Alpha One Foundation

ANNUAL REPORT 2012

Alpha One Foundation Annual Report 2012

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1. Executive Summary

This year the Alpha One Foundation made great strides in increasing awareness and detection of Alpha-1 Antitrypsin Deficiency. Our endeavours to raise public awareness are closely linked to the promotion of basic and clinical research into Alpha-1 and to the early diagnosis and improved care of Alpha-1 individuals. We are the national referral centre for Alpha-1 and have a dedicated Alpha-1 clinic in Beaumont Hospital. Our research has shown that 1 in 25 individuals in Ireland carry a gene that causes Alpha-1, which is twice as prevalent as previously estimated. This indicates that there are over 2,000 individuals with the severe form of Alpha-1 (ZZ) and over 200,000 individuals with a milder form of Alpha-1 who may also be at risk of lung or liver disease.

This year the Alpha-1 Foundation joined with other lung health charities and established the Irish Lung Health Alliance group. This group launched the 'Love your Lungs' campaign which highlights the fact that lung disease affects people of all ages, all socio-economic backgrounds and non-smokers as well as smokers. Olympic legend Dr Ronnie Delany was an ambassador for the campaign which underlined the importance of healthy lungs to an active lifestyle and vice-versa. Lung testing took place on World Spirometry Day June 27th in 10 locations throughout the country.

The National Alpha-1 Targeted Detection Programme received samples from over 25 Hospitals, GP practices, and family members of known Alpha-1 individuals. Since 2004 we have screened over 8,500 individuals for Alpha-1. Upon diagnosis individuals can be fast-tracked to our dedicated Alpha-1 Clinic in Beaumont Hospital. The National Alpha-1 Registry continues to successfully capture demographic and medical data for all Alpha-1 patients. We earnestly encourage all our Alpha-1 individuals to register on the National Alpha-1 Registry. Clinical trials for replacement therapy in Alpha-1 are also ongoing at our centre. We continue our research study in MZ (carrier) individuals; this study's aim is to clarify if MZ patients are at a greater risk of developing COPD in conjunction with Dr Edwin Silverman at Harvard University.

The Alpha One Foundation continues our active participation with the Medical Research Charities Group, (Registry Working Group, Communications Group), Rare Diseases Toward 2013 Taskforce, Irish Donor Network, Irish Platform for Patient Organisations Science and Industry (IPPOSI), Irish Lung Health Alliance, the European Organisation for Rare Diseases (EURORDIS) and ALFA Europe.

The Alpha One Foundation hosted an information stand and presented novel research at the Irish Thoracic Society (ITS) conference in November 2011 in Dublin. Our research was also presented at the American Thoracic

Society Meeting in San Francisco in May 2012. Throughout the year the Alpha One Foundation presented research and promoted awareness of the condition in over 15 centres. These included hospital- and GP-based respiratory meetings, Immunology and Biochemistry laboratory meetings and COPD patient meetings. Our annual Alpha-1 conference was held in October 2011 in the Marino Institute of Education. We were delighted to have Angela McBride, Development Officer from the Alpha-1 Foundation in the US present at the meeting. This was a well-attended conference and provided an excellent opportunity for Alpha-1 patients and family members to meet other Alpha's in a relaxed environment.

Our support group held an Alpha-1 Charity Night in Swords, featuring the band 'Pomp' in February and a Mid-Summer's Night Ball was held in the Spa Hotel in Lucan in June. These events were a tremendous success and raised valuable funds for the Foundation. The group also successfully raised funds by running the Dublin City Marathon and Flora's Women's Mini Marathon. The Alpha One Foundation held the inaugural Chopin Award competition in the Mansion House in November 2011, with students from three universities competing for the award. During Chopin's life he suffered from chronic respiratory illness and probably Alpha-1. We felt it appropriate to celebrate Chopin's life and draw attention to respiratory disease, particularly Alpha-1 Antitrypsin Deficiency. We were delighted to congratulate Louis Murphy representing Trinity College as the winner of the inaugural Alpha-1 Chopin award.

This brief overview may give you some idea of the work being done and the progress being made by the Alpha One Foundation. This work is collaborative and I wish to thank all my colleagues, past and present, for their diligence and dedication throughout the year.

Kitty O'Connor
Chief Executive Officer

2. An Update from the National AATD Targeted Detection Programme

TESTING

Guidelines from the World Health Organisation (WHO), American Thoracic Society (ATS), and European Respiratory Society (ERS) advocate targeted detection programmes for AATD. Together these guidelines recommend targeted screening of patients with COPD, non-responsive asthma, cryptogenic liver disease and also first-degree relatives of known AATD individuals (Table 2.1).

***TABLE 2.1:** ATS/ERS recommendations for diagnostic testing for AATD (type A recommendations)*

ATS/ERS Recommendations for Diagnostic Testing
Adults with symptomatic emphysema or COPD (regardless of age or smoking history)
Adults with asthma with airflow obstruction that is incompletely reversible after aggressive treatment with bronchodilators
Asymptomatic individuals with persistent obstruction on pulmonary function tests with identifiable risk factors (e.g. cigarette smoking, occupational exposure)
Adults with necrotising panniculitis
Siblings of individuals with AATD
Individuals with unexplained liver disease, including neonates, children, and adults, particularly the elderly

A diagnosis of AATD gives the clinician a unique opportunity for early medical intervention and the possible prevention of lung disease in both the affected individual and first-degree relatives. Unfortunately, despite huge strides in awareness and understanding of this condition, this opportunity is too often missed. Large variability exists in the clinical course of lung disease in AATD and therefore all COPD patients should be tested for AATD, regardless of age or smoking history. For example, we propose that any management strategy for COPD should include testing for AATD (Figure 2.1).

In May 2004, a national targeted detection programme for AATD was launched by the Alpha One Foundation and is based in the RCSI Education and Research Centre at Beaumont Hospital. AATD can be diagnosed by a simple blood test, but despite this, as a condition it remains vastly under-diagnosed both in Ireland and worldwide.

Our principal diagnostic method analyses serum samples from suspected AATD individuals (Figure 2.2) and employs the Hydragel 18 AAT isofocusing method (Sebia). This is designed for the qualitative detection and identification of the different variants of alpha-1 antitrypsin (AAT) circulating in human blood, a method known as phenotyping (Figure 2.3A). The procedure involves isoelectricfocusing on agarose gel, performed on the semi-automatic HYDRASYS system, followed by immunofixation with AAT antiserum. This method is sensitive, rapid and simple to perform (Zerimech *et al*, Clinical Chemistry and Laboratory Medicine 2008). It is the most accurate method of testing for AATD and improves the identification of not only the most common phenotypes but also rare AAT phenotypes.

A DNA genotyping system has been developed to detect the two mutations (S and Z) responsible for over 95% of all cases of AATD (Figure 2.3B). 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 and stored as a dried blood spot (DBS). DNA isolated from this DBS sample is then used to genotype the patient by RT-PCR (Real-Time Polymerase Chain Reaction), using primers and probes specific to the S and Z 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.

Since September 2010 quantification of AAT levels by the Alpha One Foundation is performed in collaboration with Dr Bill Tormey, Consultant Chemical Pathologist and Pat O'Brien and Eric Mahon of the Department of Chemical Pathology in Beaumont Hospital. Measurement of AAT levels is performed by immune turbidimetry on the Olympus AU4500 instrument, an automated system for plasma protein determinations. The Department of Chemical Pathology in Beaumont

FIGURE 2.1: *Proposed management strategy for COPD which incorporates testing for AATD*

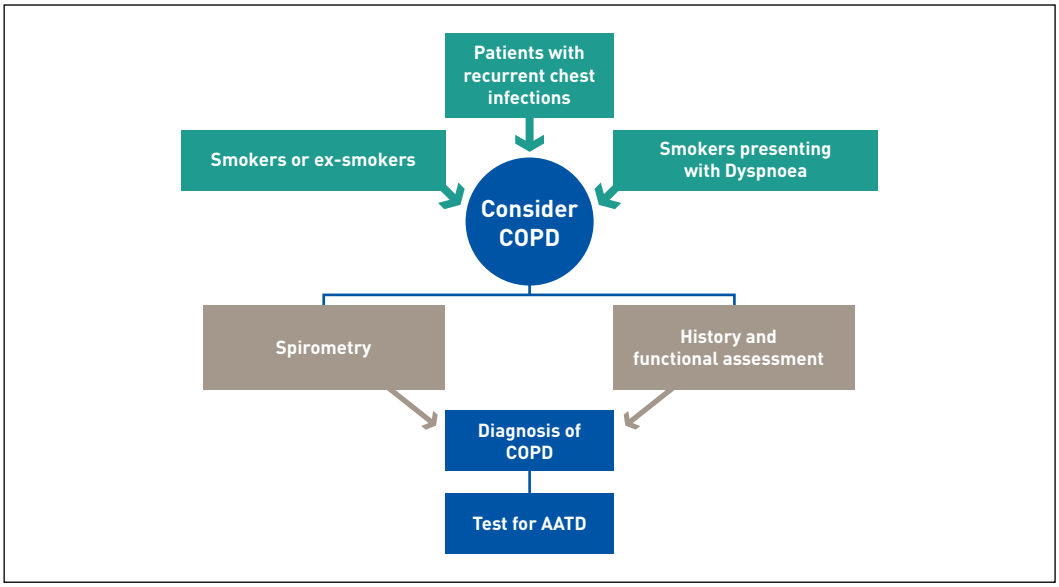


FIGURE 2.2: *Diagnostic algorithm used for testing venous blood samples for AATD.* *It is vital that diagnosis is confirmed by a combination of both AAT level and AAT phenotype*

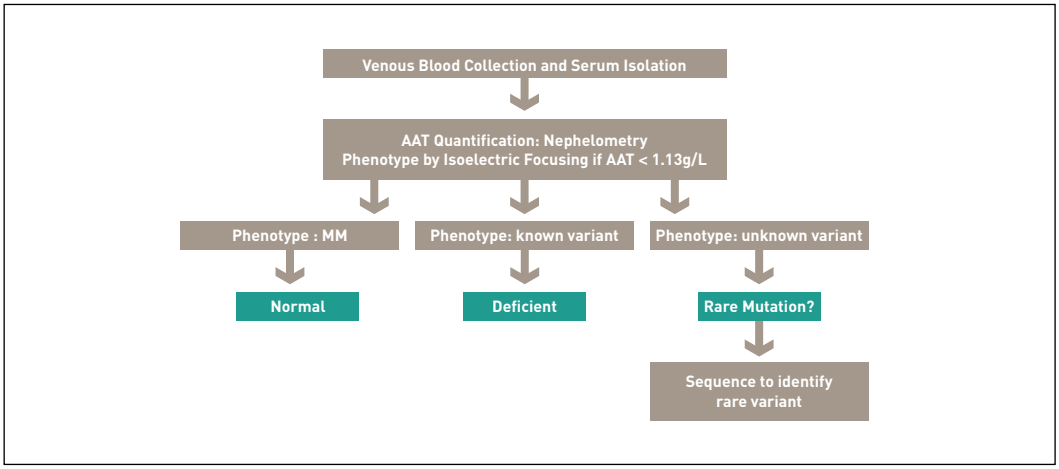
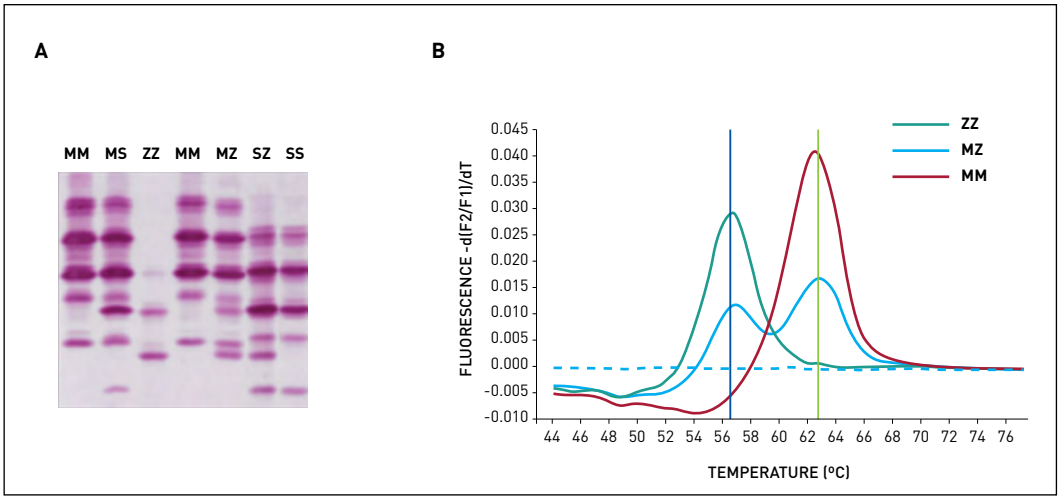


FIGURE 2.3: *Principal methods employed for diagnosis of AAT mutations.* ***A:*** *Typical isoelectric focusing gel for AAT phenotype identification with the most common phenotypes included.* ***B:*** *Genotyping assay using DNA extracted from DBS samples for the Z mutation*



Hospital has recently attained CPA accreditation which means that our AAT measurements are performed to the highest internationally accepted standards. In addition, we have been participating in the UKNEQAS quality assurance scheme for alpha-1 antitrypsin phenotyping since 2007 and have achieved 100% compliance to date.

RESULTS TO DATE

So far over 8,500 individuals with COPD, asthma, and liver disease, as well as asymptomatic first-degree relatives of known AATD individuals have been screened in our national AATD targeted detection programme since its inception in May 2004. A total of 118 ZZ (severe AATD) individuals have been identified, we have also detected 123 SZ, who are also at risk of developing lung and liver disease (Figure 2.4). In addition, a large number of other clinically significant phenotypes have been detected including 42 SS, 1249 MZ, 11 IZ, and 6 IS phenotypes. The percentage of deficiency alleles (approximately 34%) detected has been quite high, even allowing for the fact that this is a targeted population and would be expected to contain a high percentage of deficient alleles. Several rare AAT mutations were also identified in the Irish population, including I, F, V, $X_{christchurch}$, $Z_{bristol}$, M_{malton} and 2 novel Null mutations. Further studies will reveal the degree of predisposition to lung or liver disease associated with these rare mutations.

However, the main outcome of this national screening programme is that diagnosed individuals have the opportunity to receive appropriate care and management of their condition, and are offered fast referrals to our dedicated Alpha-1 clinic in Beaumont Hospital under the care of Professor Gerry McElvaney. In addition, family screening allows the identification of younger relatives with AATD in whom no significant lung damage has occurred. These individuals benefit from behavioural changes such as smoking cessation and closer medical observation which can ultimately prevent or postpone the development of lung disease. In the 10 years since the screening programme began we have identified 118 new ZZ individuals. In addition to newly diagnosed ZZ individuals, a further 59 ZZ individuals have been referred to Beaumont Hospital by other centres and physicians (Figure 2.5).

The current total of symptomatic individuals tested in the targeted detection programme to date is approximately 8000, with a further 745 individuals tested as part of family screening. Family screening means that these individuals were tested for AATD because a family relative was previously diagnosed with the condition. Requests are received from over 25 hospitals in Ireland as well as from GP practices. In the Dublin area almost 50% of all requests received have been from Beaumont Hospital (Table 2.2). Beaumont Hospital was the sole participating centre in the first year of the programme. After the first year, the screening programme has steadily expanded to include a majority of Dublin hospitals. The largest participating centre in Dublin after Beaumont Hospital is St Vincent's University Hospital, accounting for 22% of all Dublin requests.

