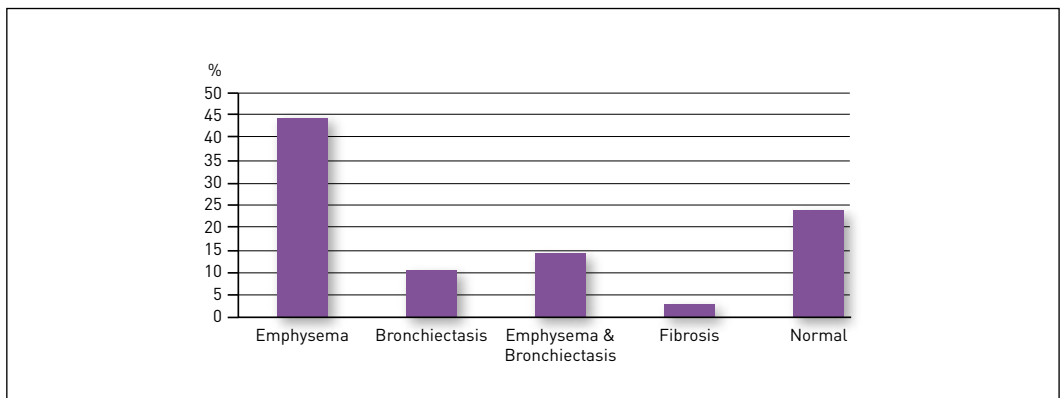
The main finding of our PFT data collection and analysis is that ZZ AATD individuals identified by family screening have significantly increased FEV<sub>1</sub> (85.3 +/- 6.5%) compared to ZZ AATD individuals identified by targeted screening (54.38 +/- 3.99%, *p* = 0.0008) (Figure 6.4).

ZZ AATD individuals that smoked had significantly decreased lung function compared to non-smoking ZZ AATD individuals (mean FEV<sub>1</sub> of ZZ smokers 50.42 +/- 3.9% v non-smokers 90.55 +/- 4.9%, *p* < 0.0001) (Figure 6.5).

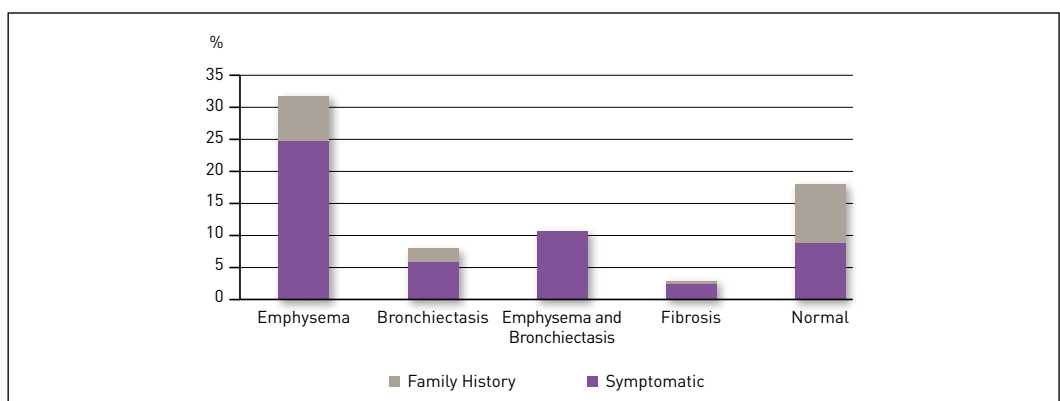
**FIGURE 6.1: Reason for the Diagnosis of ZZ patients**



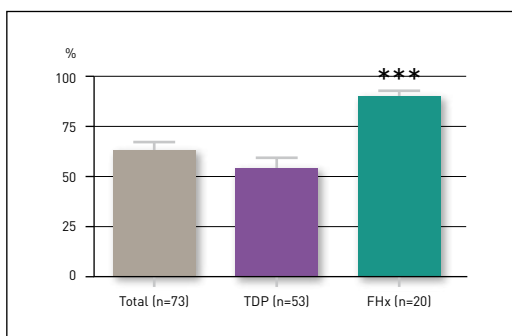
**FIGURE 6.2: HRCT scans of ZZ AATD individuals**



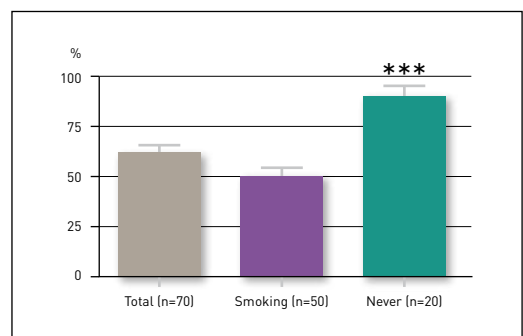
**FIGURE 6.3: HRCT scans of Symptomatic and Family Screened ZZ AATD individuals**



**FIGURE 6.4: FEV<sub>1</sub> of ZZ AATD individuals identified by symptomatic screening compared to family screening.**



**FIGURE 6.5 (Far Right): FEV<sub>1</sub> versus smoking status in ZZ AATD individuals**





## 7. Is there a Difference in Health Related Quality of Life between Family Screened Alpha-1 Antitrypsin Deficiency Individuals and Symptomatically Screened Individuals?

### AUTHORS

C O'Connor, Dr Z Moore, Prof NG McElvaney

### OBJECTIVE

The two methods of diagnosis of Alpha-1 Antitrypsin Deficiency (AATD) are symptomatic screening and family screening. The American Thoracic Society and European Respiratory Society Guidelines recommend family screening for all first-degree relatives of known AATD patients. The objective of this quantitative, cross-sectional study was to determine differences in Health Related Quality of Life (HRQoL) between family screening AATD patients and symptomatically screened individuals attending an Alpha-1 Clinic.

### METHOD

In total 35 AATD ZZ patients attending the Alpha-1 Clinic participated in this study. All participants were above the age of 18 years, and varied from 21 years to 69 years of age with a mean age of 49 years SD  $\pm$ 9 years. The patients recruited in the study were diagnosed as a result of family screening or symptomatic screening. The family screened group had n=15 individuals and the symptomatically screened group had n=20 individuals.

Data collected on all participants were related to age, gender, HRQoL (St. George Respiratory Questionnaire), number of respiratory exacerbations, vaccination uptake (Influenza, Pneumococcal, H1N1) smoking history, and spirometry (FEV<sub>1</sub>, FEV<sub>1</sub> % predicted, FEV<sub>1</sub>/FVC ratio). Data collection was carried out at a dedicated Alpha-1 Clinic. Data were collected over a three-month period from December 2009 to February 2010 and were analysed using descriptive and inferential statistics as appropriate.

### FINDINGS

#### 1. Health Related Quality of Life

The mean SGRQ 'symptoms' score in the family screened group was 32.45 SD 6.59, demonstrating that 'symptoms' have a 32% effect on HRQoL of AATD family screened individuals. The mean SGRQ 'impact' score for family screened patients was 21.78 SD 5.55. This indicates that AATD has a 21% 'impact' on HRQoL. The SGRQ 'activity' mean score was 43.98 SD 7.49. This demonstrates that 'activity' affects HRQoL by 43%. The 'overall' mean score of SGRQ in the family screened group was 30.79 SD 5.77, giving an 'overall' 30% effect on HRQoL (Figure 7.1).

The symptomatically screened group had a mean SGRQ 'symptoms' score of 53.50 SD 4.85, this representing a 53% affect of symptoms on HRQoL. The SGRQ 'impact' mean score in this group was 34.78 SD 4.52, meaning that AATD had a 34% 'impact' on HRQoL. The SGRQ 'activity' mean score was 62.02 SD 6.4, representing a 62% effect of 'activity' on HRQoL. The 'overall' mean score of SGRQ was 45.66 SD 5.03, showing an 'overall' 45% effect on HRQoL (Figure 7.1).

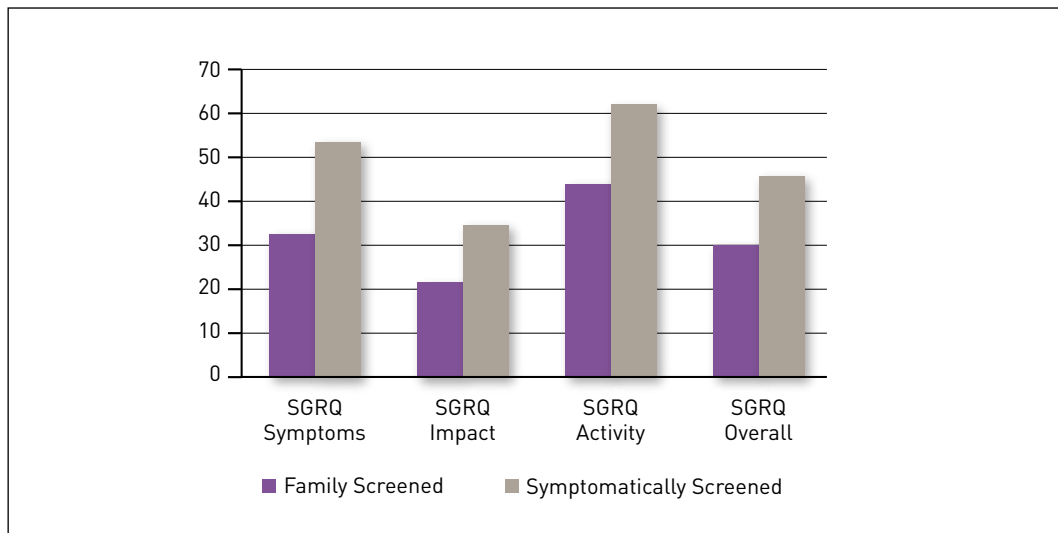
Results demonstrate a statistically significant difference in 'symptom' score between the groups (mean difference -21.05; 95% CI 37.31 to -4.78;  $p=0.013$ ).

