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1. Executive Summary

We are delighted to be celebrating the 10th anniversary of the Alpha One Foundation. During the past decade we have established a National Screening Targeted Detection Programme, National Alpha-1 Registry and commenced the first clinical trials for replacement therapy for Alpha-1 in Ireland. We are now the national referral centre for Alpha-1 and have a dedicated Alpha-1 clinic in Beaumont Hospital.

Our recently published research has shown that 1 in 25 individuals in Ireland carry the severe Z mutation of the Alpha-1 gene, 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 almost 200,000 individuals with a milder form of Alpha-1 who may also be at risk of lung or liver disease. The past ten years has seen the Alpha One Foundation steadily increase awareness of Alpha-1 amongst the medical community and this has led to increased detection of the condition. We have endeavoured to raise public awareness of Alpha-1, to promote basic and clinical research, and most importantly, to ensure early diagnosis and appropriate care of Alpha-1 patients.

The National Screening Targeted Detection Programme receives test requests from over 25 hospital laboratories, GP practices, and family members of known Alpha-1 individuals. Since 2004 we have screened over 6,500 individuals for Alpha-1. Upon diagnosis patients 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 on all our Alpha-1 patients. We earnestly encourage more Alpha-1 individuals to enrol on the National Alpha-1 Registry. Clinical trials for replacement therapy in Alpha-1 are also ongoing in the centre. We continue our research study in MZ (carrier) individuals, and the aim of this study is to clarify if MZ patients are at a greater risk of developing COPD. The study is in collaboration with Dr. Edwin Silverman and his team in 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), the European Organisation for Rare Diseases (EURORDIS), and ALFA Europe.

We were delighted to welcome Dr. Ilaria Ferrarotti from the University of Pavia in Italy during September. Dr. Ferrarotti received a prestigious travelling fellowship from the European Respiratory Society to come and work in the McElnaney research group and is carrying out research into Alpha-1. We also welcomed Dr. Timm Greulich from the alpha-1 antitrypsin laboratory in the University of Marburg in Germany who visited our facility in January.

The Alpha One Foundation hosted an information stand and presented novel research at the Irish Thoracic Society (ITS) conference in November 2010 in Cork. Our research was also presented at the American Thoracic Society (ATS) meeting in Denver in May 2011. 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 journal clubs, immunology and biochemistry laboratory meetings and COPD patient meetings. Our annual patient conference was held in October 2010 in the Marino Institute of Education, and
this is an excellent opportunity for Alpha-1 patients and family members to meet other patients.

Our patient support group held a Pub Quiz in Ballyporeen, Tipperary in February and a Slim-a-thon/Exercise-a-thon was held in Maynooth last April. These events were tremendously successful and raised valuable funds for the Foundation. The group also successfully raised funds through the Dublin City Marathon and the Flora Women’s Mini Marathon. The Alpha One Foundation held a Chopin Anniversary Recital in the Mansion House in November 2010. During Chopin’s life he suffered from chronic respiratory illness probably due to Alpha-1. We felt it appropriate to celebrate Chopin’s life and draw attention to respiratory research especially into Alpha-1 Antitrypsin Deficiency. We were delighted to welcome John Walsh CEO of the US Alpha-1 Foundation to the event. John presented about the importance of Alpha-1 detection, increasing awareness of the condition, and patient advocacy to a rapt audience of doctors, nurses, and scientists at Beaumont Hospital.

This brief overview may give you some idea of the work being done and the progress being made by the Alpha One Foundation. This work is collaborative and I wish to thank my former and present colleagues for their diligence and dedication which made the past ten years such a success for the Foundation.

Kitty O'Connor
Chief Executive Officer
2. Ten years of the Alpha One Foundation

The Alpha One Foundation was founded in 2001 by Prof N.G. McElvaney and based in the RCSI Clinical Research Centre at Beaumont Hospital. Through dedication and resilience then Chief Executive Officer Larry Warren succeeded in acquiring funding from the Department of Health and Children in 2004 to commence the National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme.