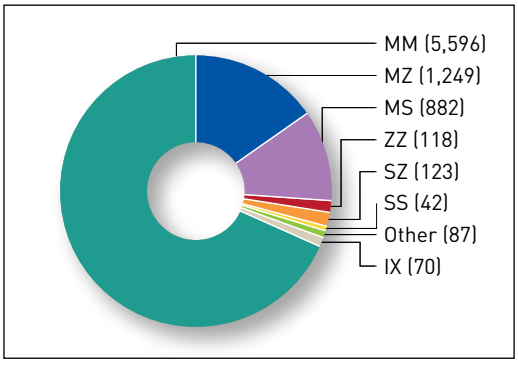


***FIGURE 2.4:** All phenotypes identified in National AATD Targeted Detection Programme*

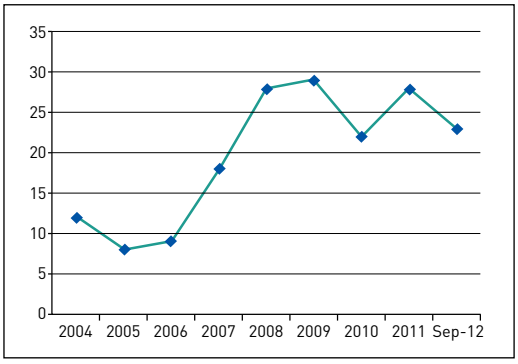


***FIGURE 2.5:** All new ZZ cases diagnosed by or referred to the Alpha One Foundation since 2004*

***TABLE 2.2:** Requests from Dublin area hospitals*

Dublin	Requests
Beaumont Hospital	1811
St Vincent's University Hospital	929
Bon Secours Dublin	389
St James's Hospital	314
Connolly Hospital Blanchardstown	341
Peamount Hospital	184
Mater Misericordiae University Hospital	122
The Adelaide and Meath Hospital, Dublin	97
St Columcille's Hospital	13
Temple Street Children's University Hospital	7
Mount Carmel Hospital	1
Rotunda Hospital Dublin	1
National Maternity Hospital, Holles Street	1
Blackrock Clinic	1
Total	**4210**

***TABLE 2.3:** Requests from hospitals nationwide (excluding Dublin)*

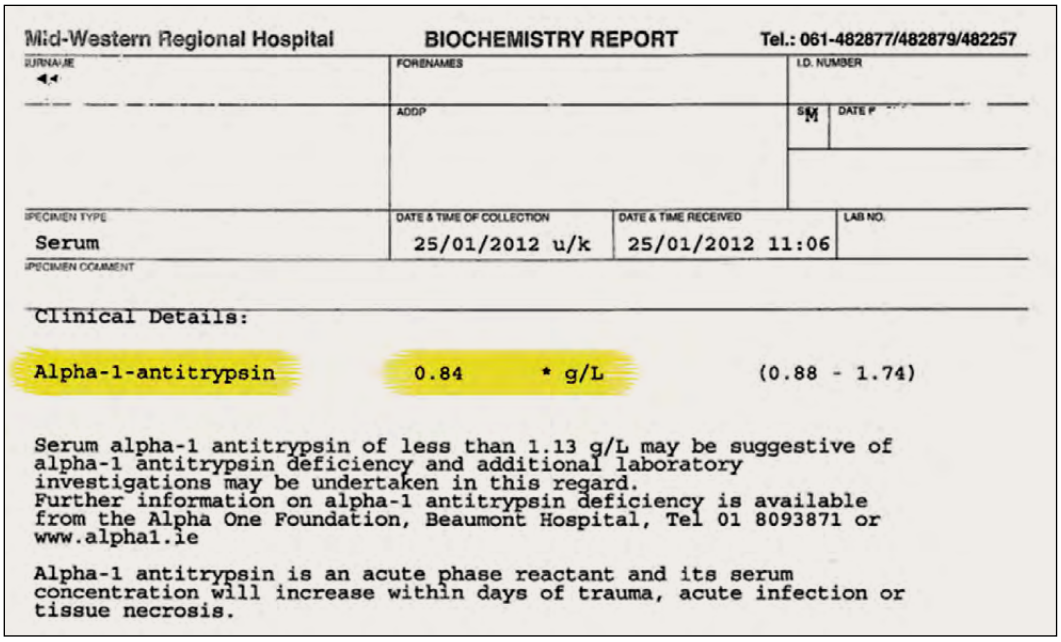
Nationwide	Requests
Cork University Hospitals	891
Sligo General Hospital	700
Letterkenny General Hospital	649
Cavan General Hospital	347
Bon Secours Tralee	233
Midland Regional Hospital Mullingar	177
Galway University Hospitals	126
Our Lady of Lourdes Hospital Drogheda	103
Mid-Western Regional Hospital Limerick	79
Midland Regional Hospital Tullamore	60
Roscommon County Hospital	43
Waterford Regional Hospital	31
Mayo General Hospital	15
Monaghan General Hospital	8
Louth County Hospital	2
Clane General Hospital	1
Bon Secours Galway	1
Total	**3467**

The targeted detection screening programme has received 3467 requests nationwide (excluding Dublin). The largest participating centre is Cork University Hospital (CUH), and 25% of all test requests from outside the Dublin area are from CUH (Table 2.3). The second biggest participating centre outside of Dublin is Sligo General Hospital (20%), closely followed by Letterkenny General Hospital (18%).

In the past 12 months we have presented results from our screening programme to the respiratory teams in Sligo and Tallaght Hospitals, and at grand rounds in Cork University Hospital and UCH Galway. In addition, we have presented to TCD postgraduate students from the M.Sc. in Clinical Chemistry course. The main aim of these presentations is to increase awareness of AATD amongst the respiratory and paramedical community. While the respiratory teams are dealing with the patient populations most at risk due to AATD, the Immunology, Biochemistry, and Clinical Chemistry Departments often measure AAT levels as a routine test during normal blood investigations.

Furthermore, in an excellent example of joined-up thinking several laboratories have adopted a "red flag" system for AAT testing. This system means that if AAT concentrations are measured by a routine laboratory and found to be below a certain threshold (1.13 g/L), an automatic suggestion or "red flag" is included on the laboratory report which recommends testing for AATD (Figure 2.6). This threshold has been calculated in a large Swiss study to achieve the greatest sensitivity, specificity, and cost-efficiency in the detection of deficient phenotypes (Zorzetto et al, Clinical Chemistry 2008). It is hoped that an electronic prompt system will lead to earlier diagnosis of AATD cases. The ultimate goal would be the adoption of this red flag system on AAT reports in every hospital in Ireland.

FIGURE 2.6: *Example of an electronic prompt on a laboratory AAT report*



Mid-Western Regional Hospital — BIOCHEMISTRY REPORT — Tel.: 061-482877/482879/482257

SURNAME | FORENAMES | I.D. NUMBER

ADDR | SEX | DATE OF BIRTH

SPECIMEN TYPE: Serum | DATE & TIME OF COLLECTION: 25/01/2012 u/k | DATE & TIME RECEIVED: 25/01/2012 11:06 | LAB NO.

SPECIMEN COMMENT

Clinical Details:

Alpha-1-antitrypsin	0.84	* g/L	(0.88 - 1.74)

Serum alpha-1 antitrypsin of less than 1.13 g/L may be suggestive of alpha-1 antitrypsin deficiency and additional laboratory investigations may be undertaken in this regard.
Further information on alpha-1 antitrypsin deficiency is available from the Alpha One Foundation, Beaumont Hospital, Tel 01 8093871 or www.alpha1.ie

Alpha-1 antitrypsin is an acute phase reactant and its serum concentration will increase within days of trauma, acute infection or tissue necrosis.

FIGURE 2.7: *AAT test requests received from August 2011 - August 2012*

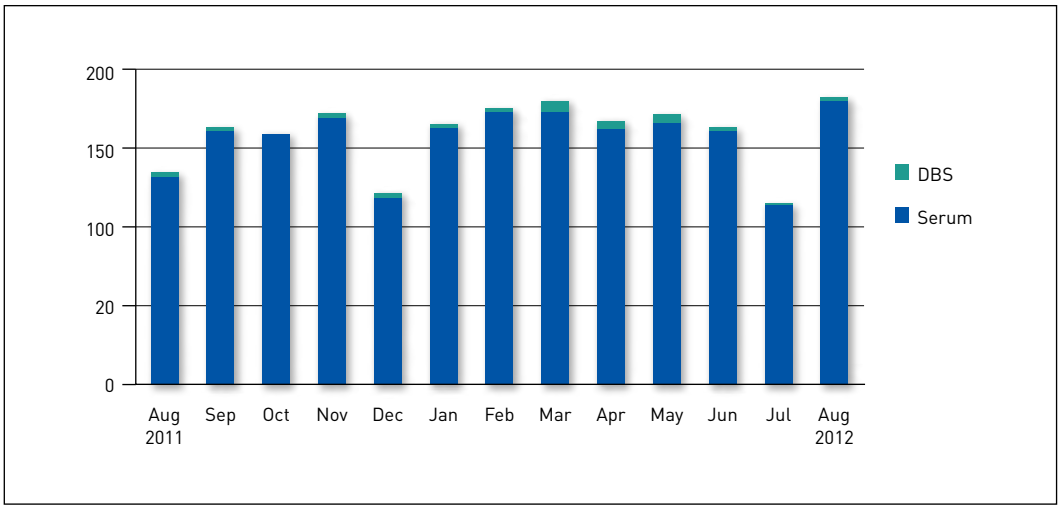


FIGURE 2.8: *Example of a typical alpha-1 antitrypsin test report*

Alpha-1 Antitrypsin Deficiency Report - 0811,

Report Details

Patient ID:	2340
Name:	0811,
Gender:	Female
Date Of Birth:	Unknown
MRN:	Not Available
Laboratory Number:	
Consultant:	Dr UK NEQAS
Hospital:	
Date Sample Taken:	13/02/2008
Genotype:	
Phenotype:	MZ
AAT Level:	0.74 g/L (Normal range 1.13 - 2.0g/L)
AAT % of normal:	44 % (Normal range 65-125%)

Comments:

All data reported by Dr. Tomas Carroll and Laura Fee

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.

Referrals can be made to the dedicated Alpha-1 Clinic in Beaumont Hospital under the care of Professor N.G. McElvaney, Respiratory Physician, Beaumont Hospital, Dublin 9.

Alpha-1 Antitrypsin (AAT) Deficiency

AAT-deficient individuals are at risk of developing life-threatening lung and liver disease because they have reduced amounts of this key antiprotease. The normal AAT protein is called the M variant which is synthesised in the liver and present in sufficient amounts to provide a protective antiprotease screen in the lung. As AAT is an acute phase protein it can be elevated during infection and inflammation. The most common severely deficient variant is Z, which causes decreased circulating levels of AAT. The Z AAT protein folds incorrectly and accumulates within the liver, preventing its release and leading to reduced blood and lung levels. Z AAT accumulation can also cause liver disease. ZZ individuals who inherit 2 defective AAT genes have 5-15% of normal AAT levels, while MZ individuals (1 normal, 1 deficient) have 50-70% of normal AAT levels. The less severe S AAT variant is associated with a milder deficiency, and is only clinically significant when co-inherited with another deficient variant. SS individuals are predisposed to developing lung disease but not liver disease, while MS carriers (1 normal, 1 deficient) possess almost normal AAT levels. Another clinically significant genotype is SZ (2 deficient AAT variants) with circulating AAT levels decreased to 25-40% of normal, and these individuals have an increased risk of lung and/or liver disease. There are at least 50 other rare variants of the AAT protein, such as I, F, and Null variants, which confer varying degrees of deficiency. These variants usually become clinically significant when co-inherited with other deficient variants.

The most important thing to remember is that cigarette smoke is the single biggest risk factor for lung disease in AAT-deficient individuals.

Status	AAT Phenotype / AAT Genotype*	What Does It Mean?
Normal	MM	Does not have the disorder and does not have any altered AAT genes
Carrier	MS	Evidence suggests no increased risk of disease but does carry altered AAT gene.
Carrier	MZ	Has a mild AAT deficiency - may possibly develop disease symptoms (particularly if smoking) and does carry altered gene.
AAT Deficiency	SS/SZ/ZZ	Moderate to severe AAT deficiency - will probably develop symptoms (particularly if smoking) and carries two altered AAT genes

*A phenotype is reported when testing is on serum – a genotype is reported when testing is on DNA.

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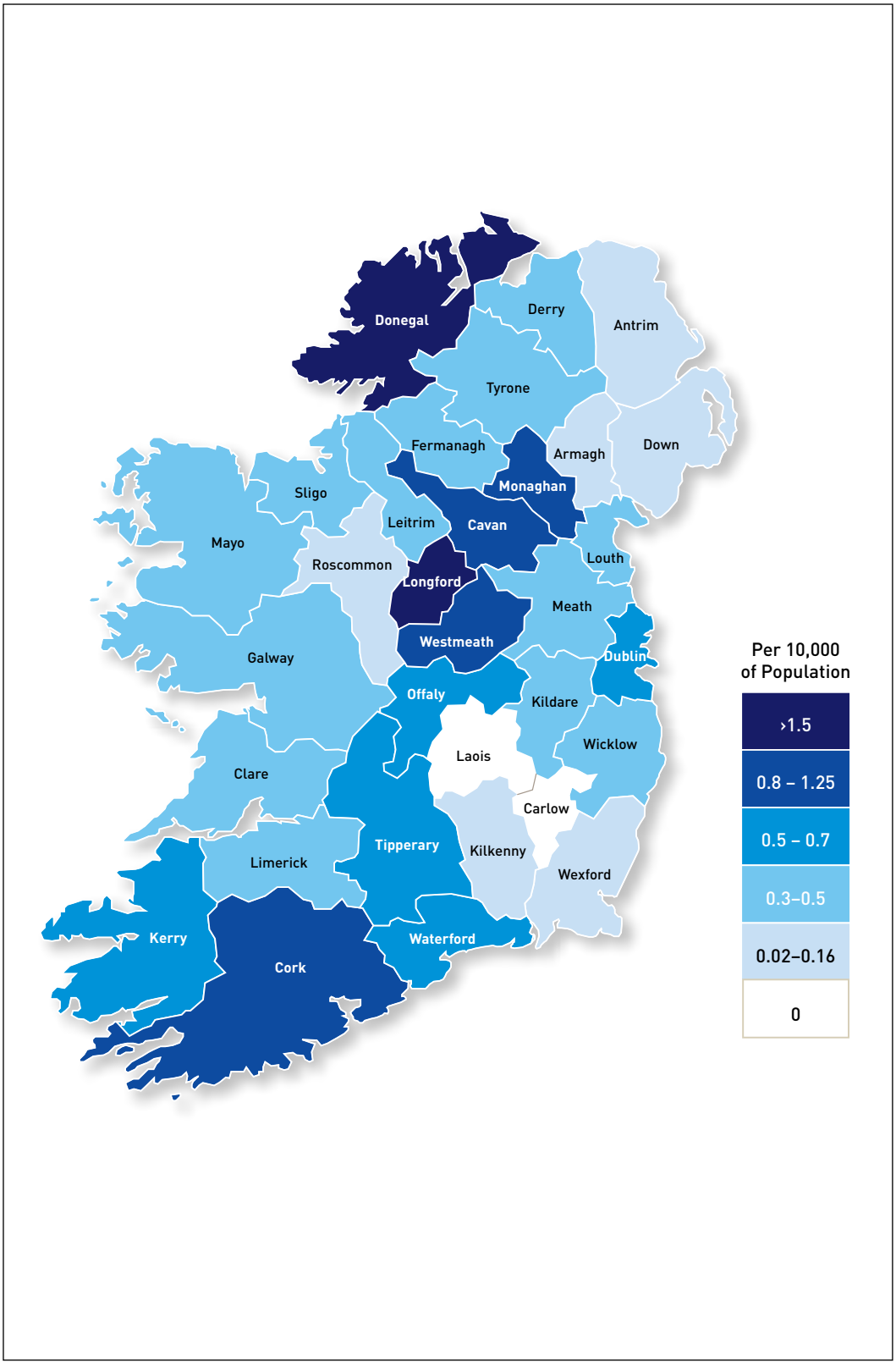
Web: www.alpha1.ie
Participant in the UK NEQAS for Alpha-1 Antitrypsin & Phenotype programme.

FIGURE 2.9: *Geographical distribution of severe AATD cases (ZZ and SZ) detected per 10,000 of population (a bias may exist for some counties depending on the number of samples received for testing)*



3. Rare Alpha-1 Antitrypsin Variants 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, over 100 other rare mutations have been identified in the AAT gene and a number of these rare variants have been discovered in the Irish population (Tables 3.1 and 3.2). It is precisely because these mutations are so rare that difficulties arise in their correct identification and this can affect the diagnosis of suspected AATD individuals. In addition, we have discovered 2 completely novel Null (Q0) mutations. Null mutations are extremely rare and lead to a complete absence of AAT in serum.

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 prevalence of cystic fibrosis and haemochromatosis in Europe. Therefore, it is not surprising that we also have a high prevalence of AATD in Ireland compared to many other countries in Europe. For example, we now know that 1 in 25 Irish individuals carry the Z AAT variant and 1 in 10 carry the S AAT variant. In addition, evidence is emerging that we have a high prevalence of rare and novel AAT mutations in Ireland.

MOLECULAR BASIS OF AATD

The majority of individuals carry two copies of the normal AAT gene, termed M, and are designated as having the genotype MM. The technique of starch gel electrophoresis originally used to separate AAT variants is responsible for the nomenclature used to

TABLE 3.1: *Selected AAT variants associated with lung and/or liver disease.*

Varient	Molecular Basis	Effect	Disease Risk
Z	GAC - AAG, Glu342Lys	Polymerisation, impaired secretion and severe plasma deficiency	Lung and liver
Siiyama	TCC - TTC, Ser53Phe	Polymerisation, impaired secretion and severe plasma deficiency	Lung and liver
Mmalton	ΔTTC, ΔPhe52	Polymerisation, impaired secretion and severe plasma deficiency	Lung and liver
Null (Q0)	Mutations causing gene deletion, premature stop codon or mRNA degradation	No AAT production	Lung
S	GAA - GTA, Glu264Val	Impaired secretion and mild plasma deficiency	Lung and liver (in compound heterozygotes e.g. SZ)
I	CGC - TGC, Agr39Cys	Impaired secretion and mild plasma deficiency	Lung and liver (case reports in compound heterozygotes e.g. IZ)
F	CGT - TGT, Agr229Cys	Defective neutrophil elastase inhibition	Lung (case reports in compound heterozygotes e.g. FZ)
Zbristol	ACG - ATC, Thr85Met	Defective glycosylation and impaired secretion	Lung (case report in compound heterozygote Z/Zbristol, unpublished observation)

identify the earliest described variants. These variants were originally designated according to their migration speed, for example M (medium), S (slow), and F (fast). As technology advanced and proteins began to be separated on the basis of their isoelectric point, the nomenclature system was revised so AAT variants were designated with earlier letters of the alphabet if displaying anodal migration and later letters of the alphabet if displaying cathodal migration. Furthermore, as the letters of the alphabet were exhausted, places of origin began to be used in addition to the letter of the closest anodal allele that a new variant resembled. More precisely, the birthplace of the first individual to carry a novel variant is used, for example Q0cairo was used to describe a novel Null mutation found in the first recognised case whose birthplace was Cairo (Zorzetto et al. 2005).