#### 2. Spirometry

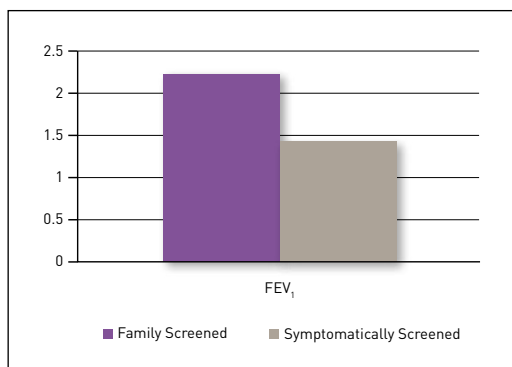
The mean FEV<sub>1</sub> in the family screened group measured 2.23 L SD  $\pm$ 1.10 L and the mean FEV<sub>1</sub> % predicted measured 72.93% SD  $\pm$ 32.49%. The FEV<sub>1</sub>/FVC ratio mean in this group measured 59.87% SD  $\pm$ 18.22%, indicating Stage II moderate lung disease (GOLD Guidelines, 2007).

The mean FEV<sub>1</sub> in the symptomatically screened group measured 1.44 L SD  $\pm$ 0.76 L and the mean FEV<sub>1</sub> % predicted in this group measured 47.90% SD  $\pm$ 26.76%. The FEV<sub>1</sub>/FVC ratio mean measurement in this group was 45.70% SD

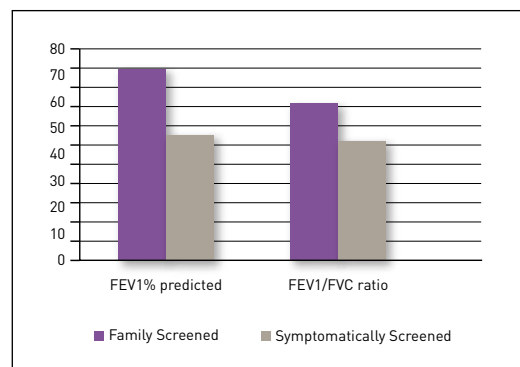
**FIGURE 7.1: SGRQ scores in Family Screened and Symptomatically Screened participants**



**FIGURE 7.2: FEV<sub>1</sub> in Family Screened and Symptomatically Screened participants**



**FIGURE 7.3 (Far Right): FEV<sub>1</sub> % predicted and FEV<sub>1</sub>/FVC ratio in family screened and symptomatically screened participants**



±18.31%. indicating Stage III severe lung disease (GOLD Guideline, 2007) (Figure 7.2 & 7.3).

An independent *t*-test identified a statistically significant difference in FEV<sub>1</sub> measurements between the two groups (mean difference 0.786; 95% CI 0.14 to 1.42; *p*= 0.018). In addition the independent *t*-test identified a statistically significant difference in FEV<sub>1</sub> % predicted between family screened patients and symptomatically screened participants, showed, mean difference 25.03; 95% CI 4.65 to 45.41; *p*= 0.018. In comparing the FEV<sub>1</sub>/FVC ratio between the two groups an independent *t*-test identified a statistically significant difference (mean difference 14.16; 95% CI 1.46 to 26.87; *p*= 0.03).

**CONCLUSION**

Family screened AATD individuals have better HRQoL compared to symptomatically screened

individuals. This is the first study investigating HRQoL within the Irish AATD population. Earlier diagnosis and intervention of AATD through family screening could potentially lead to reduced demands on respiratory services within the already overburdened Irish health care. It is hoped this study will contribute additional knowledge for respiratory community by providing evidence that family screening improves HRQoL.

**NOTE:** This research forms part of a thesis in a Masters in Nursing.

**REFERENCES**

Global Initiative for Chronic Obstructive Lung Disease, (2007) *Pocket guide to COPD Diagnosis, Management and Prevention: A guide for health care professionals*, Updated 2007, GOLD Guidelines, National Heart, Lung and Blood Institute and World Health Organisation.

## 8. The Alpha One Foundation – Pioneering research into Alpha-1 Antitrypsin Deficiency

(Beaumont Hospital, Connections, Staff Publication, April 2010)

**Alpha-1 antitrypsin (AAT)** is a protein produced by the liver. In most people, the protein does its job effectively and they never know about it. But those who are deficient in the enzyme might experience a range of distressing symptoms such as shortness of breath and wheezing and may develop emphysema in their 30s and 40s, sometimes even without ever having smoked.

The normal form of AAT is the MM form with the M gene coming from both parents. AAT travels from the liver throughout the body and specifically protects the lungs from the potentially destructive effects of naturally occurring enzymes which can, if unopposed, cause severe damage. Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder and Ireland has an unusually high level of sufferers. "AATD is not a rare disease but a disease that

is rarely diagnosed," says Professor Gerry McElvaney, professor of medicine at the RCSI and the director of the AATD programme at Beaumont Hospital. "Because its symptoms can look similar to a number of other recurrent respiratory illnesses, it tends to be significantly under diagnosed. Recent research in the US shows that it takes an average of six years from the time the symptoms first appear to accurate diagnosis. The Alpha One Foundation was established in 2001 to promote research into the condition and to improve its diagnosis and treatment. We are now one of the leading centres for AATD research in the world."

Men and women are equally affected by the disorder, which typically presents when people are in their 30s and 40s as a respiratory problem such as bronchitis or sometimes as asthma. Instances of the disorder are spread nationwide, and McElvaney says that being an island nation with a relatively small and still largely homogenous population may be the reason for the high incidence of the disorder here. Those with severe AATD get the abnormal Z gene from both parents and have what is known as the "ZZ" phenotype. The presence of this phenotype can be detected by a simple DNA test which is carried out in Beaumont Hospital, the National Centre for AATD. McElvaney estimates that there are up to 2,000 ZZ individuals in Ireland, although only 150 have been positively identified. In addition, there could be up to 200,000 people with the "MZ" type, meaning they are carriers of the disease. This group may also be susceptible to development of early emphysema. Around 600 MZs have

**BACK:** Prof Gerry McElvaney, Geraldine O'Brien (Research Scientist, Alpha One Foundation), Dr Tomás Carroll (Scientist, Alpha One Foundation), Kitty O'Connor (CEO Alpha One Foundation).

**FRONT:** Helen McHugh (Research Nurse) and Orla Keane (Patient) from Maynooth, County Kildare.



been identified by the Beaumont programme. "We're not unusual in under diagnosing the condition. It's the same wherever the disease exists and the majority of AATD individuals with emphysema are erroneously diagnosed as having chronic obstructive pulmonary disease (COPD) or non-responsive asthma," McElvaney says. "It is important to identify the disease in those who have it because it has implications for their families as well as for the management of their own health. We test family members for potential lung and liver complications and try to reach them before they start losing function. Those with Alpha-1 who smoke are almost certain to develop severe early-onset emphysema so it is important that we identify them and try to help them to stop smoking. We are currently working with around 25 hospitals on the programme in an effort to identify cases as early as possible." The national targeted detection programme for AATD was launched by the Alpha One Foundation in Beaumont Hospital in 2004. Funded directly by the Department of Health, the programme provides free testing to patients with COPD, non-responsive asthma and cryptogenic liver disease and to relatives of AATD patients. If someone is diagnosed with the disorder, the Foundation provides a range of ancillary services such as counseling, expert advice and opportunities to enroll in clinical trials and to join the Alpha-1 patient support group. Since November last year, those diagnosed with the condition have been fast-tracked to a dedicated clinic at Beaumont, where they receive appropriate therapy.

Four people work directly on the Alpha-1 project: its director Professor McElvaney, chief executive Kitty O'Connor, senior scientist Dr. Tomás Carroll, and technician Geraldine O'Brien. In addition, the project is supported by a team of 14 research scientists who work with Professor McElvaney on a number of respiratory disorders, including Alpha-1 and Cystic Fibrosis.

The project's financial support comes from the State and through grant aid from agencies such as the Medical Research Charity, the Science Foundation Ireland, and the American Alpha-1 Foundation and from occasional philanthropic donations. However, Professor McElvaney says the Foundation must constantly fundraise to

fund its research and other activities. Being diagnosed with AATD naturally comes as a shock for people, but Professor McElvaney says it can also be a relief as it explains their symptoms. "It can be very frightening for someone who is still relatively young to find themselves with breathing problems," he says. "AATD is the fourth most common cause of lung transplantation. It is an aggressive disease but if we are able to treat people at an early stage it would have the added benefit of freeing up lung transplantation slots."