This enabled the Foundation to employ a senior scientist and research nurse to begin working on the programme. A pilot study commenced in Beaumont Hospital in 2004 and now 7 years later over 25 hospitals in Ireland participate in the National Targeted Detection Programme. Our research has shown Alpha-1 is a common inherited disease with over 2,000 individuals in Ireland at risk of lung and liver disease due to severe deficiency (ZZ). In addition, almost 200,000 individuals are also at risk of lung disease due to a milder deficiency (SZ and MZ). Early diagnosis is vital for health and welfare of our patients, and this is our main objective.

In March 2010 the first Alpha-1 Clinic in Ireland commenced in Beaumont Hospital. Alpha-1 individuals can be fast tracked to this clinic, were they are medically assessed, enrolled in clinical trials, and can avail of genetic counselling. To date this clinic has seen over 550 Alpha-1 patients and family members. The Alpha One Foundation also hosts the National Alpha-1 Patient Registry, several clinical trials, and continues its awareness campaign for Alpha-1 Antitrypsin Deficiency.

Throughout the 10 years of the Alpha One Foundation we have collaborated with national and European scientific and patient groups. We continue our active participation with the Medical Research Charities Group, Irish Donor Network, Rare Diseases Taskforce, Irish Platform for Patient Organisations Science and Industry (IPPOSI), the European Organisation for Rare Diseases (EURORDIS) and ALFA Europe.

We hosted the International Patient Congress in 2004 in Dublin which was attended by over 300 Alpha-1 patients from across the globe. The Alpha One Foundation played a lead role in supporting Ireland’s successful ban on smoking in the workplace in 2004. We also played a role in the H1N1 vaccination programme and are frequent contributors to World COPD Day. We work in close alliance with the Alpha-1 Foundation in the United States in the areas of research, screening and detection to improve life possibilities for those with the condition.

The Alpha One Foundation presents at respiratory journal clubs, hospital laboratory seminars, and patient meetings throughout the year. Finally, we continue to support our vital Alpha-1 patient support group which promotes understanding and awareness of the condition among patients and their families.

**FUNCTIONS OF THE ALPHA ONE FOUNDATION:**

**National Targeted Detection Programme**
The World Health Organisation (WHO) recommends the following patient groups should be screened for Alpha-1:

- All COPD patients
- All non-responsive asthmatics
- All cryptogenic liver disease patients
- All first-degree relatives of known Alphas
- Individuals with reduced serum levels of AAT
- Panniculitis patients
There are over 1,300 Alpha-1 blood tests performed free by the Alpha One Foundation every year. Over 25 hospitals currently send us blood samples for Alpha-1 testing. This is the only national Alpha-1 screening programme in the world.

**Diagnostic Services Provided**

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

- Phenotyping
- Genotyping
- Quantification of serum AAT

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

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

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

1. **Family Screening**

   Family screening provides an opportunity for early diagnosis of Alpha-1 and therefore can potentially reduce the risk of developing lung disease. We provide a genetic counselling for family members considering testing for Alpha-1. We then offer them rapid access to our outpatient clinic facilities. Alpha-1 patients are also provided with access to the Alpha-1 patient support group and to our website [www.alpha1.ie](http://www.alpha1.ie).

2. **Evaluation of New Treatments**

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

3. **National Alpha-1 Referral Centre**

   As the national referral centre for Alpha-1 we provide a rapid access Alpha-1 clinic for newly-diagnosed Alphas. The Alpha-1 patient is seen by a team involving doctors, nurses, and physiotherapists and international best practice standards of care are followed. We host an annual patient information day for Alpha-1 individuals and family members.

4. **National Advocacy Role**

   The Alpha One Foundation presents research and promotes awareness of the condition at hospital- and GP-based respiratory team meetings, immunology and biochemistry laboratory meetings and patient meetings. The Alpha One Foundation played a lead role in supporting successful ban on smoking in the workplace. We also played a role in the H1N1 vaccination programme and are frequent contributors to World COPD (chronic obstructive pulmonary disease) Day and Rare Disease Day.