Mutations in the AAT gene that confer an increased risk for the development of COPD and/or liver disease are those in which deficiency or Null alleles are combined in homozygous or heterozygous states. The two most common mutations associated with disease in populations of European descent are the Z (Glu342Lys, rs28929474) and S (Glu264Val, rs17580) mutations, both caused by a single amino acid replacement of glutamic acid at positions 342 and 264 of the mature protein, respectively.

RARE AAT VARIANTS DETECTED IN THE IRISH POPULATION

A number of rare SERPINA1 mutations including I, F, Zbristol, and Mmalton were detected by the screening programme. The I mutation (Arg39Cys) is present at a relatively high frequency (0.0043) in Ireland. 72 cases have been identified, including heterozygotes (MI) and compound heterozygotes (IS and IZ). The F mutation (Arg223Cys) has been found in 21 cases. In addition, 2 novel Null mutations were identified, Q0dublin and Q0cork, as well as the previously described Q0bolton variant, discovered in the first Null homozygous individual to be reported in Ireland.

The effect of the I and F mutations on the AAT molecule has been described, but to date any COPD risk associated with these mutations is limited to case reports describing compound heterozygotes (for example IZ and FZ phenotypes). The I (Arg39Cys) variant is associated with a milder plasma deficiency. The point mutation underlying this variant causes less disruption compared to the Z mutation. Thus, the rate of polymer production is much slower than Z AAT, leading to less retention of protein within liver cells, milder plasma deficiency, and a minimal risk of disease in heterozygotes. However, there is a risk of disease in compound heterozygotes. If a mild, slowly polymerising I variant of AAT is inherited with a rapidly polymerising Z (or Mmalton) variant, the two variants when co-expressed can interact to form heteropolymers within hepatocytes, leading to cirrhosis and plasma deficiency (Mahadeva et al. 1999).

The point mutation in the F (Arg223Cys) variant introduces a cysteine instead of an arginine, the same amino acid substitution

***TABLE 3.2:** Rare AAT mutations identified in the national targeted detection programme*

Varient	Molecular Basis	Cases	Cellular Effect	Disease Association
I	Arg39Cys	72	Intracellular accumulation	Lung, liver
F	Arg223Cys	21	Reduced antiprotease activity	Lung
Mmalton	ΔPhe51 or 52	5	Intracellular accumulation and polymerisation	Lung, liver
Q0dublin	cod370Phe - ΔT - 373Stop	4	No AAT produced	Lung
Zbristol	Thr85Met	1	Intracellular accumulation and defective glycosylation	Lung, liver
Q0cork	cod180Thr - ΔAC - 190Stop	1	No AAT produced	Lung
Q0bolton	cod362Pro - ΔC - 373Stop	2	No AAT produced	Lung

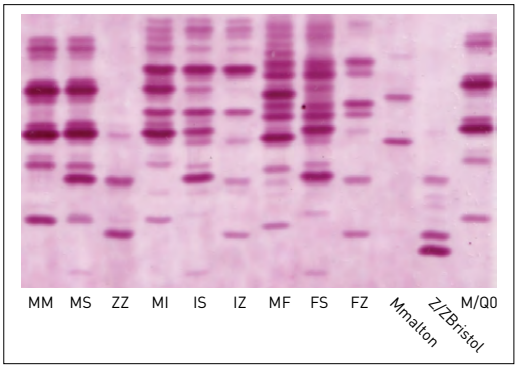


FIGURE 3.1: *Rare AATD phenotypes detected in the Irish population on an isoelectric focusing (IEF) gel (Sebia). MM, MS, and ZZ standards are included for comparison. The Mmalton variant depicted is from an individual homozygous for this mutation*

that underlies I variants. The normal M AAT protein has a single cysteine residue and the introduction of a second cysteine potentially favours the formation of disulphide bonds both intramolecularly and intermolecularly with other AAT molecules. Interestingly, and possibly a reflection of the extra cysteine residue, the major F bands run as doublets on IEF gels (Figure 3.1). In the disease context, the inhibitory activity of F AAT protein against neutrophil elastase (NE) is reduced. This suggests that individuals who co-inherit the F allele with another severe deficiency allele such as Z or Null would have a significant risk for the development of COPD. The rate of polymerisation of the F variant has not been investigated but it may well exhibit a higher rate of polymerisation than M AAT. A case report from an Irish group in 1989 described finding hepatomegaly and globules positive for AAT in a liver biopsy from an FZ individual with emphysema (Kelly *et al.* 1989). Unfortunately, there have been no reports published to date describing F homozygotes (or I homozygotes) as these might shed some light on the polymerigenicity of the F protein and associated risk of lung and liver disease.

The Mmalton mutation causes the AAT protein to accumulate within liver cells, and this leads to a plasma deficiency. This in turn leads to a high risk of both lung and liver disease. In this regard, Mmalton is very similar to the more common Z mutation. Interestingly, Mmalton is relatively common on the island of Sardinia in the Mediterranean. The Zbristol mutation is extremely rare but is thought to cause defective glycosylation of the AAT protein.

The class of AAT mutations termed "Null" or Q0 cause a complete stop in AAT production and while ultra rare, confer a particularly high risk of emphysema. As these mutations do not cause polymerisation of the AAT protein they pose no risk of liver disease. Most frequent among this class are those mutations that introduce a premature stop codon, and the three Null variants discovered in Ireland belong to this category (Q0dublin, Q0cork, and Q0bolton).

CONCLUSION

The high prevalence of rare AAT mutations in Ireland highlights the importance of a comprehensive diagnostic work up of all

FIGURE 3.2: Rare AATD phenotypes and corresponding AAT concentrations

Phenotype	Cases	Mean AAT (g/L)	Range AAT (g/L)
MI	55	1.39	0.86–2.93
IZ	11	0.60	0.49–0.90
MF	16	1.50	1.09–2.26
IS	6	1.00	0.80–1.31
FZ	4	0.89	0.74–1.16
FS	1	1.3	n/a
M/Q0dublin	4	0.80	0.64–1.11
M/Q0bolton	1	0.73	n/a
M/Q0cork	1	0.70	n/a
Z/Z0bristol	1	0.50	n/a
Mmalton/Mmalton	1	0.19	n/a
Q0bolton/ Q0bolton	1	Not detectable	n/a

patients with low AAT levels. A low AAT level (< 1.13 g/L) indicates the presence of a mutation in the AAT gene and should be phenotyped to identify the mutation(s). However, rare mutations often require a more comprehensive genetic analysis. To confirm the presence of a rare mutation, DNA from the individual should be analysed by sequencing the complete AAT gene. This procedure is performed in the University of Pavia in Italy by Dr Ilaria Ferrarotti, Dr Stefania Ottaviani, and Prof Maurizio Luisetti, and is the leading European laboratory in the identification of rare and novel AAT mutations. Often, rare mutations can be missed or incorrectly identified as M using some of the more readily available commercial genotyping assays which detect only Z and S. On account of our fruitful collaboration with the University of Pavia, the Alpha One Foundation is in a strong position to diagnose rare and novel AAT mutations in the Irish population.

4. The National Alpha-1 Antitrypsin Deficiency Patient Registry

The Alpha-1 registry is a confidential database that collects information on individuals with alpha-1 antitrypsin deficiency. It gathers information on all aspects of the condition, for example lung and liver test results and smoking history. When patients attend the Alpha-1 clinic their permission is sought to allow their medical chart details to be entered on the Alpha-1 registry. Patients from 29 counties in Ireland have been included on the registry to date. The process is ongoing and we hope to include as many Alpha-1 individuals as possible.

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 withdraw their consent at a later date. This registry is a very important tool for clinical research and increasing our understanding of Alpha-1.

WHO PARTICIPATES IN THE ALPHA-1 REGISTRY?

All patients with Alpha-1 and Alpha-1 carriers residing in Ireland can participate in the registry. The more individuals that participate, the greater the quality of the data that can be generated from the registry.

WHAT IS THE REGISTRY?

The Alpha-1 registry is a confidential database, containing information from individuals diagnosed with alpha-1 antitrypsin deficiency (Alpha-1) and 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 as a condition and facilitate the development of new treatments. For example a well managed patient registry can be used to design a clinical trial to test a new drug.

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

The registry is encrypted so it cannot be accessed by anyone unless they have an encryption key. The registry is also password protected. Alpha One Foundation staff members 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 relevant medical 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 while you give consent. These questions cover family history, for example if close family members have been tested and if Alpha-1 has affected your occupation.

WHAT HAPPENS IF YOU DON'T GIVE CONSENT?

Participation in the registry is voluntary. If you prefer not to be on the registry there will be 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 already captured on the registry will be removed.

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

Laura Fee, Clinical Research Associate, Alpha-1 Suite, Beaumont Hospital, Dublin 9. Telephone: (01) 809 3702. Email: *alpha1@rcsi.ie*

UPDATE ON THE NATIONAL ALPHA-1 PATIENT REGISTRY

The National Alpha-1 Patient Registry was established in 2007 to collect information on Alpha-1 individuals throughout Ireland. This database stores valuable clinical and demographic information on AATD individuals which improves our understanding of the condition, facilitates clinical research, and helps in the design of clinical trials.

DEMOGRAPHIC CHARACTERISTICS

There are currently 219 individuals enrolled on the Alpha-1 Patient Registry including ZZ, SZ, MZ, SS, and MS individuals, as well as some rare phenotypes. The largest group on the registry is the ZZ cohort (n = 98) of which 58% are male and 42% are female. The mean age at symptom onset was 37.6 +/- 1.6 years, while the mean age of diagnosis was 44.1 +/- 1.6 years (range 0.3 - 80) (Table 4.1). The following sections will describe some of the findings in the ZZ and SZ AATD populations.

***TABLE 4.1:** Demographic and clinical characteristics of ZZ individuals on the Alpha-1 Registry*

ZZ Individuals	Number	%
Mean age (years)	51	n/a
Gender (n=98)		
Male	43	43.9
Female	55	56.1
Ascertainment (n=98)		
Pulmonary symptoms	52	53.0
Family	33	30.7
Other	13	16.3
Mean age at symptom onset	37.6 +/- 1.6 years	n/a
Mean age at AATD diagnosis	45.3+/- 1.7 years	n/a
Smoking Status (n=94)		
Never	28	29.8
Ex-smoker	62	65.9
Active	4	4.3
Average FEV1 % predicted (n=92)	59.43	
Average DLCO % predicted (n=34)	17.6	
Liver Function Tests (n=15)		
Normal	7	46.7
Elevated liver enzymes	8	53.3
Transplant Recipients (n=3)		
Lung	1	33.3
Liver	2	66.7

FIGURE 4.1: *Reason for Diagnosis in ZZ individuals on the National Registry*

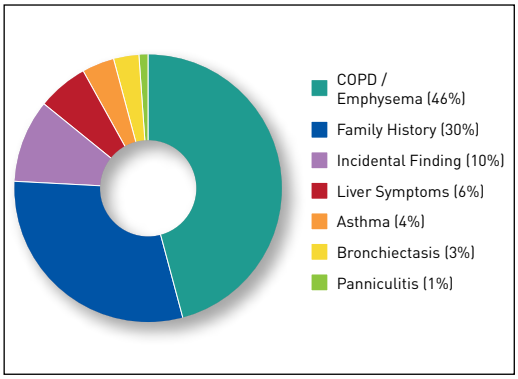


FIGURE 4.2: *High resolution CT scan findings among ZZ AATD individuals*

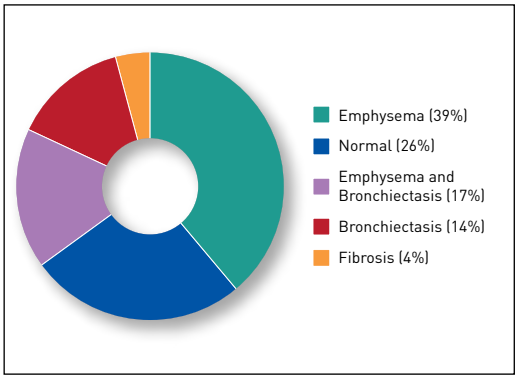


FIGURE 4.3: *High resolution CT scan findings among SZ AATD individuals*

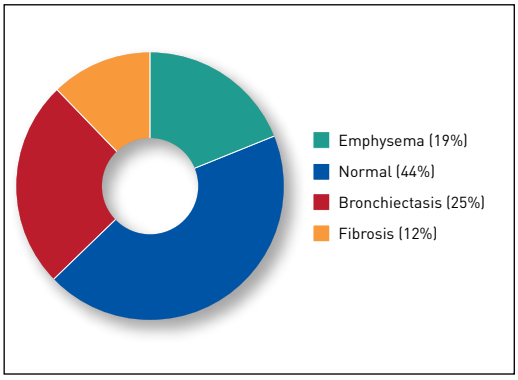
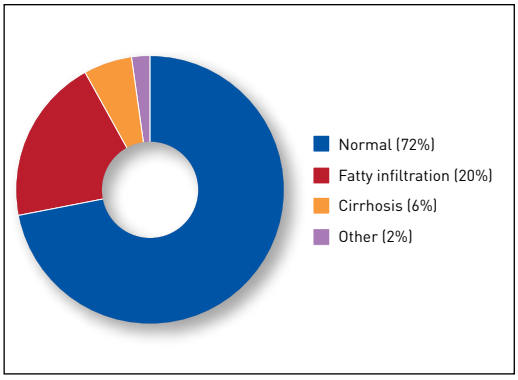


FIGURE 4.4: **Liver ultrasound findings among ZZ AATD individuals**



ASCERTAINMENT

The mode of ascertainment for ZZ AATD individuals on the registry was analysed. Pulmonary symptoms (53%) and family screening (30%) were the predominant reasons for a diagnosis of ZZ AATD. Other reasons for a diagnosis of AATD include liver disease, elevated liver function tests and panniculitis (Figure 4.1).

HIGH RESOLUTION COMPUTERISED TOMOGRAPHY FINDINGS

High Resolution Computerised Tomography (HRCT) of the thorax is performed to analyse lung structure and to confirm the presence of emphysema. HRCT data was available for 93 ZZ AATD individuals on the registry. The predominant HRCT findings were emphysema (39%), bronchiectasis (14%) and fibrosis (4%) (Figure 4.2). 26% of ZZ AATD individuals had normal HRCT scans.

Of the 28 SZ individuals on the registry, HRCT data was available for 16 individuals (Figure 4.3). 44% (n=7) had normal scans and showed no signs of lung disease while the remaining 56% (n=9) showed varying degrees of bronchiectasis, emphysema, and changes consistent with fibrotic lung disease.

LIVER ULTRASOUND FINDINGS

Liver ultrasound findings were available for 66 ZZ AATD individuals on the registry. 73% were normal, however, 28% had pathological evidence of liver disease (Figure 4.4).

PULMONARY FUNCTION TEST FINDINGS

Pulmonary function testing (PFT) is a formal examination carried out to measure lung function and is routinely conducted on all patients. The 'forced expiratory volume in one second per litre' (FEV1) in a patient is measured using a spirometer and FEV1 % predicted is a measurement which provides evidence of airflow obstruction, a hallmark of COPD.