The Alpha-1 team is at the cutting edge in the research and development of pioneering treatments for Alpha-1. Its research has already been successfully translated from the laboratory to the patient, and Professor McElvaney says the high calibre of the team's members is key to this. "Our researchers rank up there with the best in the world," he says. "We have critical mass within the department which is very important in terms of being able to bounce new ideas off each other and there are spin offs from their research into other areas. For example, it is giving us an insight into what causes emphysema in non-AATD patients."

The team is currently the leading centre for a trial on a group of ZZ AATD patients being given a weekly intravenous infusion (at home) of the normal MM protein to see if this can raise their levels of AAT sufficiently high to protect the lung. "We have proved that this works biochemically but need to support that data with clinical studies to show we can stabilise lung function and reduce the number of respiratory exacerbations. We can assess this through a variety of ways, including regular CT scans," Professor McElvaney says. Within the next few months, the team hopes to start a gene therapy study in association with colleagues in the US, and it is also leading the way in the development of an aerosol, which will work along similar lines to an asthma inhaler, to deliver noninvasive treatment to those with Alpha-1 antitrypsin deficiency.

## 9. Research Studies/Programmes

### CLINICAL TRIALS

Alpha-1 Augmentation Therapy Clinical trial: This study is being conducted in Beaumont Hospital by Professor McElvaney and his team.

This is a placebo-controlled, double blind, multicentre phase III / IV study to compare the Efficacy and safety of the drug Zemaira® in patients with Emphysema due to Alpha 1 proteinase inhibitor deficiency. The duration for each patient is 2 years.

So far there has been 22 patients recruited onto this study and they are all at various stages in the trial. The trial involves having weekly intravenous infusions of Zemaira®, an Alpha-1 proteinase inhibitor or a Placebo which is a dummy treatment that looks like the real thing but is not. As the study is Double-Blinded, neither the participating patients nor our study staff know which therapy has been assigned to them. There is equal chance of receiving either treatment. As of August 2010 we have 9 patients who have continued onto the extension phase of the study. This is where each patient receives Zemaira® for at least two years.

The infusions are given either in Beaumont Hospital or in the patient's own home and take on average 20 minutes once a week.

Every three months patients are required to attend Beaumont hospital so that routine tests can be carried out. These include:

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

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

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

- Diagnosis of Alpha-1 antitrypsin deficiency.
- Non smokers or Ex-Smokers who have stopped at least 6months prior to screening.
- Age range of 18 – 65 years of age, male and female.
- Emphysema with an FEV<sub>1</sub> of 35-70% predicted range.

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

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

**Grace Mullins,**  
Research Nurse,  
Study Co-Coordinator,  
RCSI Building  
Beaumont Hospital,  
Dublin 9.  
Tel: 01 8093864

### Clarification of the Risk of COPD in Alpha-1 antitrypsin (MZ) Individuals

**Funding Body:** Talecris Pharmaceuticals

**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 FEV<sub>1</sub> <50% predicted; FEV<sub>1</sub>/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.

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 PIMZ 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. Kevin Molloy**, MB, Bch, BAO  
Clinical Researcher  
Alpha One Foundation,  
RCSI Building,  
Beaumont Hospital,  
Dublin 9.  
Tel: +353-1-809-3976  
Mob: +353-86-776-3943  
Email: kmolloy@rcsi.ie

## GRANTS AWARDED

**Funding Body: US Alpha One Foundation, July 1, 2009 - June 30, 2011**

**PROJECT TITLE: AUTOANTIBODIES IN Z ALPHA-1 ANTITRYPSIN DEFICIENCY**

**Principal Investigator:** C Greene

**Other partners:** T Boon Low, RCSI (Co-applicant/Researcher), NG McElvaney RCSI (Co-applicant/Consultant), E Reeves RCSI (Co-applicant/Advisor), D Murphy RCSI (Collaborator).  
<http://www.alpha-1foundation.org/researchers/>

**Project Abstract:** Z Alpha-1 antitrypsin (ZAAT) deficiency is a genetic disorder that can affect either the lungs or liver. The liver disease arises as a result of accumulation of a misfolded protein (ZAAT) in liver cells whereas the lung disease occurs as a result of too little ZAAT in the airways. This 'deficiency' in the lungs allows proteases to cause damage to proteins in the lung. These damaged proteins, in turn, can lead to the generation of 'autoantibodies' which are likely to be important in the disease process. Previously autoantibodies have been shown to be important in smoking-induced emphysema – a disease that shares many similarities with ZAAT deficiency. Three sets of proteins in particular are likely to be damaged (i) elastin, (ii) collagen and (iii) neutrophil granule proteins. This project will search for and quantify autoantibodies against these three sets of protein in serum from individuals with ZAAT deficiency. Overall these studies will determine



whether ZAAT deficiency is a disease with an autoimmune component and will identify novel autoantibodies in ZAAT deficiency which will enhance our understanding of the mechanisms regulating disease severity in ZAAT deficient individuals and may point towards potential new treatments for the disorder.

**Funding Body: Medical Research Charities Group MRCG/2008/3, Oct 1, 2009-Sept 30, 2012**

**PROJECT TITLE: PI3 KINASE: A MOLECULAR TARGET OF Z ALPHA-1 ANTITRYPSIN?**

**Principal Investigator:** C Greene

**Other Partners:** T Carroll, RCSI (Co-applicant)  
<http://www.mrcg.ie/>

**Project Abstract:** 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 accumulation of a misfolded protein (ZAAT) within a compartment inside the liver cells. This 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. Individuals with ZAATD are more prone to viral infections of the liver due to the stress their condition puts on this organs. This project investigates the consequence of accumulation of misfolded ZAAT within cells on a key enzyme called PI3K; a central regulator of multiple events within cells. These studies will generate important new information regarding why liver disease occurs in ZAATD and may point towards potential new treatments for the disorder.

**Funding Body: Health Research Board (HRB)/ Medical Research Charities Group (MRCG), 3 years from January 2009**

**PROJECT TITLE: ANTI-INFLAMMATORY EFFECT OF ALPHA-1 ANTITRYPSIN ON THE PHAGOCYTTIC NEUTROPHIL.**

**Principal Investigator:** Prof NG McElvaney

**Co-applicant:** EP Reeves

**Project Abstract:** Alpha-1 antitrypsin (A1AT) is a secretory protease inhibitor produced primarily in the liver. The functional A1AT molecule is found in abundance within human plasma, with normal concentrations in the range of 20-53mmol/L. Despite its name, A1AT is the major physiological inhibitor of a range of serine proteases and within the lung it can protect the alveolar matrix from destruction by neutrophil elastase (NE) and thus maintains a protease-antiprotease balance. A1AT deficiency is a lethal hereditary disorder characterized by low plasma levels of A1AT and accumulation of the misfolded protein within hepatocytes and colangiocytes. Polymerised aggregates of A1AT are implicated in liver cirrhosis and chronic hepatitis and loss of natural anti-protease screen results in early onset and pathogenesis of emphysema. A clear understanding of the molecular mechanisms that regulate inflammation in the lung in A1AT deficiency is a priority.

The anti-inflammatory effects of A1AT are generally thought to be mediated by its anti-protease activity, however recent data indicate alternative functions. A1AT has been reported to inhibit neutrophil NADPH oxidase activity, control lipopolysaccharide (LPS)-induced cytokine and chemokine release in monocytes and regulate IgE and IgG4 production by human B cells. In addition, an *in vivo* murine study revealed that, A1AT can protect against TNFa or endotoxin induced lethality. The aim of this study is to understand the relationship between A1AT and the phagocytic neutrophil. We propose that neutrophil-associated A1AT exerts immunomodulatory activities and in the context of tissue homeostasis A1AT moderates neutrophil activation. Our preliminary data clearly show that A1AT is a genuine membrane/secretory vesicle protein of neutrophils, which is released from the cell immediately in response to physiologically relevant concentrations of pro-inflammatory cytokines with increased levels of molecular A1AT detected in the extracellular milieu. The relevance of A1AT binding to the neutrophil membrane will be investigated in respect to its ability to moderate the NADPH oxidase cascade of the circulating cell. Our initial studies have shown that A1AT exhibits

distinct inhibitory effects on fMLP receptor-mediated  $O_2^-$  production. Ensuing experiments will shed light on the signalling steps affected by A1AT. Experiments will centre on testing whether A1AT prevents targeting of the NADPH oxidase components p67<sup>phox</sup>, p47<sup>phox</sup>, p40<sup>phox</sup> and p21<sup>rac</sup> to the membrane bound flavocytochrome b<sub>558</sub> thereby averting oxidase activation. Alternatively, inhibition may occur via the cAMP pathway. Recently it has been shown that A1AT exerts *in vitro* anti-inflammatory activity in human monocytes by elevating intracellular cAMP. For this reason experiments will investigate whether the inhibitory effect of A1AT on fMLP-induced  $O_2^-$  production runs in parallel with altered intracellular cAMP levels.