EFFECT OF SMOKING ON LUNG FUNCTION

Registry PFT data informs us that ZZ AATD individuals with a history of smoking had

***FIGURE 4.5:* Effect of smoking status on FEV_1 (% predicted) in ZZ AATD individuals (n = 90).** *There was no significant difference between mean age of the ever-smoking cohort (n = 62, 50.9 +/- 1.4 years) and mean age of the never-smoking cohort (n = 28, 50.0 +/- 2.9 years)*

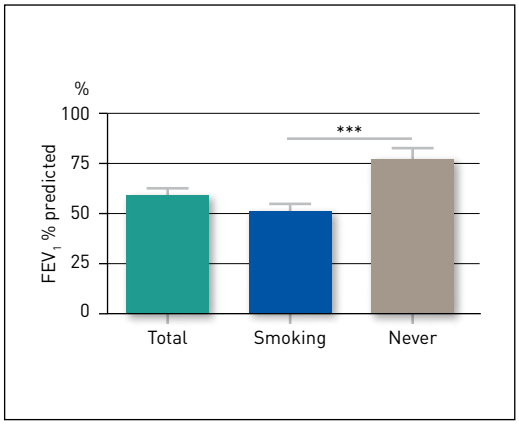


***FIGURE 4.6:* Effect of smoking status on FEV_1 (% predicted) in SZ AATD individuals (n = 23).** *The mean FEV_1 of the ever-smoking cohort was 77.9 +/- 3.4% (n = 10) and mean FEV_1 of the never-smoking cohort was 102.0 +/- 3.7 (n = 13)*

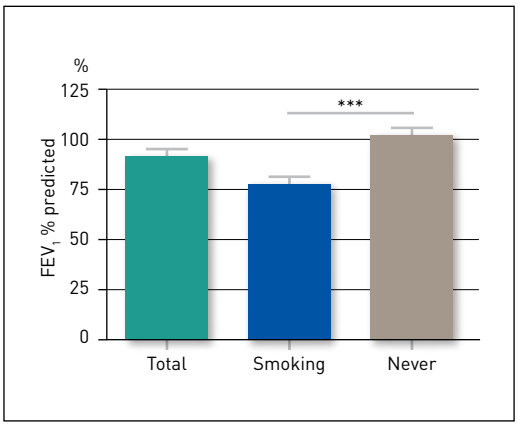
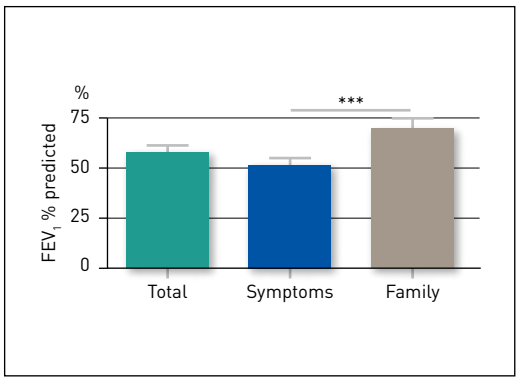


***FIGURE 4.7:* FEV_1 of ZZ AATD individuals identified by symptomatic screening compared to family screening.** *The mean age of the symptomatic cohort was 46.1 +/- 1.4 years compared to 41.2 +/- 2.8 years in the family screened cohort, while 77% of the TDP cohort was ever-smoking compared to 65% of the family screened cohort*



significantly decreased lung function compared to never-smoking ZZ AATD individuals (mean FEV1 of ever-smokers 51.4 +/- 3.6% v never-smokers 77.5 +/- 5.3%, p<0.0001) (Figure 4.5). A similar trend was observed for SZ AATD individuals, with cigarette smoke significantly impairing lung function (Figure 4.6).

This data highlights the key role of cigarette smoke in the development of lung disease in AATD individuals. It has been shown that smoking can reduce the life expectancy of a ZZ patient by up to 25 years (The US Alpha-1-Antitrypsin Deficiency Registry Study Group, 1998). All AATD patients, including ZZ, SZ, and MZ phenotypes, need to be educated about the harmful effects of cigarette smoke. Smoking cessation and the avoidance of occupational and environmental exposures (for example particulate matter, chemical vapours, and agricultural dusts) is paramount in AATD patient education (ATS/ERS guidelines, 2003). AATD individuals without apparent lung disease should also be encouraged to quit smoking as this cohort offers the most realistic chance of delaying or in some cases preventing the development of COPD. Another important benefit in diagnosing a COPD patient with AATD is that he/she is twice as likely to attempt to quit smoking compared to an AAT-replete, smoking-related COPD patient (Carpenter et al. 2007). Carpenter et al. demonstrated that knowledge of AATD motivates smokers towards cessation when compared with COPD patients. For this reason, the most important decision a newly-diagnosed ZZ individual can make is to give up smoking.

However, even in the absence of smoking history there exists a significant risk for COPD. The first study to investigate a non-smoking ZZ cohort observed marked variability in both clinical course and lung function decline (Black and Kueppers 1978). Another US study showed that exposure to second-hand tobacco smoke in childhood can accelerate the onset of Symptoms in ZZ AATD individuals (Mayer et al. 2006). A study from the Swedish registry demonstrated that while non-smoking ZZ individuals may not develop COPD until later in life, this cohort still displays a decline in lung function (FEV1) with age, especially after the age of 50 (Piitulainen,

Tornling, and Eriksson 1997). A follow up study by the same group found that an agricultural occupation was associated with decreased lung function in non-smoking ZZ individuals (Piitulainen, Tornling, and Eriksson 1998). Passive smoking was also associated with an increased frequency of chronic bronchitis, but not with impaired lung function in this study. It is clear that the ZZ individuals at highest risk due to occupation include farmers, welders, chemical factory workers, painters, and firemen.

EFFECT OF ASCERTAINMENT ON LUNG FUNCTION

ZZ AATD individuals identified through family screening have significantly increased FEV1 % predicted (70.0 +/- 5.3%) compared to ZZ AATD individuals identified by targeted screening (50.8 +/- 4.2%, p= 0.0072) (Figure 4.7). While impaired lung function would be expected in the symptomatic group, the preserved lung function in the family screened cohort despite similar age and smoking history, highlights the importance of family screening as a tool for early detection and possible prevention of COPD in ZZ individuals. The findings from the Irish registry are supported by a study from the Danish registry which found that non-index ZZ individuals had longer estimated life expectancies when compared to index (symptomatic) cases (Seersholm, Kok-Jensen, and Dirksen 1994). More recently, data from the Swedish registry showed that ZZ individuals identified by family screening had longer median survival times compared to ZZ individuals detected by symptomatic (respiratory and non-respiratory) screening (Tanash et al. 2010).

This data underlines how important an early diagnosis can be in preventing or postponing the development of lung disease. Many of the early guidelines for AATD screening advocated testing younger COPD patients and this is to the detriment of the larger COPD population of all ages. The age at which manifestations of airway obstruction, pulmonary emphysema, or chronic bronchitis appear in ZZ individuals is highly variable (The Alpha-1-Antitrypsin Deficiency Registry Study Group, 1998). While a common presentation of AATD is indeed early onset COPD, a subset of ZZ patients do not develop symptoms until much later in life, particularly if non-smokers (Campos, Alazemi, Zhang, Salathe, et al. 2009). In fact, among ZZ never-smokers the risk of liver disease increases with age (Tanash et al. 2008; Willson, Seow, and Zimmerman 2004). Numerous case reports have described AATD in elderly individuals with COPD who were lifelong never-smokers (Jack and Evans 1991). Taken together, it is clear that screening for AATD should be performed in all patients with COPD regardless of advanced age or smoking history, especially as failure to do so has clinical repercussions for undiagnosed family members.

FOOTNOTE

This section has been prepared with the grateful assistance of Seshma Ramsawak and Ashling Ní Chinnéide, RCSI undergraduate medical students who carried out SSC projects in 2012.

5. Research Studies and Clinical Trials

CLINICAL TRIALS IN ALPHA-1 ANTITRYPSIN DEFICIENCY

Alpha 1 antitrypsin intravenous augmentation therapy clinical trial

This study is being conducted at Beaumont Hospital by Professor N.G. McElvaney and his research team. This is a placebo-controlled, double blinded, multicentre, phase III/IV study. The aim of this study is to assess the safety and efficacy of the drug Zemaira® in patients with Emphysema due to Alpha 1 antitrypsin deficiency. The duration of this study is 2 years.

There have been 24 patients who have participated in this study.

The trial involves patients receiving weekly infusion of Zemaira®/placebo. The placebo is a dummy treatment that looks like the real thing but it has no active ingredient. Neither the patients nor the staff knows which treatment is being given (double-blinded). There was an equal chance for everyone to receive either Zemaira® or placebo.

The infusion is given either at Beaumont hospital or in the patients own home and takes on average 20 minutes to infuse.

All participants on the clinical trial are seen at Beaumont Hospital every 3 months so they can be routinely monitored. Monitoring assessments include:

- Blood tests
- Pulmonary function tests
- Physical examination by a physician
- Cotinine test (urine test to detect nicotine)
- CT scans
- Quality of life questionnaire.

These measurements allow the effect of Zemaira®/placebo to be measured and assess the effect it has on the patient's health, most notably their emphysema.

Following the completion of the blinded phase of the study all patients are invited to continue for 2 more years on the open label phase of the study. In this phase all participants receive Zemaira®. Recruitment for this study has now closed.

Alpha 1 antitrypsin inhaled augmentation therapy clinical trial

Professor N.G. McElvaney and his team are conducting a clinical trial looking at the safety and efficacy of inhaled alpha 1 antitrypsin (AAT) replacement therapy/placebo. This is a double blinded study, therefore neither the patients nor the staff know whether the participants are getting Kamada AAT® or placebo.

15 patients have participated in this study to date.

Kamada AAT® is a liquid preparation of human AAT that can be aerosolised (inhaled). The inhalation is given using an eflow device. The human AAT protein is taken from human blood plasma sources collected in the USA. The blood is processed and filtered according to strict standards to produce the human AAT protein used in Kamada AAT®. The administration of AAT in this way is thought to reach the target organ i.e. the lungs directly and require a much lower dose of therapeutic AAT. Self administration by inhalation is also much simpler and less stressful for the patients than that of the administration of an IV infusion.

The study lasts for 54 weeks with participants having 9 visits in that time frame. At the patient visits a number of assessments will be carried out such as:

- Blood tests
- Pulmonary function tests
- Physical examination by a physician
- Quality of life questionnaires

These assessments allow for the effect of Kamada AAT®/placebo to be measured and assess the effect it has on the patients health.

For further information on the clinical trials please contact the Alpha 1 research nurse, Grace Mullins on 01-8093864 or *gracemullins@rcsi.ie*

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

Project Description: This clinical research study, to clarify the risk of COPD in MZ individuals,

commenced in July 2007 and is supervised by Professor N.G. 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 II-IV COPD into this study. The inclusion criteria for PIMZ carriers are as follows:

- Age >30
- GOLD Stage II-IV COPD (post-bronchodilator FEV1 <80% predicted; FEV1/FVC ratio 0.7)
- Confirmed PIMZ genotype
- No other lung diseases that would affect pulmonary function testing (PFT)

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

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

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

Our goal is to include as many siblings and parents from each family to participate in this ground-breaking clinical research study. We will determine whether the PI MZ carrier status is associated with an increased risk of COPD and whether cigarette smoking confers an increased risk of COPD in carriers of Alpha-1 antitrypsin deficiency.

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

Dr Kevin Molloy, MB, Bch, BAO, Clinical Researcher, Alpha One Foundation, RCSI Building, Beaumont Hospital, Dublin 9

Tel: +353-1-809-3801
Mob: +353-86-776-3943
Email: kmolloy@rcsi.ie

GRANTS AWARDED

Title: **The role of alpha-1 antitrypsin deficiency heterozygosity in COPD**

Funding body: **American Thoracic Society and the US Alpha-1 Foundation**

Period: *2 years from October 2011*

Principal Investigator: *Dr Tomás P. Carroll*

Co-applicants: *Professor N. G. McElvaney & Dr David Bergin*

Abstract: Alpha-1 antitrypsin deficiency (AATD) is a hereditary disorder characterised by low levels of the antiprotease alpha-1 antitrypsin (AAT) and is associated with chronic obstructive pulmonary disease (COPD). This is characterized by neutrophil-dominated airway inflammation and elevated intra-pulmonary protease levels. The two most common

SERPINA1 mutations associated with AATD are the Z and S mutations, however, the vast majority of AATD individuals with COPD are ZZ homozygotes. Crucially, there is a lack of clarity pertaining to MZ and SZ heterozygotes and their risk of COPD. This is a vital clinical and public health question, as there are over 7.7 million MZ and 250,000 SZ individuals in the US alone.

The central hypothesis of this research proposal is that MZ and SZ heterozygotes are at increased risk of developing COPD through immune derangement. Although the majority of AAT is derived from the liver, both monocytes and neutrophils secrete AAT. Previously, we have demonstrated that monocytes from ZZ individuals display abnormal immune responses and exhibit ER stress and ZZ neutrophils display increased chemotaxis. These findings raise the possibility of aberrant immune function in AATD heterozygotes.

We plan to evaluate immune function and quantify ER stress indices in peripheral blood monocytes and neutrophils isolated from healthy MZ and SZ individuals. Importantly, this approach does not require invasive biopsy or bronchoalveolar lavage procedures.

The ZZ AATD individual is relatively well-studied; however, there is a paucity of information regarding MZ and SZ individuals. The precise functional consequences of heterozygosity in cells producing AAT are unknown. An important gap in our existing knowledge will be addressed by this study which could explain the predisposition of MZ and SZ individuals to COPD. If ER stress and aberrant immune responses are observed in heterozygotes, strategies targeting ER stress pathways may become viable therapeutic options.

Title: **Control of lipid induced signalling in neutrophils: therapeutic properties of alpha-1 antitrypsin**

Funding Body: **MRCG/HRB Joint Funding Scheme (3 years funding commencing November 2011)**

Principal Investigator: *Dr Emer Reeves, PhD MSc*

Co-Investigator: *Prof Noel G McElvaney*

Alpha-1-antitrypsin (AAT) deficiency (AATD) is largely unrecognized and under diagnosed. This hereditary disorder results in the rapid progression of lung disease, especially in smokers. Specific treatment for this disorder is available in the form of weekly intravenous injections of AAT. This is referred to as replacement or augmentation therapy and studies have shown that such treatment restores the concentration of AAT in the blood and may slow down the course of lung disease. We hypothesize that as a result of AATD and low serum levels of AAT, neutrophils from AATD individuals release excessively high levels of cytotoxic molecules in response to lipid activators. This project will assess whether AAT augmentation therapy impacts upon the excessive activity pattern of the ZZ-AATD circulating cell.

Title: **The role of carbohydrate residues in Alpha-1 antitrypsin anti-protease and anti-inflammatory properties.**

Funding Body: **MRCG/HRB Joint Funding Scheme (3 years funding commencing November 2011)**

Principal Investigator: *Dr David Bergin, PhD, MSc*

Co-investigators: *Prof N.G.McElvaney*

Lay summary: Alpha-1 antitrypsin (AAT) deficiency (AATD) has been previously classified as a rare disease but more recently, evidence points towards it being a disease that is relatively common but rarely diagnosed. It is estimated that the most severe form of this genetic disease, the ZZ type, affects around 2000 people in Ireland. People with AATD are at high risk of developing early onset emphysema, a destructive irreversible lung disease which impairs the affected individual's ability to get oxygen from the air into the bloodstream. If unchecked this eventually leads to respiratory failure and death.

The major contributors to the lung disease associated with AATD are; 1) the lack of AAT protein in the lungs where it inhibits enzymes produced by inflammatory cells and 2) the high amount of inflammation within the lungs of AATD

patients. Current therapies available to patients with AATD include AAT augmentation therapy where plasma purified AAT from non-AATD individual's blood is infused into the circulation of AATD patients once weekly in order to raise the levels of AAT in the blood and lungs. Another potential treatment is aerosolization of AAT into the lungs in order to prevent inflammatory enzymes destroying the lung tissue.

AAT is primarily an inhibitor of these inflammatory enzymes called proteases, but recent information highlights that AAT has other novel anti-inflammatory properties. Key to its anti-inflammatory capabilities, are sugar residues that coat the AAT protein. In this study we will investigate the importance of the different sugar residues in AAT anti-protease and anti-inflammatory effect. The result of this research will point the direction towards developing more efficient therapy for the treatment of AATD by indentifying potential modification of the sugar residues on AAT to enhance its anti-inflammatory function while maintaining its antiprotease activity.

CONFERENCE PRESENTATIONS

Trends in Diagnosis and Clinical Presentation of Alpha-1 Antitrypsin Deficiency in Ireland

Poster Presentation: **Irish Thoracic Society Annual Scientific Meeting, Dublin, November 2011; Second prize in the ANÁIL Respiratory Nurses Award**

Poster presentation: **American Thoracic Society Conference, San Francisco, May 2012; received Travel Award from ATS Nursing Assembly**

C.O'Connor, T. Carroll, G. O'Brien, Prof N.G. McElvaney.Department of Medicine, RCSI, Beaumont Hospital, Dublin 9

Rationale: Alpha-1 Antitrypsin Deficiency (AATD) is an autosomal co-dominant genetic disorder associated with a substantially increased risk for the development of chronic obstructive pulmonary disease (COPD) and liver disease. The most common mutation associated with AATD is the Z mutation (Glu342Lys) and 1 in 25 individuals carry this variant in the Irish population (Carroll et al 2011). AATD is a notoriously under-diagnosed and under-recognized condition. ATS/ERS guidelines recommend testing of all individuals with COPD and poorly controlled asthma. The objective of the study was to investigate the diagnostic experiences of ZZ AATD individuals in Ireland.

Method: 60 ZZ AATD individuals completed questionnaires at an Alpha-1 clinic in relation to their diagnostic experiences and clinical presentation.

Results: The total mean age of symptom onset was 36.7 +/- 1.7 years (range 4-60); mean age of diagnosis was 42.9 +/- 1.6 years (range 4-68). The total interval between onset of symptom and diagnosis was 6.2 years. The total mean number of physicians seen prior to a diagnosis of AATD was 2.6 +/- 0.3 (range 1-13). The interval between onset of symptoms and diagnosis was significantly different ($p<0.05$) between symptomatically and family screened ZZ individuals. The initial symptoms reported were shortness of breath on exertion (57%), recurrent chest infection (42%) and shortness of breath at rest (30%).

Conclusion: Our results in the Irish population further underline the need for increased awareness and early detection of symptomatic ZZ AATD. The significant differences observed between symptomatically screened and family screened ZZ AATD individuals highlight the importance of a comprehensive family screening program within the AATD population.

Characteristics of an Irish Population of Alpha-1 Antitrypsin Deficiency Patients with the SZ Phenotype

Poster Presentation: **Irish Thoracic Society Annual Scientific Meeting, Dublin, November 2011**

Ní Chinnéide A, Carroll T, O'Connor C, O'Brien G, McElvaney NG.

Department of Respiratory Research, RCSI Education and Research Building, Beaumont Hospital, Dublin 9

Alpha-1 antitrypsin (AAT) is a serine protease inhibitor produced by hepatocytes and functions as the most important antiprotease in the lungs. AAT deficiency is a hereditary disorder leading to increased risk of pulmonary and/or liver disease. ATS/ERS guidelines advocate screening all COPD, poorly-controlled asthma, and cryptogenic liver disease patients, as well as first degree relatives of known AATD patients, however, AATD is still under diagnosed and delays in diagnosis are common. The two most common mutations associated with AATD in Ireland are the Z and S mutations.

Using the National Alpha-1 Antitrypsin Deficiency Registry individuals with the SZ phenotype were identified and available clinical and biochemical data was collected and analysed.

The mean age at diagnosis for SZ individuals was 45.03 +/- 3.12 years for males and 51.8 +/- 2.94 years for females. The mean AAT level in the SZ cohort was 0.56 g/L (range 0.44 – 0.83 g/L). We demonstrate that SZ individuals identified as a result of family screening have significantly increased FEV1 (95.56 +/- 5.9%) compared to SZ patients identified by targeted symptomatic screening (92.1 +/- 4.67%, p=0.0062). SZ patients who smoked had significantly decreased lung function compared to non-smoking SZ with a positive correlation between pack years and FEV1.

Current laboratory guidelines include diagnostic criteria that often fail to identify SZ individuals. This group is at increased risk of lung and liver disease. The results of this study emphasize the need for increased awareness and early detection of SZ AATD individuals. This would allow preventative interventions such as smoking cessation to be implemented prior to development of severe lung disease.

The Irish National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme

Oral Presentation: **Irish Thoracic Society Annual Scientific Meeting, Dublin, November 2011**

Poster presentation: **American Thoracic Society Conference, San Francisco, May 2012**

T.P. Carroll, C. O'Connor, G. O'Brien, S. J. O'Neill and N. G. McElvaney

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

AAT deficiency (AATD) results from mutations in the SERPINA1 gene, classically presenting with early-onset emphysema and liver disease. The most common mutation causing AATD is the Z mutation, with the S mutation weakly associated with lung disease. AATD is under-diagnosed and prolonged delays in diagnosis are common. ATS/ERS guidelines advocate screening all COPD, poorly-controlled asthma, and cryptogenic liver disease patients, as well as first degree relatives of known AATD patients.

Over 7,000 individuals have been screened following ATS/ERS guidelines in the national targeted detection programme. A combination of serum AAT quantification by nephelometry, phenotyping by isoelectric focussing (IEF), and genotyping of DNA isolated from dried blood spot samples was employed.

We have identified 115 ZZ, 110 SZ, 40 SS, 1100 MZ, and 700 MS individuals. This yields gene frequencies of 0.052 and 0.094 in a targeted population for S and Z, respectively. A number of rare genotypes have also been identified, particularly containing the I (Arg39Cys) and F (Arg223Cys) variants. More than 25% of the targeted population screened to date contains at least one deficient AAT allele.

The targeted detection approach is the most effective and cost-efficient method of identifying AATD. Our results underline the need for increased awareness and earlier detection of AATD. Our data highlight the maxim that AATD is not a rare disease but a disease that is rarely diagnosed.

Rare alpha-1 antitrypsin mutations in the Irish population

Poster Presentation: **American Thoracic Society Conference, San Francisco, May 2012; European Respiratory Society Congress, Vienna, September 2012; Irish Thoracic Society Annual Scientific Meeting, Dublin, November 2011**

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AAT deficiency (AATD) results from mutations in the SERPINA1 gene, classically presenting with early-onset emphysema and liver disease. The most common mutation responsible for AATD is the Z mutation. AAT deficiency is under-diagnosed and prolonged delays in diagnosis are common. ERS and ATS guidelines advocate the screening of all COPD, poorly-controlled asthma, and cryptogenic liver disease patients, as well as first degree relatives of known AATD patients.

8,000 individuals have been screened following ATS/ERS guidelines as part of the Irish national targeted detection programme. AAT levels were determined by immunonephelometry. AAT phenotyping was performed by isoelectric focussing. Rare and novel mutations were identified by DNA sequencing of the SERPINA1 gene.

A number of rare SERPINA1 mutations including I, F, V, $X_{christchurch}$, $Z_{bristol}$, and M_{malton} were identified. The I mutation (Arg39Cys) was present at a relatively high frequency (0.0038) in the targeted population, with over 60 cases described. Three Null SERPINA1 mutations were detected, including two novel mutations. In addition, the first individuals in Ireland homozygous for a Null mutation and for the Mmalton mutation were identified.

Current testing of suspected AATD cases is often limited and can miss rare and novel clinically significant SERPINA1 mutations. The rare mutations described in this study were not detected by a commonly used genotyping assay; however, the low AAT levels prompted their correct identification using more detailed genetic analysis. Our findings underline the need for a comprehensive diagnostic work up of all patients with low AAT levels including phenotyping, genotyping and if necessary, sequencing of the SERPINA1 gene.

Characteristics of ZZ Alpha-1 Antitrypsin Deficiency Patients on the Irish National Registry

Poster Presentation: **American Thoracic Society Conference, San Francisco, May 2012**

G. O'Brien, C. O'Connor, T. P. Carroll, and N. G. McElvaney

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

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. Individuals with this deficiency classically present with lung disease in adulthood. WHO guidelines advocate a targeted strategy in screening COPD, non-responsive asthma, cryptogenic liver disease patients and relatives of known AATD patients.

The most common AAT phenotype associated with lung disease is ZZ. A chart review of AATD patients on the National AATD Registry was performed on ZZ individuals (n=100). Our registry collects data on pulmonary function tests, GOLD guidelines, smoking history, complications, and initial reason for screening.

We found that ZZ individuals identified as a result of family screening have significantly increased FEV1 (85.3 +/- 6.5%, 40.8 +/- 2.8 years) compared to ZZ individuals identified by targeted symptomatic screening (54.38 +/- 3.99%, 44.86 +/- 1.8 years, p=0.0008). ZZ individuals with a history of smoking had

significantly decreased lung function (FEV1, 54.8 +/- 3.9%, 43.71 +/- 1.6 years) compared to never-smoking ZZ individuals (FEV1, 88.24 +/- 4.8%, 43.39 +/- 3.6 years p <0.0001).

Our results highlight the role of cigarette smoke in the pathogenesis of lung disease in AATD and 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.

The therapeutic potential of alpha-1 antitrypsin to include inflammatory lung disease associated with leukotriene B_4.

Poster Presentation: **Irish Thoracic Society, Dublin 2012**

Reeves EP, Bergin DA & McElvaney NG

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

Neutrophil driven airway inflammation is a major factor in the pathology of chronic obstructive pulmonary disease (COPD) associated with alpha-1 antitrypsin (AAT) deficiency (AATD). There are several types of inflammatory chemoattractants that mediate neutrophilic infiltration within the airways and recent *in vitro* and *in vivo* research findings from our laboratory have demonstrated that serum AAT coordinates both CXCR1 and soluble immune complex receptor mediated neutrophil migration (1). The aim of the present study was to investigate the ability of AAT to inhibit a third major neutrophil stimulant namely leukotriene B_4 (LTB_4), which activates neutrophils through BLT_1 and BLT_2 (BLT_{1+2}) receptors. The biological consequence of the described AAT induced inhibition was investigated at the level of neutrophil migration and release of azurocidin, a potent activator of human monocytic cells.

Purified neutrophils from healthy control donors or clinically stable AATD patients (n=4) were exposed to LTB_4 (100nM/2.5x10^5) and neutrophil migration was quantified employing a multiwall chemotaxis chamber. The level of neutrophil released azurocidin was compared by Western blot analysis.

Our in vitro data has shown that low serum levels of AAT leads to a significant increase in LTB_4 induced mean chemotactic index of AATD neutrophils compared to healthy control cells ($P<0.05$). Additionally, densitometry of immuno-bands revealed that neutrophils obtained from AATD individuals release significantly higher levels of azurocidin from cytoplasmic secretory vesicles, an effect reversed by inclusion of AAT (27.5μM; $P<0.05$).

The results of this study indicate that AAT can inhibit LTB_4 signaling and proposes AAT augmentation therapy as an effective treatment not only for AATD, but also for other LTB_4 associated pulmonary diseases including cystic fibrosis and severe asthma.

The ability of Alpha 1-antitrypsin to inhibit leukotriene b_4 signalling and the potential for aerosolised alpha 1 antitrypsin in the treatment of cystic fibrosis

Oral Presentation: **European Cystic Fibrosis Society. Dublin, June 2012**

O´ Dwyer CA, McElvaney NG and Reeves EP

RCSI, Beaumont Hospital, Dublin

Cystic Fibrosis (CF) is characterised by neutrophil-dominated lung inflammation attributable to release of a potent chemotactic agent leukotriene b_4.The aim of this study was to investigate the ability of exogenous alpha 1 antitrypsin (AAT) to inhibit leukotriene B_4 (LTB_4), a potent neutrophil (PMN) agonist. The biological consequence of the AAT induced inhibition was investigated at the level of PMN migration and release of azurocidin, a potent activator of human monocytic cells.

PMN isolated from healthy control volunteers (n=4) were stimulated with LTB_4 (100nM/ 2 x 10^7) in the presence and absence of AAT (27.5mM) for increasing increments of time (0,

5, 10 and 20 min). The level of degranulated proteins in surrounding supernatants was determined by western blot analysis. Proteins investigated included myeloperoxidase, LL-37 and MMP9 as markers for primary, secondary and tertiary granule release respectively. Levels of azurocidin released from primary granules and secretory vesicles was examined. PMN migration in response to LTB_4 was determined and the ability of AAT to bind LTB_4 was assessed.

In vitro data has shown that levels of degranulated MPO, LL-37 and MMP-9 were significantly decreased in the presence of AAT ($P<0.05$). Densitometry of immuno-bands revealed that PMNs release azurocidin in response to LTB_4, an effect reversed by inclusion of AAT (27.5μM; $P<0.05$). The mechanism of inhibition involved direct binding of AAT to LTB_4 as reduced vibrational fine structure of the LTB_4/AAT UV absorbance spectrum indicated complexation of the two molecules in solution.

The results of this study indicate that AAT can inhibit LTB_4 signaling and further justifies the use of aerosolised AAT as an effective treatment for CF.

The ability of alpha-1 antitrypsin to inhibit leukotriene B_4 induced neutrophil signalling and the potential to reduce bronchial inflammation

Poster Discussion: **American Thoracic Society. San Francisco, 21 May 2012**

O' Dwyer CA, McElvaney NG and Reeves EP

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

Neutrophil driven airway inflammation is a major factor in the pathology of chronic obstructive pulmonary disease (COPD) associated with alpha-1 antitrypsin (AAT) deficiency (AATD). There are several types of inflammatory chemoattractants that mediate neutrophilic infiltration within the airways and recent in vitro and in vivo research findings from our laboratory have demonstrated that serum AAT coordinates CXCR1 and soluble immune complex receptor mediated neutrophil migration[1]**.** The aim of this study was to investigate the ability of AAT to inhibit a third major neutrophil stimulant, leukotriene B_4 (LTB_4), which activates neutrophils through BLT_1 and BLT_2 (BLT_{1+2}) receptors. The biological consequence of the described AAT induced inhibition was investigated at the level of neutrophil migration and release of azurocidin, a potent activator of human monocytic cells.

Neutrophils isolated from healthy control volunteers and clinically stable AATD individuals (n=4) were stimulated with LTB_4 (100nM/ 2 x 10^7) in the presence and absence of AAT (27.5mM) for increasing increments of time (0, 5, 10 and 20 min). The level of degranulated proteins in surrounding supernatants was determined by western blot analysis. Proteins investigated included myeloperoxidase (MPO), LL-37 and matrix metalloprotease 9 (MMP-9) as markers for primary, secondary and tertiary granule release respectively. Levels of azurocidin released from primary granules and secretory vesicles was electrophoretically examined. Neutrophil migration in response to LTB_4 was determined using a multiwall chemotaxis chamber. The ability of AAT to bind LTB_4 was assessed specrophometrically with UV spectra recorded on a Jenway 6405 spectrophotometer at 25°C.

Our *in vitro* data has shown that low serum levels of AAT leads to a significant increase in LTB_4 induced mean chemotactic index of AATD neutrophils compared to healthy control cells ($P<0.05$). Levels of degranulated MPO, LL-37 and MMP-9 were significantly decreased in the presence of AAT ($P<0.05$). Additionally, densitometry of immuno-bands revealed that neutrophils obtained from AATD individuals release significantly higher levels of azurocidin in response to LTB_4, an effect reversed by inclusion of AAT (27.5μM; $P<0.05$). The mechanism of inhibition involved direct binding of AAT to LTB_4 as reduced vibrational fine structure of the LTB_4/AAT UV absorbance spectrum indicated complexation of the two molecules in solution.

The results of this study indicate that AAT can inhibit LTB_4 signaling and proposes AAT

augmentation therapy as an effective treatment not only for AATD but also for other LTB_4 associated pulmonary diseases including cystic fibrosis and severe asthma.

Alpha-1 antitrypsin regulates tumour necrosis factor alpha autocrine signalling through inhibition of NF-κB activation

Poster Presentation: **Irish Thoracic Society, Dublin, November 2011**

Jameel R, Bergin DA, McElvaney NG and Reeves EP

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

Alpha-1-antitrypsin (AAT) deficiency (AATD) can lead to chronic obstructive pulmonary disease (COPD), yet is largely unrecognized and under diagnosed. This hereditary disorder results in the rapid progression of lung disease, especially in smokers. Tumour necrosis factor alpha (TNF-α) is an inflammatory cytokine which is elevated in the sputum and serum of AATD patients and is a driving factor of airway inflammation. The aim of this study was to examine the impact of AAT on TNF-α self-regulated gene expression.

The human promyelocytic HL-60 cell line which can be induced to differentiate to neutrophil-like cells was employed in this study. To examine the effect of AAT on TNF-α gene expression, HL-60 cells (10^7/ml) were incubated with TNF-α (2.5ng) ± AAT (27μM) for 6 or 24 hours. TNF-α gene expression was evaluated by real time RT-PCR and standardised to GAPDH. To determine NF-κB activation, western blot analysis of IκB-α degradation was performed on HL-60 cells treated with TNF-α ± AAT over 60 minutes or neutrophils lysates of control donors (n=5) or stable AATD individuals (n=5).

Our results demonstrate that AAT can down-regulate TNF-α autocrine signalling processes and can function to significantly reduce TNF-α gene expression in HL-60 cells. Mechanisms of inhibition were shown to involve the ability of AAT to prevent TNF-α induced activation of NF-κB by preventing IκB-α degradation. In support of these results we observed increased NF-κB activation in AATD neutrophils when compared to healthy control cells.

This study supports the role AAT as an important autocrine regulator in regards to TNF-α signalling and highlights the potential use of AAT augmentation therapy in treatment of TNF-α related diseases other than hereditary COPD.

Alpha-1 antitrypsin modulates neutrophil reactive oxygen species production by inhibiting key players of the respiratory burst oxidase stytem

Poster Presentation: **Irish Thoracic Society, Dublin, November, 2012**

O'Flynn E, Bergin DA, McElvaney NG & Reeves EP

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

Activation of neutrophils sequestered in the alveolar milieu can cause the release of reactive oxygen species (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_2) to superoxide (O_2^-). Alpha-1 antitrypsin (AAT) deficiency (AATD) provides us with the most definitive evidence for the physiological and clinical importance of AAT and in this study we examined the immunomodulatory activity of AAT and investigated whether neutrophil ROS production was regulated by AAT.

Neutrophil O_2 consumption and O_2^- production in response to fMLP (10^{-6}M) and TNF-α (10ng) was measured using a Clark type oxygen electrode and cytochrome C reduction assays respectively. Translocation of essential respiratory oxidase cytosolic components (p67phox and p47phox) to the neutrophil plasma membrane was quantified by western blot analysis.