There is a tremendous need to understand the molecular and cellular events that influence the course of lung disease within A1AT deficient patients. We propose to characterise in depth the anti-inflammatory effect of A1AT on neutrophil activity and study the implications of A1AT deficiency with respect to neutrophil regulation

This study will enhance and develop our knowledge of the role of the phagocytic neutrophil in pulmonary inflammation, and will not only provide a further understanding of the essential steps in neutrophil immunity but ultimately may lead to the identification of new therapeutic targets for A1AT deficiency.

**Funding Body:** US Alpha-1 Foundation, 2 years from June 2009

**PROJECT TITLE:** CAN REPLACEMENT THERAPY INFLUENCE THE NEUTROPHIL?

**Principal Investigator:** Prof NG McElvaney

**Co-applicant:** EP Reeves

**Project Abstract:** Individuals with severe hereditary alpha-1 antitrypsin (AAT) deficiency (AATD) are at risk of developing early-onset emphysema. Replacement (augmentation) therapy has become a standard treatment for lung disease associated with AATD. Clinical studies have shown that augmentation therapy is associated with a reduction in frequency and severity of lung infections and a marked

slow down in the course of lung deterioration. Neutrophils are the primary effector cells responsible for the pathological manifestations of AATD lung disease and for this reason the important translational research of this project will investigate the effect of AAT augmentation therapy on neutrophil function and activity. Our central hypothesis is that neutrophil membrane-associated AAT exerts immunomodulatory activities by sequestering neutrophil cellular processes. Our compelling preliminary data clearly show that AAT is a genuine outer membrane protein of neutrophils associated with lipid raft micro-domains via binding to a glycosylphosphatidyl-inositol (GPI) linked membrane protein. Physiological concentrations of AAT were found to modulate formyl-methionine-leucine-phenylalanine induced NADPH oxidase activity and interleukin-8 chemotactic responses of normal neutrophils. In contrast, neutrophils of AATD individuals illustrated enhanced NADPH oxidase and migratory activity, indicative of a primed or sub-activated cellular state. This innovative study will focus on the clinical relevance of AAT augmentation therapy and with respect to assigning an AAT anti-inflammatory role, will challenge the hypothesis that infused AAT in AATD individuals effectively binds circulating neutrophils *in vivo* efficiently modulating cellular activity. Technically we aim to combine a well developed clinical framework with quantitative proteomics specifically of neutrophil membranes pre- and post- AAT augmentation therapy and marry the results of the proteomic studies examining the proteins comprising the outer cellular membrane with modifications to cellular activity. The long-term objective of this research is to develop the means to control lung disease associated with AATD. The potential ramifications of AAT as a modulator of neutrophil activity will add a new understanding to the role of AAT in health and disease.

**Funding Body:** US Alpha-1 Foundation, 1 year from June 2010

**TITLE:** ALPHA-1 ANTITRYPSIN CONTROLS AKT & P47<sup>PHOX</sup> ACTIVATION

**Principal Investigator:** Prof NG McElvaney

**Co-applicant:** EP Reeves

**Project Abstract:** The hereditary condition of alpha-1 antitrypsin (AAT) deficiency (AATD) provides us with the most definitive evidence for the physiological and clinical importance of AAT. The protease-antiprotease imbalance theory was accepted as a reason for the pulmonary emphysema associated with AATD. However, activation of neutrophils sequestered in the AATD alveolar milieu can also cause the release of free radicals and reactive oxygen (ROS), increasingly regarded as key substances modulating epithelium dysfunction and disruption. These oxidants are generated by the neutrophil respiratory burst oxidase system that reduces molecular oxygen (O<sub>2</sub>) to superoxide (O<sub>2</sub><sup>-</sup>). In this study we aim to demonstrate using *in vitro* and *in vivo* models that AAT controls neutrophil oxidase activity by inhibiting Akt kinase activation. By analyzing clinically stable AAT deficient patients, homozygous for the Z allele, our preliminary data has shown that low serum and cell membrane associated AAT, leads to an increase in neutrophil O<sub>2</sub><sup>-</sup> production. Additionally, our *in vitro* results have shown that exogenous AAT inhibits neutrophil O<sub>2</sub><sup>-</sup> consumption in a dose dependent manner. The pioneering significance of this proposal lies in its potential to elucidate the signaling mechanism by which AAT modulates neutrophil oxidase activity. We hypothesize that AAT inhibits Akt activation, and as a result, phosphorylation and membrane translocation of the cytosolic protein p47<sup>phox</sup> is impaired, two essential steps involved in oxidase activation. This innovative study will focus on the clinical relevance of AAT augmentation therapy and will potentially increase the data assigning an anti-inflammatory role to AAT. The ultimate goal of this study is to investigate whether infused AAT in AATD individuals, modulates cellular oxidase Akt signaling mechanisms. The potential of AAT as a regulator of neutrophil activity will add a new understanding to the role of AAT in health and disease.

#### CONFERENCE PRESENTATIONS:

**44th Annual Scientific Meeting of the European Society for Clinical Investigation, Bari, Italy 24 - 27 February 2010**

**Category:** Oral presentation

**Presenters:** EP Reeves, DA Bergin, P Meleady, M Henry, M Clynes, SJ O'Neill and NG McElvaney.

**Respiratory Research Division, Department of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin 9, Ireland.**

**TITLE:** ALPHA-1 ANTITRYPSIN REGULATES NEUTROPHIL CHEMOTAXIS THROUGH ITS EFFECT ON FCGAMMARIIIB.

**Background:** Manifestations of chronic lung disease frequently involve excessive mobilisation of neutrophils into the lung. Alpha-1 antitrypsin (AAT) deficiency is a disease that is characterized by severe lung inflammation, in which neutrophil-derived factors play a crucial pathological role. The aim of this study was to investigate the effect of AAT on neutrophil function.

**Materials and Methods:** Localization of AAT was performed by sub cellular fractionation and lipid raft isolation. Membrane bound AAT was evaluated by FACs and western blot analysis. FPLC, immunoprecipitation and mass spectrometry was performed to identify the AAT membrane binding partner.

**Results:** Our results show an inhibitory effect of AAT on neutrophil chemotaxis and illustrate that a low AAT environment, such as occurs in the circulation of ZZ-AAT deficient individuals, correlates with enhanced neutrophil chemotaxis. We demonstrate that neutrophil migration is dependent on opposing gradient concentrations of both chemokine (IL-8) and AAT. We further show that AAT is associated with neutrophil membrane lipid rafts, interacting with the glycosylphosphatidyl-inositol (GPI) linked membrane protein FcgammaRIIIb. Results illustrate that AAT can control neutrophil chemotaxis by inhibiting release of FcgammaRIIIb from the cell.

**Conclusion:** The ramification of AAT as an anti-inflammatory modulator of neutrophil chemotaxis, adds a new understanding to the role of AAT in health and disease. These results provide strong insight into a mechanism for the therapeutic effect of AAT augmentation therapy in pulmonary disease.

#### American Thoracic Society presentation New Orleans 2010

**Category:** Poster presentation

**Presenters:** TP Carroll, PhD, C O'Connor, RGN, G O'Brien, MSc, NG McElvaney, MB FRCPI FRCPC.

*Royal College of Surgeons in Ireland, Beaumont Hospital - Dublin/IE*

#### **TITLE: THE IRISH NATIONAL ALPHA-1 ANTITRYPSIN DEFICIENCY TARGETED DETECTION PROGRAMME**

**Rationale:** Alpha-1 antitrypsin deficiency (AATD) is a hereditary disorder and classically presents as emphysema and/or liver disease. The most common mutation presenting with clinical evidence is the Z variant, causing decreased levels of circulating AAT due to retention of the aberrantly folded protein in the liver. AAT deficiency is under-diagnosed with prolonged delays in diagnosis common. World Health Organisation guidelines advocate targeted detection programmes of patients with COPD and asthma. We have previously shown that the prevalence of the Z and S AAT mutations in Ireland is among the highest in Europe. We initiated a national targeted detection programme to investigate whether we could identify AATD patients in Ireland.

**Method:** 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 AATD patients and carriers.

**Results:** 4,000 individuals with COPD, asthma, cryptogenic liver disease and first degree relatives of known AATD patients were screened in a national targeted detection programme.

Targeted screening identified 70 ZZ, 70 SZ, 22 SS, 600 MZ, 400 MS, and 16 MI individuals, yielding gene frequencies of 0.055 and 0.09 for S and Z respectively. Several rarer AAT mutations were also identified.

**Conclusion:** The targeted detection approach is the most effective and economical method of identifying AATD. In our targeted population the Z mutation has a four-fold higher frequency than the general population, highlighting its role in the pathogenesis of lung and liver disease. Interestingly, the frequency of the S mutation in the targeted population was not significantly higher than in the general population. Our data suggests the S mutation is only clinically significant when co-inherited with another AAT mutation.