In this study we demonstrate using in vitro models that AAT modulates neutrophil O_2

consumption and O_2^- production elicited by fMLP and TNF-α (P<0.05). Mechanisms of inhibition were investigated and in vivo studies revealed that in AATD individuals, infused AAT functions to bind the circulating neutrophil membrane and decreased translocation of p67phox and p47phox from the cytosol to the plasma membrane.

The potential of AAT as a regulator of neutrophil ROS production adds a new understanding to the role of AAT in health and disease.

Alpha-1 antitrypsin: a novel TNF-α blocker?

Poster Presentation: **Irish Thoracic Society, Dublin, November 2011, Winner of Best Poster Award**

Wolfe R, Bergin DA, Reeves EP and McElvaney NG

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

Pro-inflammatory cytokines including TNF-α play an important role in perpetuating lung inflammation associated with chronic obstructive pulmonary disease (COPD) and alpha-1 antitrypsin (AAT) deficiency (AATD). Neutrophil and neutrophil-derived factors are implicated in the pathophysiology of these inflammatory diseases with TNF-α being a key stimulus inducing release/degranulation of neutrophil proteolytic enzymes. The aim of this study was to evaluate whether AAT could modulate neutrophil degranulation in response to TNF-α.

ELISA was employed to investigate the ability of AAT to inhibit TNF-α binding to the TNF receptors TNF-R1 and TNF-R2. Isolated neutrophils were treated with TNF-α (10ng) in the presence and absence of physiological concentrations of AAT (27.5mM) and release of proteolytic enzymes was evaluated by western blot analysis of extracellular supernatants employing antibodies against cathelicidin (hCAP-18) and MMP-9. Levels of hCAP-18 and MMP-9 were quantified in AATD patient serum pre- and 2 days post-AAT augmentation therapy (n=5) by zymography and ELISA respectively.

Our results demonstrate the ability of glycosylated AAT to bind to TNF-α thereby significantly inhibiting TNF-R1 and TNF-R2 engagement (p<0.05). As a direct result AAT inhibited TNF-α induced release of hCAP-18 and MMP-9 *in vitro*, and *in vivo* significantly lower levels of hCAP-18 and MMP-9 were detected in serum of AATD patients post AAT augmentation therapy.

This study has raised the possibility of broadening the therapeutic spectrum of AAT to include treatment of other diseases involving TNF-α induced inflammation.

Alpha-1 Antitrypsin and Cell-specific miRNA Expression Profiling in Three Cell Lines

Poster Presentation: **Irish Thoracic Society, Dublin November 2011 and American Thoracic Society, San Francisco, May 2012**

T. Hassan, I. Oglesby, S. O'Neill, N.G. McElvaney, C.M. Greene

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New methods to identify microRNAs (miRNAs) that regulate a single mRNA are of major interest. We developed an experimental method to search target miRNAs of the Alpha-1 Antitrypsin (AAT) mRNA.

Using our affinity isolation method and the nCounter miRNA Expression Assay, we compared and validated AAT-specific with cell-specific miRNAs in three cell lines; monocytic (THP-1), bronchial epithelial (16HBE14o-) and hepatocytes (HepG2)

Appreciable detection (normalized data) occurred for 252 miRNAs across the 3 cell lines. Expression differed in non-AAT and AAT-specific miRNAs. miRNAs selected for validation included miR-455-3p (expressed in 3 cell lines), miR-328, miR-769-5p and miR 295-5p expressed exclusively in THP-1, 16HBE140- and HepG2 respectively. Pre-miR co-transfection

resulted in a significant decrease in luciferase gene expression from the reporter vector containing the AAT-3'UTR. Overexpression of pre-miR 455-3p significantly decreased AAT mRNA and protein in all cell lines whilst overexpression of pre-miR 328 inhibited AAT expression in THP-1 cells only. Similar results were seen with miR-769-5p in 16HBE14o- and miR-296-5p in HepG2.

Our novel method identified multiple miRNAs targeting AAT mRNA. We provide direct evidence of cell-specific miRNAs targeting and modulating the AAT gene which is important in the future development of gene therapies for AAT-deficiency.

miRNAs Expression Profile Differences in PiMM and PiZZ Monocytes in Alpha-1 Antitrypsin Deficiency

Poster Presentation: **Irish Thoracic Society, Dublin November 2011 and American Thoracic Society, San Francisco, May 2012**

T. Hassan, S. O'Neill, C.M. Greene, N.G. McElvaney

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

Alpha-1 antitrypsin deficiency (AATD) is a genetic disorder associated with emphysema. Mutant Z protein accumulation has been shown to induce endoplasmic reticulum stress in blood monocytes. miRNAs are short endogenous RNAs that regulate gene expression. We studied miRNA expression in monocytes of normal (PiMM) and AATD (PiZZ) individuals.

Peripheral blood monocytes were isolated (PiMM=3 and PiZZ=3) , and total and AAT-specific miRNAs were profiled using the nCounter miRNA Expression Assay. pre-miR transfections and pMIR-REPORT-AAT 3'UTR studies were performed

60 miRNAs were differentially expressed in PiMM versus PiZZ monocytes. 46 and 14 were over and under-expressed in PiZZ subjects respectively. 42 were AAT-specific. miR455-3p and -328 (both under-expressed) were selected and proven to be direct targets of AAT mRNA using (i) the luciferase gene assay and (ii) pre-miR overexpression in PiMM monocytes, which significantly reduced both AAT mRNA and protein expression. Similar reduction of AAT mRNA was also found for PiZZ monocytes.

In conclusion, miRNA expression differs in PiMM and PiZZ monocytes. Overexpression of miR-455-3p and -328 can modulate AAT in PiMM monocytes. Whether altered miRNA expression is related to AATD or ER stress remains to be determined.

Altered programmed cell death of neutrophils in individuals with alpha-1 antitrypsin deficiency

Winner of the European Alpha-1 Antitrypsin Deficiency Laurell's Training Award (eALTA)

Oral Presentation: **European Respiratory Society Conference, Amsterdam, September 2011**

Hurley K, Reeves EP, McElvaney NG

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

The eALTA research project "Altered programmed cell death of neutrophils in individuals with Alpha-1-Antitrypsin Deficiency" focuses on comparing the rates of AATD and normal neutrophil apoptosis and to uncover the extracellular and/or intracellular mechanism by which AAT modulates neutrophil cell death. Importantly for patients on alpha-1 antitrypsin augmentation therapy the effect of AAT on neutrophil survival will be investigated as well.

Neutrophils are the primary effector cells responsible for the pathological manifestations of AATD lung disease and therefore an important immune cell to study. Regulation of the neutrophil life span by apoptosis provides a fine balance between their function as effector cells of host defence and a safe turnover of these potentially harmful cells. Accelerated neutrophil apoptosis has been previously linked

with liver cirrhosis and observed within sputum neutrophils of COPD patients. Within this project we hypothesizes that AAT directly impacts upon the half-life of the circulating neutrophil and the goal of this innovative study is to demonstrate that circulating neutrophils of AATD individuals display accelerated apoptosis.

This translational research aims to fully characterise the anti-apoptotic effect of AAT on the circulating neutrophil and to study the implications for AATD with respect to neutrophil apoptosis. Potential patient benefits include evaluating whether AAT augmentation therapy corrects the accelerated rate of neutrophil apoptosis in ZZ-AATD individuals.

Dr Killian Hurley *received the European Alpha-1 Antitrypsin Laurell's Training Award (eALTA) at the European Respiratory Society Annual Congress in Amsterdam in September 2011*

Altered polymorphonuclear leukocyte apoptosis in individuals with alpha-1 antitrypsin deficiency is associated with endoplasmic reticulum stress and IL-6 expression

Poster Presentation: **American Thoracic Society, San Francisco, May 2012**

Hurley K, Reeves EP, Bergin DA, McElvaney OJ, McElvaney NG.

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

Alpha-1 antitrypsin (AAT) deficiency (AATD) is the most frequent form of genetically determined emphysema. AAT opposes the destructive effects of neutrophil serine proteases in the lung and new evidence suggests that AAT may play a role in regulation of alveolar cell apoptosis. Endopalsmic reticulum (ER) stress is known to influence apoptosis and is found to be increased in hepatic and alveolar cells of individuals with AATD. The aim of this study was to determine the rate of polymorphonuclear leukocyte (PMN) apoptosis in asymptomatic AATD individuals homozygous for the Z allele (ZZ-AATD) compared to healthy control cells (MM) and to uncover intrinsic or extrinsic factors which may augment apoptosis.PMN were isolated from ZZ-AATD and MM individuals (n=5), and were incubated at time intervals between 0 and 22 hours, with and without AAT at physiological concentration (27.5μM). Apoptosis was determined by caspase-3 cleavage by Western blotting and by annexin V staining and CD16b expression by FACs analysis. ER stress was determined at the gene and protein level by real time polymerase chain reaction and Western blotting respectively. Cytokine production was determined by cytokine array of cell supernatants and confirmed by ELISA.

In ZZ-AATD neutrophils both the rate and the total amount of caspase-3 cleavage was greatly increased compared to MM control cells ($p<0.05$). This increased rate was supported by annexin V staining and reduced CD16b expression. The addition of exogenous AAT greatly reduced caspase-3 cleavage and annexin V staining in ZZ-AATD neutrophils ($p<0.05$).

Markers of ER stress GRP78 and cleaved ATF6 were found to be significantly upregulated in ZZ neutrophils compared to MM control cells ($p<0.05$). Levels of the pro-survival cytokine IL-6 was decreased at both the gene and protein level in the ZZ-AATD PMN ($p<0.05$). In addition, treating ZZ-AATD PMNs with AAT restored normal apoptosis rates and up-regulated the gene and protein expression of IL-6 ($p<0.05$). Our data shows that PMNs undergo accelerated cell death in AATD possibly secondary to ER stress and that the addition of AAT to the ZZ PMN attenuates this apoptotic rate. We have also identified a novel association of decreased autocrine production of IL-6 and accelerated PMN apoptosis in ZZ-AATD individuals. We hypothesise that AAT augmentation therapy may normalise neutrophil apoptosis thereby reducing inflammation and recurrent infections in patients with AATD.

PUBLISHED RESEARCH

JOURNAL ARTICLE

A novel neutrophil derived inflammatory biomarker of pulmonary exacerbation in cystic fibrosis

Reeves EP, Bergin DA, Fitzgerald S, Hayes E, Keenan J, Henry M, Meleady P, Vega-Carrascal I, Murray MA, Low TB, McCarthy C, O'Brien E, Clynes M, Gunaratnam C, McElvaney NG

Journal of Cystic Fibrosis. 2012 Mar;11(2):100-7

Respiratory Research Division, Department of Medicine, Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland. emerreeves@rcsi.ieThe focus of this study was to characterize a novel biomarker for cystic fibrosis (CF) that could reflect exacerbations of the disease and could be useful for therapeutic stratification of patients, or for testing of potential drug treatments. This study focused exclusively on a protein complex containing alpha-1 antitrypsin and CD16b (AAT:CD16b) which is released into the bloodstream from membranes of pro-inflammatory primed neutrophils.Neutrophil membrane expression and extracellular levels of AAT and CD16b were quantified by flow cytometry, Western blot analysis and by 2D-PAGE. Interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α) and AAT:CD16b complex were quantified in CF plasma (n=38), samples post antibiotic treatment for 14 days (n=10), chronic obstructive pulmonary disease (n=10), AAT deficient (n=10) and healthy control (n=14) plasma samples by ELISA.Cell priming with IL-8 and TNF-α caused release of the AAT:CD16b complex from the neutrophil cell membrane. Circulating plasma levels of IL-8, TNF-α and AAT:CD16b complex were significantly higher in patients with CF than in the other patient groups or healthy controls ($P<0.05$). Antibiotic treatment of pulmonary exacerbation in patients with CF led to decreased plasma protein concentrations of AAT:CD16b complex with a significant correlation with improved FEV1 ($r=0.81$, $P=0.003$).The results of this study have shown that levels of AAT:CD16b complex present in plasma correlate to the inflammatory status of patients. The AAT:CD16b biomarker may become a useful addition to the clinical diagnosis of exacerbations in CF.

BOOK CHAPTER

Alpha-1 Antitrypsin Deficiency – A Genetic Risk Factor for COPD

In: **Chronic Obstructive Pulmonary Disease – Current Concepts and Practice**

Tomás P. Carroll, Catherine A. O'Connor, Emer P. Reeves and Noel G. McElvaney (2012), Kian-Chung Ong (Editor)

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PUBLISHED REVIEWS

Mechanisms of protein misfolding in conformational lung diseases

McElvaney NG, Greene CM

Curr Mol Med, 2012 Aug 1;12(7):850-9

Genetic or environmentally-induced alterations in protein structure interfere with the correct folding, assembly and trafficking of proteins. In the lung the expression of misfolded proteins can induce a variety of pathogenetic effects. Cystic fibrosis (CF) and alpha-1 antitrypsin (AAT) deficiency are two major clinically relevant pulmonary disorders associated with protein misfolding. Both are genetic diseases the primary causes of which are expression of mutant alleles of the cystic fibrosis transmembrane conductance regulator (CFTR) and SERPINA1, respectively. The most common and best studied mutant forms of CFTR and AAT are ΔF508 CFTR and the Glu342Lys mutant of AAT called ZAAT, respectively. Non-genetic mechanisms can also damage protein structure and induce protein misfolding in the lung. Cigarette-smoke contains oxidants and other factors that can modify a protein's structure, and is one of the most significant environmental causes of protein damage within the lung. Herein we describe the mechanisms controlling the folding of wild type and mutant versions of CFTR and AAT proteins, and explore the consequences of cigarette-smoke-induced effects on the protein folding machinery in the lung.

The role of proteases, endoplasmic reticulum stress and SERPINA1 heterozygosity in lung disease and alpha-1 antitrypsin deficiency

Greene, C. MT. Hassin, K. Molloy, McElvaney, NG.

Expert Rev Respir Med. 2011 Jun;5(3):395-411

The serine proteinase inhibitor alpha-1 antitrypsin (AAT) provides an antiprotease protective screen throughout the body. Mutations in the AAT gene (SERPINA1) that lead to deficiency in AAT are associated with chronic obstructive pulmonary diseases. The Z mutation encodes a misfolded variant of AAT that is not secreted effectively and accumulates intracellularly in the endoplasmic reticulum of hepatocytes and other AAT-producing cells. Until recently, it was thought that loss of antiprotease function was the major cause of ZAAT-related lung disease. However, the contribution of gain-of-function effects is now being recognized. Here we describe how both loss- and gain-of-function effects can contribute to ZAAT-related lung disease. In addition, we explore how SERPINA1 heterozygosity could contribute to smoking-induced chronic obstructive pulmonary diseases and consider the consequences.

Alpha-1 antitrypsin: a potent anti-inflammatory and potential novel therapeutic agent

Bergin DA, Hurley K, McElvaney NG, Reeves EP.

Arch Immunol Ther Exp (Warsz). 2012 Apr;60(2):81-97

Alpha-1 antitrypsin (AAT) has long been thought of as an important anti-protease in the lung where it is known to decrease the destructive effects of major proteases such as neutrophil elastase. In recent years, the perception of this protein in this simple one dimensional capacity as an anti-protease has evolved and it is now recognised that AAT has significant anti-inflammatory properties affecting a wide range of inflammatory cells, leading to its potential therapeutic use in a number of important diseases. This present review aims to discuss the described anti-inflammatory actions of AAT in modulating key immune cell functions, delineate known signalling pathways and specifically to identify the models of disease in which AAT has been shown to be effective as a therapy.

6. Being An Alpha-1 Carrier – Mary's Story

In 2005 I started to attend Pilates classes as my back had begun to grumble again and I knew that the exercises would be helpful. I soon realised that when I had to lie on my back or on my left side and do the recommended breathing for the exercises that I had a wheezy and noisy breathing pattern. I was also aware that I always started to cough after the class began and that was I the only one coughing. Eventually after some investigations over a few months in the Spring of 2006 the consultant initially diagnosed asthma. He changed that diagnosis some time later to COPD. I have been using inhalers since then.

In the early months of 2009 I was admitted to hospital complaining of a severe headache. As my sister had a brain haemorrhage the previous year I was put through a series of tests. The pain subsided and I was lucky that nothing serious was found. When I was being discharged some days later the consultant mentioned that there was a query about a blood result showing a liver issue. I said that having had jaundice over 30 years earlier I had always assumed that these odd liver blood results were because of that. He said that he could not afford to assume that and that I should not assume that either. I then mentioned to him that I had read about Alpha 1 in someone with COPD and liver problems in the Irish Times Health supplement some months earlier and at the time of reading it I had wondered about myself and whether it had anything to do with me. He said he could have that checked out.

On 22nd February 2012 I answered the phone at home. Dr Kevin Molloy introduced himself as a doctor from Beaumont Hospital and told me that I was an Alpha 1 carrier. He asked what I knew of Alpha 1 and as I tried to remember what I had read 3 years earlier he explained something about the condition of being a carrier. I had a fair idea of where I might find the Irish Times article and I found it later that evening. Needless to say I re-read it with a greater interest then. Dr Kevin recommended that my family should be tested. This meant my five adult children and my three brothers and two sisters. One brother had died suddenly in 1982 aged 34 years. Some weeks later two of my brothers, one sister and two sons and one daughter who all live in or around Sligo gathered to be tested by Dr Kevin. Some remaining family members have yet to be tested.

We have learnt from those tests that I am not the only one in the family who is a carrier and those of us who are carriers are all learning more about being a carrier. It was confirmed that it came via my mother's side of the family so I began a one woman information campaign to let all my many first cousins know about Alpha 1. My late mother was one of seven. Only two members of that family now survive. Between them they had over forty offspring so I made a point of informing at least one cousin in each family over a number of weeks and asked them to inform their brothers and sisters and direct them to the website. As I have three grandchildren now and as some of those cousins are now grandparents the knowledge that this is in the family is very important.

Some months away from that phone call in February what has changed for me as a result of knowing that I am an Alpha 1 carrier?

When I was having the lung function tests in early 2006 the staff doing the tests were surprised to learn that I had never smoked. However when I told them that I had been exposed to passive smoking during my teenage years while serving in the bar of our small seaside hotel and also as many of my friends smoked at that time we presumed that my difficulties arose from passive smoking. Now I know that may not be the reason. I surprised myself recently by politely telling a smoker that I needed to stay away from him and this was outdoors!

Forgotten memories have come to the fore. Childhood memories have been reawakened and I realised that plenty of smoking took place in our tiny kitchen when I was a child. My father and his visiting friends smoked while we were playing there. A sister of my fathers who lived with us for years smoked many cigarettes over those years! I remember being a member of my school choir singing at our local Feiseanna and having to slip off the bench while singing as I began a bout of coughing and was struggling to breathe. I remember the piano teacher who left the room and left me to my own devices if I began to cough. I had been told that I had whooping cough when I was less than three months old and that my parents and the GP thought that I would not survive. As their first pregnancy had ended in a stillbirth my early months of life must have been a very anxious time for them. I thought my efforts to take in air when I had a cough were connected to my early bout of whooping cough.

I remembered my doctor's surprise when I developed jaundice in the early seventies. He told me that he would have expected to hear of others who had it but I was the only one in town. He said it usually presented in clusters. Was it due to my carrier status? I have always had sinus problems. I went to hospital with suspected meningitis when our first child was about three months old. It was a sinus infection. Am I more prone to sinus infections because I am a carrier?

I may have a lot of learning to do over the next few months. Meanwhile I spread the word about Alpha 1 both within the family and in those whom I meet day to day. I met a young woman recently as we had to spend about 10 minutes together on a project. I was immediately conscious of her breathing so I asked her about it. She was on several inhalers. I told her to check the Alpha 1 website and talk to her doctor. We needed an extension of our ten minutes!

When Dr Kevin spoke to me on his first phone call he was worried that he was throwing all this information at me but my response was that it was good thing be aware of and to know about. If my parents had known about Alpha 1 they would not have exposed us to cigarette smoke or any other environmental hazards. My mother who died in 2006 would have known why she sometimes had coughing and could not breathe. She told us that when coming around after surgery and a full anaesthetic she was coughing and trying to get her breath that the resuscitation team were angry with her and said "you never told us you had asthma." She said to them when she was able to breathe that nobody had ever told her that she had asthma. I still feel that it is better to know about Alpha 1 and being a carrier.

Mary
An Alpha-1 Carrier

7. Beaumont Hospital becomes a smoke-free campus

A new Tobacco-Free policy saw Beaumont Hospital become a smoke-free campus on 4 July 2012.

Beaumont Hospital became a tobacco-free campus on 4th July 2012. The hospital prohibits smoking on the campus in a move to create a healthy, clean and safer environment for patients, staff and visitors. The move to go tobacco free across the campus follows extensive work by a multi-disciplinary steering committee and working groups. The policy was designed in response to the ongoing concern about the harmful effects of tobacco use and smoking in a healthcare environment.

Pictured L-R, from Beaumont Hospital, are **Brendan Halligan**, Technical Services, **Liam Duffy**, CEO, and **Joe Costello**, Technical Services. **Josephine McGuirk**, advocate for the Alpha 1 Foundation and Beaumont Hospital patient, joins them in a moment to mark the launch of the tobacco-free campus

The HSE Corporate Plan (2008-2011) recognised the need for a shift towards prevention and better self care in respect of tobacco. Research and experience shows that this requires ongoing health awareness, illness prevention and health promotion initiatives as well as population health strategies. The Tobacco-Free steering committee chaired by Professor Gerry McElvaney, Director of the Respiratory Research Laboratory RCSI/Beaumont Hospital and supported by Beaumont CEO Liam Duffy and the Senior Management Team, have developed this policy in response to the HSE Corporate Plan as a measure to protect Beaumont Hospital Site users from the dangers associated with smoking and exposure to second-hand smoke.

Commenting on the launch, Liam Duffy, CEO Beaumont said "As a major health service provider and hospital, it is our obligation to provide a safe environment for our patients, staff and visitors. The transition to a smoke-free campus is one that Beaumont feels is a necessary and natural step in continuing to provision of safe and effective health care."

"We have been working with the steering committee and staff over a number of months to ensure that the transition will be seamless and have found great support from all involved for preparation of the launch of Beaumont Hospital as a tobacco-free campus."

Prof Gerry McElvaney, Chair of the Tobacco Free Steering Committee also said "This is a positive step for Beaumont Hospital. The rationale for the move is very simple: smoking is the single largest preventable cause of disease and premature death and there is a growing recognition throughout the developed world that allowing smoking on healthcare campuses significantly undermines the health promotion message of healthcare organisations."

TOP TEN TIPS FOR QUITTING SMOKING

1
PREPARE YOURSELF
– Make a list of reasons for quitting- health, money, family

2
SET A DATE TO STOP
– And stick to it!

3
GET SUPPORT
– Get the support of your family, friends, your GP, the National Smokers' Quitline or your local stop-smoking service

4
CHANGE YOUR ROUTINE AND PLAN AHEAD
– People often link smoking to certain times and situations. Avoid these and replace them with new activities you do not link with smoking

5
GET MOVING
– Exercise and being active makes you feel good and helps manage weight

6
THINK POSITIVE
– Withdrawal symptoms are positive signs that your body is recovering from the effects of smoking

7
LEARN TO DEAL WITH CRAVINGS
– Remember the 4D's

Delay at least 3 minutes and the urge will pass

Drink a glass of water or fruit juice (sip slowly)

Distract yourself. Move away from the situation

Deep breathe. Breathe slowly and deeply. It will help you relax

8
START SAVING MONEY
– Save the money you would have spent on cigarettes and treat yourself!

9
WATCH WHAT YOU EAT
– Avoid snacking on chocolate bars and biscuits. Try some fruit or sugar-free gum instead

10
TAKE ONE DAY AT A TIME
– Remember every day without a cigarette is good news for your health, your family and your pocket

MOTIVATION STATION

If you want to change your lifestyle... *Top 10 reasons to quit*

TOP 10 SMOKING MYTHS

1. **My other healthy habits can make up for my smoking**
Some smokers justify their smoking by insisting that eating healthy and excising are enough to keep them healthy. Research shows that having a healthy diet and exercise are excellent for your health but will not reduce the health risks associated with smoking.

2. **Switching to 'light' cigarettes will cut my risk**
Smokers who switch to brands labelled 'light' or 'mild' need to satisfy their cravings for nicotine.

3. **I've smoked for so long , the damage is already done**
Quitting smoking at any age brings health benefits but the earlier you quit, the better.

4. **I'll put on weight if I quit smoking**
Many people who quit do not gain weight. For people who do gain a few pounds, remember it is temporary and you will return to your normal weight within a few months. Don't fall into obvious trap of replacing cigarette with high-calorie foods.

5. **Quitting 'cold turkey' is the only way to go**
Some smokers think that willpower is the only effective tool for quitting. They are partly right because commitment is essential. But smokers are more likely to succeed at quitting if they get help.

6. **I'm the only one who is hurt by my smoking**
Your tobacco smoke can cause serious harm to the people around you. Second-hand smoke can increase a non-smoker's risk of getting lung cancer and heart disease. Second-hand smoke is particularly dangerous for children because their bodies are still developing. Smoking when you are with your children in the home or the car can increase their risk of cot death, asthma and chest infections, and possibly cancer later on in life.

7. **I tried quitting once and failed, so it's no use trying again**
Most smokers try several times before quitting for good. So if you have not yet succeeded, don't let that stop you from trying again.

8. **I need smoking to cope with stress in my everyday life**
Not true. Smoking makes the heart beat faster and raises blood pressure.

9. **I'm just a social smoker**
Many people think they are in control of social smoking habits when they are not. The more you smoke, the more your body learns to depend on nicotine, and this is what makes quitting so hard. There is no such thing as a safe cigarette. Every cigarette is harming you and the people around you.

10. **I only smoke menthol cigarettes**
Menthol cigarettes are just as harmful to your health as normal cigarettes. The only difference is that they are flavoured by menthol. Recent reports suggest that they may be more addictive than average cigarettes and may encourage more young people to smoke.

ASK THE EXPERTS

Roisin Thursan, Smoking Cessation Officer and **Michelle McGettigan**, Health Promotion Officer, Beaumont Hospital, Dublin 9

Dear Roisin and Michelle,
I have recently been diagnosed with Alpha-1 and I'm trying to give-up smoking. What is the best way to get help? – Mary, 40 yrs

Dear Mary,

Well done on deciding to quit! As your medical team have probably explained, it's very important that you quit smoking as soon as possible. The good news is that deciding to quit now will benefit you and the fact that you feel ready will increase your chances of success. Writing down the reasons why you want to quit can be a great motivator for people and can help you later during the initial stages of your quit attempt. Choosing a quit date is also important as it gives you a focus as well as allowing you time to develop a 'quit smoking' plan. Asking your family and friends for support can make all the difference. This may involve asking them not to smoke around you or not to offer you cigarettes: the important thing is that you feel supported.

The best way to get help is to get in touch with the smoking cessation support services. The Alpha One Foundation will have information on the local smoking cessation nurse/advisor and may have one linked to the centre. This healthcare professional will meet with you to discuss your smoking and how best to quit. The session will involve advice and suggestions on how to quit, techniques to deal with cravings as well as what nicotine replacements therapies (NRT's) will be most suitable for you. They will have a variety of stop smoking reading materials available which will help you to remember the advice given during the session. There are also community services available which can offer you support. Your GP, your dentist and your local pharmacist will usually have some quit smoking materials that they can offer. The National Smokers' Quitline (1850 201 203) is a valuable support which aids people regularly. There are also supports available online such as *www.quit.ie* and *www.facebook.com/HSEquit*

Remember getting help and advice increases your chances of success. Best of luck!

Dear Roísín and Michelle,

I smoke when I'm out with my friends at the weekend. I want to stop smoking but still go out and have fun. Have you any tips for me? – Anne, 25 yrs

Hi Anne,

Well done on deciding to quit smoking. Quitting smoking is one of the most important things you can do to improve your health so it's great that you feel motivated to make the change. It's good that your smoking is limited to the weekends but would be even better if you were smoke-free altogether! As you have been diagnosed with alpha-1 you are at an increased risk of developing severe lung disease. As a young, fun-loving lady you have lots of good reasons to quit so that you can enjoy a future full of great weekends.

As the weekends are the times when you tend to smoke, it may be a good idea to choose a quiet weekend to quit. By choosing a quit date you're committing to a plan and this will help to give your quit attempt focus as well as an opportunity to abstain with fewer temptations. Because drinking alcohol can affect your willpower it may be helpful to reduce the amount that you drink at weekends. That way, you can fight the cravings more effectively. Advising your friends that you want to quit and asking them for their support will help your quit effort. While out with friends at the weekend, I recommend that you make sure there's at least one friend that doesn't smoke so that you have company while others leave to have a cigarette. Cigarette cravings last between 3 to 5 minutes so try to distract yourself when you feel tempted and the craving will pass.

For more information you can contact the National Smokers' Quitline (1850 201 203) or view websites such as ***www.quit.ie*** or ***www.cancer.ie***. There is also smoking cessation support available on ***www.facebook.com/HSEquit***.

Staying positive will help you with your quit attempt: remembering the reasons why you're doing it and how far you've come in your attempt. Don't think 'one cigarette won't hurt' and try not to be tempted by your friends who smoke. Best of luck!

USEFUL NUMBERS

National Smoker's Quitline: For help quitting smoking, call the National Smoker's Quitline on Callsave 1850-201-203

Quit.ie: For help, information and advice on quitting smoking, visit *www.quit.ie* or *www.cancer.ie*

Get Ireland Active: For information on getting active and managing your weight, visit *www.getirelandactive.ie*

Alpha One Foundation: For information and advice on Alpha-1 Antitrypsin Deficiency visit *www.alpha1.ie* or call 01-809 3871

8. US and Irish Alpha-1 Connections

ANGELA MCBRIDE

Director of Development, US Alpha-1 Foundation

Who knows where they will end up in life? As I was growing up in Stackallen, Navan, Co. Meath Ireland I would wonder where I would end up and how I could contribute to society.

The bright blue skies of Miami, Florida is where I ended up. So far removed from the lush green grass, the river Boyne, and the friends and family I left behind.

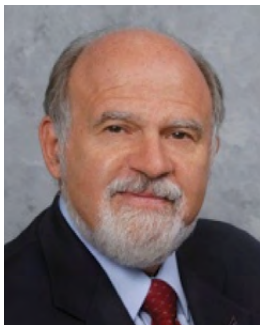
From a young age, giving back and helping one another was the normal thing to do. You helped your family and your communities succeed. So, I guess that's why I feel so at home with my "Alpha" family. Eleven years ago this month I met an Alpha, John W. Walsh, and told me that he was going to help find a cure for Alpha-1 Antitrypsin Deficiency. We talked about an Irish Professor named Gerry McElvaney and the Alpha-1 Foundation in Ireland. I was hired by the Alpha-1 Foundation and was captivated by this family of Alphas almost immediately.

John was a man on a mission and I like to think that I am on a mission as well. For me, it's helping those diagnosed with a chronic condition such as Alpha-1, understand that they too can make a difference. Each and every one of us has a part to play in finding a cure. My days are spent empowering Alphas, their friends and family members through a program called "Building Friends for a Cure." We organize walks, bowling events, golf tournaments and many others. As an Irish woman I love to party, so I am totally biased in saying that our "Celtic Connection" party in Boston is my favorite. We have raised over $1.5 million to date through this program with all the monies going directly into research and related programs.The work that has been done to move the progress of research forward is amazing. I hope you are encouraged by the progress and that you also will "Take Action and Make a Difference." Every day I have an opportunity to tell somebody about Alpha—1. It's important for everyone to simply tell their stories. One such Alpha, Joan Garry, told me her story and it turns out that we grew up in the same town and went to the same schools. Alpha-1 is not a rare condition, it's only rarely diagnoised. Alphas are all around us we just have to keep looking!

Need encouragement? I would be happy to help. Contact me at *amcbride@alpha-1foundation.org*.

JOHN WALSH

CEO, US Alpha-1 Foundation



The Alpha-1 Foundation wishes to congratulate the Alpha One Foundation in Ireland for the progress reported in this Annual Report and for your continued commitment to Alpha-1 research and improved health outcomes for individuals with Alpha-1.

Professor Gerry McElvaney has been involved with the Alpha-1 Foundation's scientific leadership since inception of the Foundation. The Board of Directors and Scientific Leadership of the Alpha-1 Foundation has recognized Professor McElvaney for his contributions and leadership to the field of Alpha-1 research. Most recently, Gerry was awarded the coveted "Golden Shillelagh" by the Alpha-1 patient community at a celebration in Boston last March. His dedication and commitment to Alpha-1 at the National

Institutes of Health (NIH), National Heart, Lung and Blood Institute (NHLBI) made a significant contribution to mapping out the natural history of Alpha-1 related lung disease and resulted in his active participation in creation of the Alpha-1 Foundation in the United States. As a member of our Medical and Scientific Advisory Committee, Grants Advisory Committee and participation in numerous Workshops and Conferences he has made an exemplary contribution to our progress in advancing the understanding of Alpha-1 through our funding of over $46m to researchers at 92 institutions in North America and Europe.

Since 2003, The Alpha-1 Foundation has provided over $1 Million in support for Alpha-1 Research to investigators affiliated with the Royal College of Surgeons in Ireland at the outstanding lab under the leadership of Professor Gerry McElvaney. This support includes funding for 5 pilot and feasibility grants, 6 research grants, and 1 matching grant partnership with the American Thoracic Society.