#### Alpha-1 Foundation Travel Award for American Thoracic Society, New Orleans 2010

**Category:** Poster Presentation

**Presenters:** DA Bergin, EP Reeves, SJ O'Neill and NG McElvaney.

*Respiratory Research Division, Dept of Medicine, Royal College of Surgeons in Ireland, Beaumont Hospital, Dublin, Ireland*

#### **TITLE: THE ROLE OF CALCIUM IN THE HYPERACTIVE STATE OF ALPHA-1 ANTITRYPSIN DEFICIENT NEUTROPHILS.**

**Rationale:** For patients with alpha-1 antitrypsin (AAT) deficiency (AATD), an autosomal recessive disorder characterized by AAT serum levels 35% below that of normals. AATD is characterized by severe lung inflammation, in which neutrophils and neutrophil-derived factors play a crucial pathological role leading to the early onset of emphysema. The most frequent AAT mutation is the Z-allele which is responsible for >95% cases of AATD. Cytosolic free Ca<sup>2+</sup> concentration is an important determinant of neutrophil activity. It has been shown that changes in cytosolic Ca<sup>2+</sup> plays an important role in regulating the onset of the neutrophil respiratory burst and chemotactic activity. In resting neutrophils Ca<sup>2+</sup> is low (approximately 100 nM), but in response to occupation of cell surface receptors, it rises

to micromolar levels, thereby activating a variety of cellular functions. The aim of this study is to determine the role AAT plays in the modulation of Ca<sup>2+</sup> flux in neutrophil activity within AATD.

**Methods:** Peripheral Blood neutrophils were isolated from MM and ZZ individuals. Quantification of AAT RNA and protein was validated by RT-PCR and western blot/FACS analysis. Neutrophil chemotaxis was performed employing a Boyden chamber and the chemokine IL-8. Quantification of reactive oxidative species was carried out by a cytochrome c reduction assay using fMLP and IL-8. Ca<sup>2+</sup> flux within neutrophils was carried out utilizing a Fluo-4 NW probe.

**Results:** We confirmed the presence of AAT at a RNA and protein level within the neutrophil. Evaluating neutrophil chemotaxis demonstrated the ZZ neutrophil to be more chemotactic upon exposure to IL-8 ( $p=0.01$ ). Superoxide production from MM neutrophils was significantly reduced when compared to ZZ neutrophils. Significantly reduced levels of AAT within the ZZ neutrophil compared to MM neutrophils ( $p<0.001$ ) was observed. Finally, we observed AAT ability to modulate the Ca<sup>2+</sup> flux during neutrophil activation with IL-8.

**Conclusion:** This study has re-evaluated and redefined Z AATD neutrophil physiology. Results demonstrate the Z AATD neutrophil is in a higher state of activity compared to MM neutrophils. Furthermore it highlights the importance of AAT as a modulator of the Ca<sup>2+</sup> flux during neutrophil activation.

#### **Irish Thoracic Society Annual Scientific Meeting, Galway, Ireland, November 2009**

**Category:** Poster Presentation

**Presenters:** T Carroll, C O'Connor, G O'Brien, O Floyd, R Costello, SJ O'Neill and NG McElvaney

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

**TITLE: THE ALPHA-1 ANTITRYPSIN DEFICIENCY NATIONAL TARGETED DETECTION PROGRAMME**

**Rationale:** 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. World Health Organisation guidelines advocate screening COPD, poorly-controlled asthma, cryptogenic liver disease patients and first degree relatives of known AATD patients.

**Method:** 3,500 individuals with COPD, asthma, or cryptogenic liver disease were screened in the national targeted detection programme. Phenotyping was performed by isoelectric focusing and genotyping performed by real-time PCR and melt curve analysis.

**Findings:** Targeted screening has identified 55 ZZ, 60 SZ, 18 SS, 535 MZ, 325 MS, and 14 MI individuals, yielding gene frequencies of 0.055 and 0.093 for S and Z respectively in a symptomatic population.

**Conclusion:** Our results underline the need for increased awareness and early detection of asymptomatic AATD. Our data shows AATD is not a rare disease but a disease that is rarely diagnosed. 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.

#### **Irish Thoracic Society Annual Scientific Meeting, Galway, Ireland, November 2009**

**Category:** Poster Presentation.

**Presenters:** DA Bergin, EP Reeves, SJ O'Neill and NG McElvaney.

*Respiratory Research, Dept of Medicine, Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland.*

**Title:** Alpha-1 antitrypsin modulates the G-coupled protein receptor activation of the NADPH oxidase in neutrophils.

**Rationale:** The manifestation of lung disease within alpha-1 antitrypsin (AAT) deficiency (AATD) results in the early onset of emphysema. Neutrophils and neutrophil derived products, such as reactive oxidative species (ROS), are implicated in the progression of lung disease associated with AATD. ROS are generated by the neutrophil NADPH-oxidase system that reduces molecular oxygen ( $O_2$ ) to superoxide ( $O_2^-$ ). In the present study we examined the anti-inflammatory activities of AAT, and investigated the ability of AAT to modulate neutrophil NADPH oxidase post activation via the G-protein coupled fMLP and IL-8 receptors.

**Method:**  $O_2$  consumption and  $O_2^-$  production by neutrophils were measured by a Clarke Type II oxygen electrode and cytochrome c reduction assay respectively. PI3kinase activation, an upstream signalling event of the NADPH-oxidase, was quantified by AKT phosphorylation (Ser-473) by Western blot analysis.

**Results:** Our results demonstrate the ability of AAT (27.5 $\mu$ M) to modulate  $O_2$  consumption post IL-8 (10ng) and fMLP ( $10^{-6}$ M) stimulation. Furthermore AAT demonstrated the capability to inhibit ROS production in a dose dependant manner. Physiological concentrations of AAT (27.5 $\mu$ M) abrogated  $O_2^-$  production post IL-8 and fMLP stimulation ( $P < 0.05$ ). AKT phosphorylation was inhibited by AAT, confirming AAT as an inhibitor of PI3kinase activation.

**Conclusion:** To summarize, this study further demonstrates the anti-inflammatory effects of AAT and implicates the importance of AAT in modulating neutrophil function.

#### Irish Thoracic Society Annual Scientific Meeting, Galway, Ireland, November 2009- Runner-Up ANÁIL Research Awards

**Category:** Poster Presentation

**Presenters:** C O'Connor, T Carroll, G O'Brien, I Hennessy, P Rowland and NG McElvaney.

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

**TITLE: CHARACTERISTICS OF ZZ ALPHA-1 ANTITRYPSIN DEFICIENCY PATIENTS ON THE NATIONAL REGISTRY**

**Rationale:** 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, cryptogenic liver disease patients and relatives of known AATD patients.

**Method:** The most common AAT phenotype associated with disease is ZZ. A chart review of AATD patients on the National Alpha-1 Registry was performed on ZZ (n=70) patients. Our registry collects data on pulmonary function tests, GOLD guidelines, initial reasons for screening, complications, and smoking history.

**Results:** We demonstrate that ZZ individuals identified as a result of family screening have significantly increased FEV<sub>1</sub> (78.5 +/- 6.9%, 47.3 +/- 2.4 years) compared to ZZ patients identified by targeted symptomatic screening (55.0 +/- 4.8%, 52.0 +/- 1.3,  $p=0.0062$ ). ZZ patients who smoked had significantly decreased lung function compared to non-smoking ZZ.

**Conclusion:** Our results underline 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.

#### PUBLISHED RESEARCH

**Respir Med. 2010 Jun;104(6):763-72. Epub 2010 Mar 20.**

#### ALPHA-1 ANTITRYPSIN DEFICIENCY

**Authors:** E Kelly, CM Greene, TP Carroll, NG McElvaney, SJ O'Neill.

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## ABSTRACT

**Objective:** To review the topic of alpha-1 antitrypsin (AAT) deficiency.

**Method:** Narrative literature review.

**Results:** Much work has been carried out on this condition with many questions being answered but still further questions remain.

**Discussion and Conclusions:** AAT deficiency is an autosomal co-dominantly inherited disease which affects the lungs and liver predominantly. The clinical manifestations, prevalence, genetics, molecular pathophysiology, screening and treatment recommendations are summarised in this review.

**European Respiratory Journal 2010 May; 35(5):1155-63.**

### ANTI-APOPTOTIC EFFECTS OF Z ALPHA-1 ANTITRYPSIN IN HUMAN BRONCHIAL EPITHELIAL CELLS.

**Authors:** CM Greene, SD Miller, TP Carroll, IK Oglesby, F Ahmed, M O'Mahony, CC Taggart, NG McElvaney and SJ O'Neill.

**Respiratory Research Division, Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin, Ireland.**

**Abstract:** Z alpha-1 antitrypsin (AAT) deficiency is a genetic disease, which manifests as early-onset emphysema or liver disease. Although the majority of AAT is produced by the liver, it is also produced, amongst others, by bronchial epithelial cells in the lung. Here, we investigate the effects of ZAAT expression on apoptosis in a human bronchial epithelial cell line (16HBE14o-) and delineate mechanisms involved. Control, MAAT- or ZAAT-expressing cells were assessed for apoptosis, caspase-3 activity, cell viability, phosphorylation of Bad, NFkB activation and induced expression of a selection of pro- and anti-apoptotic genes. Expression of ZAAT in 16HBE14o- cells, like MAAT, inhibited basal and agonist-induced apoptosis. ZAAT expression also inhibited caspase-3 activity by 57% compared to control cells ( $p=0.05$ ) and was a more potent inhibitor than MAAT.

Whilst ZAAT had no effect on activity of Bad, its expression activated NFkB-dependent gene expression above control or MAAT-expressing cells. In 16HBE14o- cells but not HEK293 cells, ZAAT up regulated expression of cIAP-1 an upstream regulator of NFkB. cIAP1 expression was increased in ZAAT versus MAAT bronchial biopsies. The data suggest a novel mechanism by which ZAAT may promote human bronchial epithelial cell survival.

**Journal of Immunology 2010 April 15; 184(8)**

### EVIDENCE FOR UNFOLDED PROTEIN RESPONSE ACTIVATION IN MONOCYTES FROM INDIVIDUALS WITH ALPHA-1 ANTITRYPSIN DEFICIENCY.

**Authors:** TP Carroll, CM Greene, CA O'Connor, AM Nolan, SJ O'Neill, and NG McElvaney.

**Respiratory Research Division, Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland.**

**Abstract:** The hereditary disorder alpha-1 antitrypsin (AAT) deficiency results from mutations in the SERPINA1 gene and presents with emphysema in young adults and liver disease in childhood. The most common form of AAT deficiency occurs due to the Z mutation, causing the protein to fold aberrantly and accumulate in the endoplasmic reticulum (ER). This leads to ER stress and contributes significantly to the liver disease associated with the condition. In addition to hepatocytes, AAT is also synthesised by monocytes, neutrophils, and epithelial cells. In this study we show for the first time that the unfolded protein response (UPR) is activated in quiescent monocytes from ZZ individuals. ATF4, XBP-1 and a subset of genes involved in the UPR are increased in monocytes from ZZ compared to MM individuals. This contributes to an inflammatory phenotype with ZZ monocytes exhibiting enhanced cytokine production and activation of the NF- $\kappa$ B pathway when compared to MM monocytes. In addition, we demonstrate intracellular accumulation of AAT within the ER of ZZ monocytes. These are the first data showing that Z AAT protein accumulation induces UPR activation in peripheral blood monocytes. These findings change the current paradigm regarding lung

inflammation in AAT deficiency, which up until now was derived from the protease-anti-protease hypothesis, but which now must include the exaggerated inflammatory response generated by accumulated aberrantly folded AAT in circulating blood cells.

**American Journal of Respiratory Critical Care Medicine. 2010 Jan 1; 181(1):31-5**

**ANTI-PROLINE-GLYCINE-PROLINE OR ANTIELASTIN AUTOANTIBODIES ARE NOT EVIDENT IN CHRONIC INFLAMMATORY LUNG DISEASE.**

**Authors:** CM Greene, TB Low, SJ O'Neill and NG McElvaney.

**Department of Medicine, Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin, Ireland.**  
cmgreene@rcsi.ie

**Abstract Rationale:** In patients with chronic inflammatory lung disease, pulmonary proteases can generate neoantigens from elastin and collagen with the potential to fuel autoreactive immune responses. Antielastin peptide antibodies have been implicated in the pathogenesis of tobacco-smoke-induced emphysema. Collagen-derived peptides may also play a role.

**Objectives:** To determine whether autoantibodies directed against elastin- and collagen-derived peptides are present in plasma from three groups of patients with chronic inflammatory lung disease compared with a nonsmoking healthy control group and to identify whether autoimmune responses to these peptides may be an important component of the disease process in these patients.

**Methods:** A total of 124 patients or healthy control subjects were recruited for the study [Z-A1AT deficiency, n = 20; cystic fibrosis, n = 40; chronic obstructive pulmonary disease, n = 31; healthy control, n = 33]. C-reactive protein, IL-32, and antinuclear antibodies were quantified. Antielastin and anti-N-acetylated-proline-glycine-proline autoantibodies were measured by reverse ELISA.

**Measurements and Main Results:** All patients were deemed stable and noninfective on the basis of the absence of clinical or radiographic evidence of recent infection. There were no significant differences in the levels of autoantibodies or IL-32 in the patients groups compared with the healthy control subjects.

**Conclusions:** Antielastin or anti-N-acetylated proline-glycine-proline autoantibodies are not evident in chronic inflammatory lung disease.

## 10. Conferences

### EUROPEAN POLICY ON RARE DISEASE GATHERS MOMENTUM AT THE EUROPEAN CONFERENCE ON RARE DISEASES ECRD KRAKOW, 14 & 15 MAY, 2010

An unprecedented number of participants attended the largest ever European Conference on Rare Diseases (ECRD), which was held in Krakow, Poland. Over 600 participants from 43 countries, a third of which came from Eastern Europe, gathered to discuss key policies and actions to improve the lives of those affected by these conditions. The Alpha One Foundation was represented by Kitty O'Connor.

Dr Andrzej RyD, Director for Public Health at the European Commission, opened the Conference by declaring that the overall number of patients suffering from rare diseases, the high European added value due to the rarity of patients and experts for each rare disease together with the limited access to information, to treatment opportunities and drugs available, constitute a challenge that justifies action from the European Union in this field. He highlighted that Member States have until 2013 to adopt rare disease plans or strategies in their own countries based on common policy recommendations.

According to Director of Orphanet, Dr Ségolène Aymé's presentation, National Plans have already been adopted in France, Portugal, Greece, Bulgaria and Spain, and are well under way in Germany, Romania and the UK. The first steps have been taken in other countries, such as Poland and Ireland. "This ECRD 2010 Krakow has served to identify those areas that need better policies in order to fulfill the objectives of the Council Recommendation and to build momentum for national plans and strategies to be implemented across Europe," declared Yann Le Cam, CEO of the European Organisation for Rare Diseases (EURORDIS). "Indeed, the momentum applies to Poland as 20 Polish patient representatives, healthcare professionals and scientists met on the first day of the Conference to sign a Common Declaration to the government calling to establish a National Plan for Rare Diseases in Poland. They also suggested following the EuroPlan guidelines to accomplish their goals."

Measures to improve accurate diagnosis and early treatment of many rare diseases were presented at the Conference, namely:

- Coding and classifying rare diseases and integrating them in the WHO's International Classification of Diseases system.
- Identification and support of centres of expertise in all European countries and pooling existing expertise through European Reference Networks.
- Sharing research infrastructures (databases, biobanks and registries), involving patient organisations in clinical trials
- Making the best use of knowledge and funds for genetic testing.

The ECRD 2010 Krakow was also the occasion to present the EU Committee of Experts on Rare Diseases. The Committee, which will include around 50 representatives of all stakeholder groups, will act as a sort of 'Parliament' of the rare disease community in order to follow up on the work initiated at the biennial Conference.

The ECRD series is a unique forum that sees patient representatives of all the rare diseases, from the majority of European countries and further afield, gather with healthcare professionals, academics, researchers, policy makers and industry representatives to discuss the most recent rare disease initiatives in the fields of research, healthcare, information and social services. ECRD 2010 Krakow is organised by The European Organisation for Rare Diseases (EURORDIS) in partnership with Rare Disorders Denmark, the National Health.

### 5TH EUROPEAN ALPHA-1 CONGRESS, LONDON 9TH & 10TH JULY 2010

Myself (Crea Crosbie Sheahan) and my sister Orla Keane (both Alpha-1 ZZ) attended as delegates alongside Kitty O'Connor - CEO Alpha One Foundation. For Orla it was once again so nice to meet fellow Alphas and their families and to catch up with everyone since they met in Vienna 2009.

The conference itself had a full programme with some wonderful speakers.

- **Professor Sabina Janciauskiene**  
MHH Hannover, Germany  
*What is Alpha1 Antitrypsin Deficiency?*
- **Doctor Nedim Hadzic**  
King's College Hospital, London  
*Liver Diseases of Children*
- **Professor David Lomas**  
University of Cambridge  
*Developing a Cure for Alpha-1*
- **Doctor Heinz Steveling**  
Ruhrklinik Essen, Germany  
*20 Years of Replacement Therapy*
- **Melissa Hillier**  
Genetic Alliance UK  
*Improving access to healthcare for families  
with rare genetic conditions*
- **John Walsh**  
Alpa-1 Foundation, USA,  
*Patient Empowerment*

All of the speakers gave a great presentation and I'm sure everyone came away with a greater understanding of Alpha-1 Antitrypsin Deficiency.

My own two favourites were firstly Sabina Janciauskiene who had a wonderful way of explaining Alpha-1, I wish I had heard it presented in this way when I was first diagnosed.

Secondly Professor David Lomas - his presentation on 'Developing a Cure for Alpha-1' was for me the highlight of the conference. I wish I had a recording of this enthralling speech, or at least a copy of the (power point) computer notes. We may not see results from this work for 10 years and it may not be successful but it certainly won't be for lack of enthusiasm...plus for me the knowledge that such research / studies are being conducted gives hope for the future!

It was my first time to attend a Congress and I was very glad to have the opportunity to do so. It was good to meet other Alphas and hear their stories and of course the conference was a great learning experience. I would encourage other patients to take the opportunity to attend next year's Congress if given the chance.

**Crea Crosbie Sheahan**  
Patient Representative

# 11. What is Alpha-1 Antitrypsin Deficiency (Alpha-1)? Should I be Tested?

## WHAT IS ALPHA-1 ANTITRYPSIN DEFICIENCY?

Alpha-1 Antitrypsin Deficiency, commonly referred to as Alpha-1, is an inherited, genetic disorder which results in low levels of a protein in the blood called alpha-1 antitrypsin (AAT). If you have Alpha-1 you may develop serious lung and/or liver disease or pass the disorder onto your children. When AAT levels are low, the body is not adequately protected from an enzyme in the white blood cells that can cause damage to the air sacs in the lungs.