Several investigators from the RCSI -Beaumont lab have presented at our International Scientific Conferences and Critical Issues Workshop Series and continue to engage in international collaborations of Alpha-1 research. Members of the Alpha-1 Foundation leadership attended the dedication ceremony for the Alpha One Foundation in Ireland at the Royal College of Surgeons with Dr James Kiley, Director of the Lung Division of the NIH-NHLBI and Dr Jack McCormick, Acting Director of the U.S. Food and Drug Administration (FDA), Office of Orphan Drug Development (OODD).Congratulations on another successful year of progress and excellence in research and patient care. As we ready for the 50th Anniversary of the discovery of Alpha-1 in 2013, we welcome an opportunity to expand our collaborations and look forward to your participation at the International Alpha-1 Scientific Conference and Global Alpha-1 Patient Congress in Barcelona on April 11th-13th.

On behalf of the Alpha-1 Foundation,
John W. Walsh
Co-Founder, President & CEO

9. Rare Disease Day

RARE DISEASE DAY 2012, 29 FEBRUARY 2012

Rare Disease Day 2012 video and postcard campaign

In December 2011, GRDO collaborated with European Rare Disease Alliance and EURORDIS, in the production of a short awareness-raising video.

The video featured six people from Ireland, each with a different rare condition. As part of International Rare Disease Day 2012, the video was launched in 12 different languages and received over 100,000 views around the world. GRDO also produced a series of postcards based on the video, carrying basic information about rare disease issues. These were sent to members of the media, and Dáil and Seanad representatives ahead of Rare Disease Day this year. For more information and to watch the video see *http://www.rarediseaseday.org/solidarity*.

1. **Lauren Shaw** is a bright and bubbly eight-year old. She lives with Friedreich's Ataxia, a rare condition that causes nerve damage and movement problems.
2. **Jamie O'Brien's** ambition is to work in music production and website design. Jamie has Ehler-Danlos Syndrome, the hypermobility type that causes his joints to dislocate constantly.
3. **Josephine McGuirk** moved to the seaside in recent years and feels healthier for it. She has Alpha-1 antitrypsin deficiency, a rare genetic condition that affects the lungs and liver.
4. **Christy Murtagh** is embarking on a new career in counselling, having left behind the building trade. Christy is affected by Retinitis Pigmentosa, which causes gradual but progressive sight loss.
5. **Clare Louise Creedon** has another form of Ataxia: Ataxia Oculomotor Apraxia-1 (AOA1), and is in a wheelchair full-time now. Despite this, she makes sure to do the things she enjoys like going to the gym, shopping, and getting the bus to visit friends.
6. **Evan O'Gorman** is a keen actor and, though still at school, already has a few paid roles under his belt. Evan has Epidermolysis Bullosa, an extremely painful skin condition affecting his feet.

UCD SCHOOL OF MEDICINE & MEDICAL SCIENCE MARK 5TH INTERNATIONAL RARE DISEASE DAY

UCD School of Medicine delivered a new and innovative module in 2012 entitled: *Rare Genetic Disorders and the Medical Healthcare Professional*. This new module was shaped by the experiences of patients, and reflects the responsive nature of the UCD curriculum, as well as the commitment to serve society through understanding and improving the patient experience. You can learn more about International Rare Disease Day 2012 on *www.rarediseaseday.org*.

UCD & IPPOSI LEADING THE WAY IN RAISING AWARENESS OF RARE DISEASES

By **Dr Paula Byrne**, *Lecturer, UCD School of Medicine & Medical Science & Module Coordinator*

The UCD School of Medicine and Medical Science continually strives to use innovative methods to equip our students with the necessary skills and knowledge to become competent and caring healthcare professionals who are life-long learners. We are responsive to evolving advances in biomedical science and technology and to the needs of society.

In line with this we have developed an innovative elective module on *Rare Genetic Disorders and the Medical Healthcare Professional* which aims to increase awareness among future medical healthcare professionals. Rare genetic disorders are those that affect less than 1 in 2000 people, but with over 7,000 different rare disorders - *it is in fact not rare to have a rare disorder*.

Though the clinical aspects of the diseases may differ, people and families affected with rare disorders face many of the same obstacles and issues. They face many ongoing challenges on a daily basis, from getting a diagnosis, having access to treatment and services, finding information about the disease and research into it.

The patients are often the driving force behind overcoming these obstacles and the role of patient support groups cannot be underestimated. Over the course of this module we have a variety of lectures from clinicians, scientists, representatives of pharmaceutical industry, patients and patient support organisations, to explore some of the clinical, political, economic and societal issues around rare disorders. A highlight of the module will be talks from patients affected with rare disorders which will give a personal insight into selected rare disorders and are bound to make a lasting impact on our future medical healthcare professionals.

RARE BUT STRONG TOGETHER - IMPROVING THE PATIENT EXPERIENCE THROUGH EMPOWERMENT, AWARENESS & UNDERSTANDING

By **Eibhlín Mulroe**, *CEO of Irish Platform for Patients' Organisations, Science and Industry & Contributor to Rare Disease Module*

February 29, 2012 marks the fifth international Rare Disease Day. On this day hundreds of patient organisations from more than 50 countries worldwide are organising awareness-raising activities converging around the slogan "Rare but strong together".

Ireland has signed up to the European Commission council recommendation in June 2009 to develop a National Plan for Rare Diseases by the end of 2013. In January 2011, IPPOSI were involved in organising the Europlan-sponsored national conference in Farmleigh, bringing together patients, patient organisations, Department of Health, HSE staff and healthcare professionals to discuss what might feed into the development of a national strategy for rare diseases.

Following the success of the Europlan meeting the Minister for Health, Dr James Reilly, TD established a National Steering Group which is tasked with developing a five-year national plan which will focus on areas concerning diagnosis, prevention, management, treatment and research. IPPOSI are represented on this Steering Group by the CEO.

Rare diseases are individually rare but in totality they account for 6 - 8% of the overall population. Ireland needs an organised approach to facilitating patients with Rare Diseases, their families and their healthcare professionals in providing, in particular; adequate care, treatment and access to new therapies. The diagnostic journey can often be long for patients with Rare Diseases and can take up to 10 years to get a diagnosis. Education of our healthcare professionals and providing them access to information on Rare Diseases is vital in challenging that time taken to diagnose.

It is with this in mind that IPPOSI developed a partnership with Dr Paula Byrne in the UCD Medical School. Together IPPOSI/UCD planned the first module of its kind in Ireland for 3rd year medical students focusing exclusively on Rare Diseases and the patient perspective. The lecturers on this module are scientists, clinicians and most interestingly, patients describing their own condition to students. We hope that this will set the bar for other medical schools in Ireland and Europe to bring patients and their patient organisations into the classroom.

Who better to explain their unique experience than the patient? Future doctors will complete

this module with an increased awareness of the issues faced by patients living with Rare Diseases and a better informed medical community can only help future patients in the system.

For more information, visit *www.ipposi.ie*

RARE DISEASE PROFILE: ALPHA-1

By **Dr Tomás Carroll**, *Department of Medicine, RCSI & Contributor to UCD Rare Disease Module*

Ireland has one of the highest prevalences of Alpha-1 in the world and 1 in 25 Irish individuals are carriers for Alpha-1. Over 2,000 Irish individuals have severe Alpha-1 and over 170,000 are carriers, making it the most common genetic lung disease in Ireland after cystic fibrosis. The National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme was established in 2004 and provides testing free of charge to individuals throughout Ireland.

Alpha-1 antitrypsin (AAT) is an important protein produced by the liver, which is released into the blood and travels to the lungs. Here it protects the lungs from harmful environmental exposures, particularly tobacco smoke.

Alpha-1 antitrypsin deficiency (Alpha-1) is a genetic disorder which results in a reduction of AAT protein in the blood. The low AAT levels leave the lungs vulnerable to cigarette smoke and bacterial infections. Alpha-1 classically presents as emphysema or chronic obstructive pulmonary disease (COPD) and sometimes liver disease. The World Health Organisation recommends Alpha-1 testing for the following groups:

- COPD & emphysema patients
- Non-responsive severe asthmatics
- Patients with liver disease of unknown cause
- Relatives of patients and carriers with Alpha-1

You can learn more about Alpha-1 on the Alpha One Foundation's website www.alpha1.ie. UCD School of Medicine & Medical Science will occasionally profile rare diseases on the homepage to highlight the excellent contributions from experts (including patients with direct experience) to the School's rare disease module.

Other diseases covered in this important educational module included epidermolysis bullosa, sarcoidosis and hereditary metabolic disorders such as Fabry's Disease, and many of the lectures were given by patients, offering the undergraduate medical students a unique perspective on what it is like to suffer from a rare disease.

10. Love Your Lungs' Campaign

A group of Irish charities teamed up with Olympic Champion Dr Ronnie Delany to promote healthy lungs by highlighting symptoms of lung disease and the importance of early intervention. The Irish Lung Health Alliance, comprised of the Alpha One Foundation, the Irish Thoracic Society, the Cystic Fibrosis Association of Ireland, the Irish Lung Fibrosis Association, Ben Bulben COPD Support Group, the Irish Sleep Apnoea Trust, the Asthma Society of Ireland, the Irish Cancer Society and the Irish Sarcoidosis Network, united officially on Valentine's Day, to launch the **'Love Your Lungs'** campaign.

The campaign carried four simple messages:

1. Lung disease can affect anyone regardless of age, health status and walk of life
2. Symptoms such as persistent cough and shortness of breath are not normal and should be checked with your GP
3. Lung disease is treatable – the earlier you treat the better.
4. Keep lungs healthy - don't smoke and stay active

The 'Love Your Lungs' campaign saw charities working with hospitals and medical centres throughout the country to offer free lung function tests on World Spirometry Day 27th June. It also incorporated a new website, *www.lunghealth.ie*, which offered helpful information and advice regarding common lung conditions as well as patient support and tips on how to keep your lungs healthy.

Olympic legend Dr Ronnie Delany was an ambassador for the campaign underlining the importance of healthy lungs to an active lifestyle and visa versa. As an Olympic Gold Medallist and the winner of an unprecedented and unsurpassed 40 straight victories "indoors" in America from 1956 to 1959 including 33 mile races, Dr Delany embodied the link between good lung function and athleticism.

As part of the global initiative to encourage early detection of lung disease hospitals across the country in Dublin, Cork, Limerick, Waterford, Clare, Sligo, Mayo and Mullingar provided free lung (spirometry) tests on Wednesday, 27th June. Lung health tests were also being offered at the Love Your Lungs mobile health unit, there was also a unit stationed at the top of Grafton Street in Dublin city centre on the day. Early diagnosis and treatment of lung disease is crucial, leading to improved symptoms and quality of life for patients as well as avoiding further irreversible lung damage. It's also important to remember that many lung diseases can be prevented by keeping your lungs healthy through avoiding tobacco smoke and staying active.

This Olympic Year was the perfect opportunity to draw attention to the fact that regular physical

activity helps boost lung health. People with lung conditions often fear that exercise will make breathlessness worse but regular physical exercise is proven to improve quality of life and fitness including for those with lung conditions.

Representatives from the Lung Health alliance and Dr Ronnie Delany

SPIROMETRY – THE GOLD STANDARD IN LUNG TESTING

In the same way that blood pressure measurements provide a simple yet effective screening method for cardiovascular disease, spirometry tests can help to unmask the early symptoms of a variety of lung diseases before any more obvious signs appear.

It is a quick, simple and non-invasive method to test lung function. It measures the amount and speed of air that can be inhaled and exhaled by the lungs.

Spirometry is used to diagnose and monitor patients with lung disease. It helps assess alpha-1, asthma, cystic fibrosis and chronic obstructive pulmonary disease (COPD).

THE IRISH LUNG HEALTH ALLIANCE

11. Recent Events and Alpha-1 Support Group Update

ANNUAL ALPHA-1 CONFERENCE 2011

The Annual Alpha-1 Conference took place in October in the Marino Institute of Education. We were delighted to welcome Angela McBride, Development Officer from the US Alpha-1 Foundation based in Miami. The Alpha-1 Support Group presented a cheque for €7,500 to the Alpha One Foundation for the Sebia testing equipment for Alpha-1.

Angela McBride, Development Officer, US Alpha-1 Foundation

Right: *Alpha-1 Support Group presenting a cheque*

Far Right: *Prof N.G McElvaney, Deborah Kelleher, Louis Ryan, Seamus Crimmins and Kitty O'Connor*

CHRISTMAS CARDS

We had another successful year of sales for our Alpha-1 Christmas cards. Thanks to all the patients and sellers of the cards. This year more Christmas cards will be on sale please contact the Alpha-One Foundation for details.

CHRISTMAS CAROL SINGING

The patient support group were Christmas Carol Singing on Grafton St Dublin in December last year. Although this was one of the coldest days of the winter the team were full of the joys of Christmas and sang carols which resulted in raising over €1,000. Many thanks to all of you who participated in this festive event.

THE ALPHA-1 CHOPIN AWARDS 2011

The Annual Alpha-1 Chopin Awards took place in the Mansion House in November last year. This year 3 colleges, Trinity College, Dublin Institute of Technology and the Ulster University of music competed for a Bursary of €1,000. Louis Ryan from Trinity College won the Chopin Alpha-1 Award. The competition was judged by Deborah Kelleher, Director of the Royal Irish Academy of Music and Seamus Crimmins, Executive Director, RTE Performing Groups.

ALPHA-1 CHARITY NIGHT IN SWORDS WITH 'POMP' IN FEBRUARY 2012

Josephine McGuirk and her family organised a charity night for Alpha-1 in Swords in February this year. Pomp provided live music and there were plenty of raffle prizes to be won. The event raised over €2,000. Congratulations to all who helped organise the event and those who attended.

FLORA WOMAN'S MINI MARATHON

A big thank you to all those who participated in the Flora Women's Mini Marathon, this was a great day out and raised valuable funds for the Foundation. If you wish to participate in next year's run t-shirts and sponsorship cards are available from the Foundation.

MID-SUMMER'S NIGHT BALL 2012

Congratulations to Orla Keane and Martin Wickham from the Alpha One Foundation and Cystic Fibrosis Association who organized a mid-summer's night ball in the Lucan Spa hotel in aid of the two charities. The night consisted of dinner, Zumba dancing and music by John Walsh and Sons. This event raised €2,000 per charity.

Catherine Whelan, Martin Wickham, Kitty O'Connor, Orla Keane, Josephine McGuirk

SKY DIVE IN THE WEST

In September 2011 a family in Sligo raised over €2,000 for the Alpha One Foundation by participating in a sponsored Sky Dive, we would like to thank all those who organised and supported this brave event.

12. Acknowledgements

We would like to thank the following:

- The Alpha-1 Patient Support Group
- Pat O'Brien, Eric Mahon, Helen Moore, and Dr Bill Tormey and the Beaumont Hospital Biochemistry Department for their continued support and advice
- John Walsh and Angela McBride of the Alpha-1 Foundation (USA)
- Professor Maurizio Luisetti, Dr Ilaria Ferrarotti and Dr Stefania Ottaviani, Centre for Diagnosis of Inherited Alpha-1 Antitrypsin Deficiency, University of Pavia, Italy
- Dr Ned Barrett, Department of Biochemistry, Mid-Western Regional Hospital, Limerick
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- Dr Joseph McPartlin, Trinity Biobank, Institute of Molecular Medicine, St James's Hospital, Dublin
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- Dr Paula Byrne and Dr Amanda McCann of the UCD School of Medicine
- Professor Dermot Kenny and the RCSI Clinical Research Centre

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

We would also like to acknowledge the contribution of the following hospitals;

- Bon Secours Hospital Tralee
- Bon Secours Hospital Dublin
- Cavan General Hospital
- Children's University Hospital, Temple Street, Dublin
- Coombe Women and Infants University Hospital
- Cork University Hospitals
- Galway University Hospitals
- James Connolly Memorial Hospital Blanchardstown
- Letterkenny General Hospital
- Mayo General Hospital
- Mercy University Hospital Cork
- Midland Regional Hospital, Tullamore
- Midland Regional Hospital, Mullingar
- Midwestern Regional Hospital, Limerick
- Our Lady's Children's Hospital, Crumlin
- Our Lady of Lourdes Hospital Drogheda
- Peamount Hospital, Dublin
- Rotunda Hospital, Dublin
- Sligo General Hospital
- St James's Hospital, Dublin
- St Vincent's University Hospital, Dublin
- The Adelaide and Meath Hospitals including National Children's Hospital Tallaght
- The Mater Misericordiae University Hospital Dublin
- Waterford Regional Hospital

- **Alpha-1 antitrypsin deficiency (Alpha-1) is a genetic condition that can cause lung and liver disease**
- **1 in 25 Irish individuals carry a gene that causes Alpha-1**
- **There are 3,000 individuals on the island of Ireland with severe Alpha-1 and over 200,000 carriers**
- **Individuals with chronic obstructive pulmonary disease (COPD), regardless of age or smoking history, should be tested for Alpha-1**
- **Less than 10% of Alpha-1 individuals have been diagnosed in Ireland**
- **Carrier individuals who smoke are also at increased risk of developing lung disease**
- **Smoking cessation and the avoidance of harmful environmental and occupational pollutants are vital to prevent lung disease in Alpha-1**
- **Smoking cessation rates are increased in individuals with Alpha-1**

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