## WHAT ARE SOME IMPORTANT FACTS ABOUT ALPHA-1?

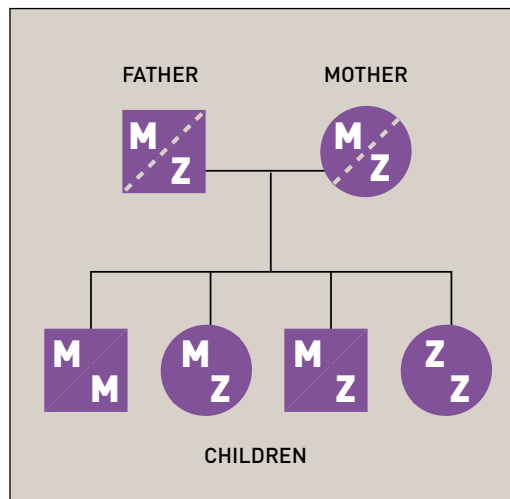
Alpha-1:

- Is an inherited genetic disorder resulting in low levels of AAT.
- May cause lung disease in adults.
- May cause progressive liver damage in adults, children and infants.
- Is often underdiagnosed.
- Is treatable, not curable.
- Is easily identified by a blood test.

## HOW IS ALPHA-1 INHERITED?

One half of your genes are inherited from each parent. Refer to Figure 11.1 to see the possible outcomes for children if both parents are carriers (having one normal and one altered AAT gene). This example applies to immediate family members only.

**FIGURE 11.1:** Possible outcomes for children if both parents are carriers



<b>Normal</b>	<b>MM</b>	Does not have the disorder and does not carry the altered AAT gene.
<b>Carrier</b>	<b>MZ</b>	Mild to moderate AAT deficiency (generally does not develop disease symptoms but does carry the altered AAT gene).
<b>AAT Deficiency</b>	<b>ZZ</b>	Severe deficiency (could develop the disease and does carry the altered AAT gene).

## WHAT ARE THE SIGNS SUGGESTING ALPHA-1?

### 1. Immediate Family History of Alpha-1, Lung or Liver Disease.

### 2. Symptoms

- Shortness of breath at rest or with exercise
- Wheezing
- Coughing
- Repeated lung infection
- Sputum (or phlegm) production
- History of suspected allergies and/or asthma.

### 3. Any of these medical problems:

- Chronic Obstructive Pulmonary Disease (COPD)
- Emphysema
- Bronchiectasis
- Chronic Bronchitis
- Asthma
- Chronic liver disease in adults
- The skin disease panniculitis
- Unexplained liver disease in infants and children.

It is important to note that people with Alpha-1 may not show any signs of the disorder for many years. This does not mean that you will not have symptoms in the future.

### WHAT IS INVOLVED IN TESTING FOR ALPHA-1?

Testing for Alpha-1 is simple, quick and highly accurate. Two types of test are used. One examines your blood for certain proteins that indicate whether or not you have Alpha-1. This test determines the amount of AAT in your blood. The second blood test determines the type of AAT protein you have. There are two ways to take the blood for these tests. One is a self administered finger prick (A growing use of this method is anticipated). The other way is to have your doctor draw blood during a check-up or clinic visit.

### ONCE THE BLOOD SAMPLE IS OBTAINED:

- It is shipped to Beaumont Hospital for analysis. There is no cost.
- You will wait a number of weeks for the confidential test results to be reported to your Doctor.
- You will need to schedule a follow-up visit with the Alpha-1 clinic in Beaumont to discuss your test results and to provide advice and support.
- All results will be confidential and only accessible by your medical doctor.

### WHAT WILL THE TEST TELL YOU?

The test will tell you the amount of AAT in your blood and whether or not you have the normal genes, are a carrier (having one normal AAT gene and one altered AAT gene), or have a severe deficiency of AAT.

It is important for your doctor to know the amount of AAT you have circulating in your blood. If the amount of AAT in your blood is low your doctor can use this information to guide plans for your future treatment.

### WHO SHOULD CONSIDER BEING TESTED?

If you have signs suggesting Alpha-1 (immediate family history of Alpha-1, specific symptoms or any of the identified medical problems) you should consider being tested. There are ways your life could be affected by learning information that may be discovered by genetic testing.

### POTENTIAL BENEFITS

- Allow you to increase your knowledge of Alpha-1 and awareness of your personal risk.
- Provide information for future lifestyle decisions.
- Allow you to take steps that may slow the progression of Alpha-1.
- Assist you and your family in making decisions about work, health and family decisions.

### POTENTIAL DRAWBACKS

- May be personally unsettling and cause anxiety
- May create some concerns in your family.

In our opinion, the benefits far outweigh drawbacks. "Your health is your wealth". These tests are voluntary. You should discuss medical and non-medical risks and benefits with your doctor, family and relevant others. You need to thoroughly understand the potential benefits and drawbacks prior to testing. This is called "informed consent".

### WHAT SHOULD I DO WITH THE RESULTS?

#### If your test results are positive:

Contact the Alpha One Foundation/GP/Consultant.

#### Ask them about:

- Interpretation of your test results.
- How the results will affect your specific medical condition.
- The impact of Alpha-1 on your current medical state.
- Your specific options for treatment
- Stop smoking and avoid second-hand tobacco smoke as much as possible.
- Avoid exposure to dusts and fumes.
- Evaluate your health behaviour (i.e. hand washing, minimizing contact with people who have respiratory infections).
- Decide whom you should inform in your family and if they should be tested.

- Develop an exercise programme (under medical supervision).
- Develop a nutritional programme (under medical supervision).
- Ask Alpha One Foundation/GP/Consultants for a copy of the brochure "Guide for the recently Diagnosed Individual".
- Contact the resources listed below for more information.

There are ways that Alphas (persons with Alpha-1 antitrypsin deficiency) can protect themselves through proper nutrition, exercise and stress management and, most importantly, not smoking. In many cases, these measures can help ward off symptoms of the disorder for many years.

#### **WHERE CAN I GO FOR MORE INFORMATION?**

##### ***Alpha One Foundation Ireland***

RCSI Building,  
Beaumont Hospital,  
Dublin 9.  
Registered Charity Number: CHY14812  
Tel: 01-8093871  
Fax: 01-8093591  
Email: [alpha1@rcsi.ie](mailto:alpha1@rcsi.ie)  
Web: [www.alpha.ie](http://www.alpha.ie)

##### ***Alpha-1 Foundation US***

[www.alpha-1foundation.org](http://www.alpha-1foundation.org)

##### ***AlphaNet***

[www.alphanet.org](http://www.alphanet.org)

##### ***Alpha-1 Association***

[www.alpha1.org](http://www.alpha1.org)



## 12. What does it mean to be an Alpha-1 Carrier?

### WHAT IS MEANT BY THE TERM ALPHA-1 CARRIER?

An Alpha-1 carrier is a person who has one normal alpha-1 gene (M) and one defective alpha-1 gene (usually S or Z). Being a carrier is very common and it is estimated 200,000 people on the island of Ireland are carriers. Most Alpha-1 carriers are MS or MZ. Carriers may have lower blood levels of alpha-1 antitrypsin protein, but their levels are rarely as low as those of people with Alpha-1.

### HOW CAN BEING AN ALPHA-1 CARRIER AFFECT YOUR LUNGS?

Alpha-1 carriers usually have only a slight risk of developing lung disease related to Alpha-1. The main type of carrier linked to increased risk for lung disease has MZ genes. Currently, there is no known risk for lung disease for MS carriers.

**Lung Disease:** The risk for emphysema may be greater for MZ carriers. However, the increased risk is small unless the carrier is a smoker or exposed to high levels of air pollution. The risk of having Chronic Obstructive Pulmonary Disease (COPD) is higher among MZ carriers who have relatives with COPD. This suggests that the COPD in these families may be due to other genetic factors. There is no significant evidence that MS carriers are at risk for lung disease.

### Lung symptoms that might be linked to being an Alpha-1 Carrier

- Shortness of breath
- Wheezing
- Chronic cough and sputum (phlegm) production (chronic bronchitis)
- Recurring chest colds
- Decreased exercise tolerance
- Non-responsive asthma or year-round allergies
- Bronchiectasis

### HOW CAN BEING AN ALPHA-1 CARRIER AFFECT YOUR LIVER?

Alpha-1 carriers usually have only a slight risk of developing liver disease related to Alpha-1. The

main type of carrier linked to increased risk for liver disease has MZ genes. Currently, there is no known risk for liver disease for MS carriers.

**Liver Disease:** The risk of chronic liver disease in Alpha-1 carriers is much less than that for people with Alpha-1. Research suggests that chronic liver disease might appear in MZ carriers only when the liver has been damaged first by something else, such as a virus, chemicals (including alcohol) or being overweight. There is no scientific evidence that MS carriers are at risk for liver disease.

### Liver symptoms that may be related to Alpha-1 Carrier status

- Eyes and skin turning yellow (jaundice)
- Swelling of the abdomen (ascites)
- Vomiting blood or passing blood in the stool
- Unexplained liver problems or elevated liver enzymes.

### CHILDREN OF ALPHA-1 CARRIERS

Alpha-1 carriers may pass their defective alpha-1 gene to their children.

- If a carrier (MZ) has a child or children with a person who has normal alpha-1 genes (MM), each child has one chance in two (50% risk) of being an Alpha-1 Carrier. There is no risk that any of the children will have the full condition.
- If a carrier (MZ) has children with another carrier (MZ), each child has one chance in two (50% risk) of being an Alpha-1 Carrier. Each child also has one chance in four (25% risk) of having Alpha-1 (ZZ) and one chance in four (25%) of having normal alpha-1 genes (MM).

### WHO SHOULD BE TESTED?

Anyone thinking about being tested for Alpha-1 should first contact the Alpha One Foundation or speak with a healthcare professional that has knowledge of genetic disease. This could be their physician or GP. Testing is advised for parents, brothers and sisters of a person with Alpha-1. Testing is also advised for anyone with the following medical conditions:

- COPD (emphysema and/or chronic bronchitis)
- Asthma where lung function is not made normal by prescribed medications
- Unexplained liver disease
- Liver disease with a family history of liver disease

### CAN BEING A CARRIER AFFECT MY HEALTH INSURANCE?

Alpha-1 is a genetic condition. Under the terms of the Disability Act 2005 part 4, section 2: it is *illegal* to disclose any information gained by genetic testing (such as testing for Alpha-1) for the purpose of insurance, assurance, pension, mortgage etc.

### HOW CAN CARRIERS PREVENT OR REDUCE THEIR RISK OF GETTING DISEASE LINKED TO ALPHA-1?

MZ carriers have only a slightly increased risk for the lung or liver disease seen in people with Alpha-1. You may prevent or reduce the risks by making changes to your lifestyle, such as:

- Do not smoke and avoid second-hand smoke
- Influenza and Pneumococcal vaccinations
- Avoid repeated exposure to dust, fumes or gases
- Quit or cut back on drinking alcohol
- Vaccinations against hepatitis A and B

If the carrier has children who are also carriers, the children should be informed about their genetic status. The importance of a healthy lifestyle should be emphasized from an early age.

### WHAT ARE THE RECOMMENDED TREATMENTS FOR CARRIERS WITH LIVER OR LUNG DISEASE?

Your doctor will determine the course of your treatment.

### TO WHOM SHOULD I REVEAL MY ALPHA-1 CARRIER STATUS?

Sharing your status with others is up to you, but you may want to inform family members so they can consider testing. Also, your physician may need to know to better plan treatment options.

### WHERE CAN I GO FOR MORE INFORMATION AND SUPPORT?

Learning that you are an Alpha-1 Carrier may confuse or upset you. It may help you to:

- Share your status with your family
- Learn as much as you can about the effects it can have on your health
- Seek support groups to answer your questions.

Also, there are organisations that can offer help and advice to you. Some of these are listed in this brochure.

### TARGETED DETECTION PROGRAMME

The Alpha One Foundation has a targeted detection programme to test patients and family members nationwide for Alpha-1 Antitrypsin Deficiency according to the World Health Organisation recommendations.

The purpose of this programme is to detect, identify and treat as many people with Alpha-1 in Ireland as possible. This programme is being carried out in a collaborative manner with respiratory clinics through the country. Early identification and focused treatment of these patients will greatly reduce their need for hospitalisation and improve their life expectancy and quality of life.

### ALPHA ONE FOUNDATION

RCSI Building, Beaumont Hospital, Dublin 9.  
Registered Charity Number: CHY14812  
Phone: 01-8093871  
Fax: 01-8093591  
Email: [alpha1@rcsi.ie](mailto:alpha1@rcsi.ie)  
Web: [www.alpha1.ie](http://www.alpha1.ie)

### OTHER RESOURCES

#### **Alpha-1 foundation (US)**

<http://www.alphaone.org/>

Link to the US Alpha-1 patient website.

#### **UK Alpha-1 Awareness**

<http://www.alpha1awareness.org.uk/welcome.htm>

Patient website maintained by the UK Alpha-1 Awareness Group.

**Alpha1 Kids (US)**

<http://www.alpha1kids.org/index.php>

Website for parents of newly diagnosed children with Alpha-1.

**Stop Smoking**

<http://www.giveupsmoking.ie/>

Site dedicated to smoking cessation.

**COPD Support**

<http://www.copdsupport.ie/>

Detailed Irish website on COPD.

**Asthma Society**

<http://www.asthmasociety.ie/>

Website for the Asthma Society of Ireland

## 13. Recent Events

*R to L: Kitty O'Connor (CEO), Cllr. Aine Clancy (Deputy Lord Mayor), His Excellency Yvon Roe D'Albert (French Ambassador), Prof Gerry McElvaney, His Excellency Dr Tadeusz Szumowski and his wife (Polish Ambassador), Larry Warren (former CEO Alpha One Foundation)*



### CHOPIN RECITAL, OCTOBER 2009

Fredric Chopin suffered from chronic respiratory disease, probably alpha-1 antitrypsin deficiency, during his short but very productive life. The Alpha One Foundation wished to celebrate his life and draw attention to respiratory research especially research into alpha-1 antitrypsin deficiency.

As his condition greatly influenced his music we thought it appropriate to celebrate Chopin's life and music each year in conjunction with the anniversary of his death. The Lord Mayor of Dublin Councillor Emer Costello kindly invited us to use her residence for the occasion. We gratefully acknowledge her generosity.



### VISIT OF MINISTER OF STATE BARRY ANDREWS TD, FEBRUARY 2010

The Alpha One Foundation was delighted to welcome Barry Andrew T.D. Minister of State for Children in February 2010. This visit highlighted our new Alpha-1 Clinic in

Beaumont Hospital and the innovative clinical and molecular research being carried out in the RCSI, Clinical Research Centre at Beaumont Hospital. The Minister met with Alpha-1 patients, researchers and medical staff.

## 14. Patient Support Group



### FEBRUARY 2010

Friendship and Community Spirit is alive and well in Maynooth, County Kildare. The wonderful success of a Charity Fashion Sale in aid of The Alpha One Foundation was a testament to everyone involved.

The event was organised by three Alpha-1 patients Jo McGuirk, Crea Sheahan and well known local woman Orla Keane.

We were overwhelmed by the amazing support of friends, family, local business and of course the community who arrived in great numbers and helped us to raise over €5,000.00 for the Alpha One Foundation.



***R to L: Josephine McGuirk (Patient Support Group), Orla Keane (Patient support Group), Geraldine O'Brien (Research Associate), and Tomás Carroll (Research Scientist)***

### JUNE 2010

Orla Keane and Josephine (Jo) McGuirk presenting the cheque of monies raised in the Charity Fashion Sale and other fundraising events... €8,000.00 in total. We are delighted that the money raised has helped to fund the continuing cost of the machine on the left of the photo – which is of great benefit to patients. The machine is a Sebia Hydrasys machine and is used for diagnosing Alpha-1 patients by a method called "phenotyping".

In 2008 The Alpha One Foundation acquired this new piece of equipment for the National Targeted Detection Programme. This has allowed them to implement a more accurate method of phenotyping. They can now identify the different alpha-1 antitrypsin phenotypes with an increased sensitivity of detection.

The Sebia Hydrigel 18 AAT isofocusing kit is designed for the qualitative detection and identification of the different phenotypes of alpha-1 antitrypsin (AAT) circulating in human blood. This new phenotyping method has been found 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.

## 15. Acknowledgements

We would like to thank the following for their invaluable help:

- The Alpha-1 Foundation in the US, John Walsh, President and Angela McBride, Director of Development
- Maurizio Luisetti MD and Dr. Ilaria Ferrarotti, Centre for Diagnosis of Inherited Alpha-1 Antitrypsin Deficiency, Laboratory of Biochemistry and Genetics Institute for Respiratory Disease, University of Pavia, Italy
- Marc Miravittles, Servei de Pneumologia (ICPCT), Hospital Clínic Villarroel, Barcelona, for protocols and technical information
- Pat O'Brien and Eric Mahon in the Beaumont Hospital Biochemistry Department for their continued support
- Dr. Joseph McPartlin, Trinity Biobank, Institute of Molecular Medicine, St James's Hospital, Dublin

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

- Alpha-1 is twice as prevalent as previously estimated and occurs in Ireland 1 in 2,100 individuals, with 1 in 24 individuals predicted to be carriers.
- Family screening provides an opportunity for early diagnosis of Alpha-1 and therefore can potentially reduce the risk of developing lung disease.
- New treatments for Irish Alpha-1 patients in clinical trials at the moment include intravenous replacement therapy, inhaled Alpha-1, and a new gene therapy treatment.
- Several rare Alpha-1 genes found in the Irish population lead to lung and liver disease. This highlights the need for comprehensive testing of suspected patients. This must include measuring Alpha-1 levels, combined with phenotype analysis.
- Alpha-1 is not a rare disease, but a disease that is rarely diagnosed. Less than 10% of known Alphas have been diagnosed in Ireland to date.