5. **National Alpha-1 Patient Registry**

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

6. **Patient Support Group**

   Our patient support group provides support and understanding to both newly-diagnosed and known Alphas and their families. The group is also actively involved in fundraising events such as Dublin City Marathon, the women’s mini-marathon, fashion sales, and pub quizzes.
3. The Prevalence of Alpha-1 Antitrypsin Deficiency in Ireland

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**INTRODUCTION**

Alpha-1 antitrypsin (AAT) deficiency is a hereditary disorder first reported in the early 1960s when emphysema was described in patients with low plasma levels of AAT protein [1]. The condition is associated with substantially increased risk for the development of pulmonary emphysema by the third or fourth decades of life and is also associated with risks for development of hepatic disease [2], cutaneous panniculitis [3], bronchiectasis [4], vasculitis [5], Wegener’s granulomatosis [6], and lung cancer [7]. AAT deficiency is characterised by misfolding of the AAT protein and belongs to a class of genetic diseases termed conformational disorders [8].

The SERPINA1 gene is highly pleiomorphic with over 100 alleles identified to date [9]. Mutations which confer an increased risk of developing pulmonary emphysema and/or liver disease are those in which deficiency alleles are combined in homozygous or heterozygous states, yielding

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**RESPIRATORY RESEARCH. 2011 JUL 13;12:91.**

**ABSTRACT**

**Background:** Alpha-1 antitrypsin deficiency (AATD) results from mutations in the SERPINA1 gene and classically presents with early-onset emphysema and liver disease. The most common mutation presenting with clinical evidence is the Z mutation, while the S mutation is associated with a milder plasma deficiency. AATD is an under-diagnosed condition and the World Health Organisation recommends targeted detection programmes for AATD in patients with chronic obstructive pulmonary disease (COPD), non-responsive asthma, cryptogenic liver disease and first degree relatives of known AATD patients.

**Methods:** We present data from the first 3,000 individuals screened following ATS/ERS guidelines as part of the Irish National Targeted Detection Programme (INTDP). We also investigated a DNA collection of 1,100 individuals randomly sampled from the general population. Serum and DNA was collected from both groups and mutations in the SERPINA1 gene detected by phenotyping or genotyping.

**Results:** The Irish National Targeted Detection Programme identified 42 ZZ, 44 SZ, 14 SS, 430 MZ, 263 MS, 20 IX and 2 rare mutations. Analysis of 1,100 randomly selected individuals identified 113 MS, 46 MZ, 2 SS and 2 SZ genotypes.

**Conclusion:** Our findings demonstrate that AATD in Ireland is more prevalent than previously estimated with Z and S allele frequencies among the highest in the world. Furthermore, our targeted detection programme enriched the population of those carrying the Z but not the S allele, suggesting the Z allele is more important in the pathogenesis of those conditions targeted by the detection programme.
AAT serum levels below a putative protective threshold of 11µM. The most common variants associated with disease are the Z [Glu342Lys] and S [Glu264Val] mutations, caused by a single amino acid replacement of glutamic acid at positions 342 and 264 of the polypeptide, respectively [8]. The class of SERPINA1 variants termed “null” mutations lead to a complete absence of AAT production and while extremely rare, confer a particularly high risk of emphysema [10].

AATD is an under-diagnosed condition with most cases misdiagnosed as COPD or non-responsive asthma. As a result, long delays between presentation of first symptoms and correct diagnosis are commonplace [11]. Guidelines issued by both the World Health Organisation and the American Thoracic Society/European Respiratory Society (ATS/ERS) recommend the establishment of targeted screening programmes for the detection of patients with AATD [12-13]. Moreover, while a large number of cohorts have been investigated, many of these studies were based on screening symptomatic patients, and performed on small groups of less than 500 individuals with accompanying high risk of error.

Apart from a few notable exceptions, such as a Swedish neonatal screening study [2], the lack of large population based studies using random sampling means the true prevalence of AATD in most European countries remains unknown. To address the paucity of data relating to AATD in the Irish setting, we analysed 1,100 individuals taken at random from the general population. In addition, we analysed a targeted population of symptomatic individuals and compared the findings with our general population to investigate whether targeted detection increased yield across all deficiency allele groups.

MATERIALS AND METHODS

Subjects
A total of 3,000 individuals were screened as part of the Irish National Targeted Detection Programme (INTDP). The detection programme is ongoing and began in May 2004 supported by funding from the Irish Government. The criteria for targeted screening were COPD, non-responsive asthma, cryptogenic liver disease, first degree relatives of known AATD patients (including ZZ, SZ and MZ) and individuals with reduced serum AAT levels according to the joint ATS/ERS guidelines (Figure 3.1). In addition, 1,100 individuals were screened from the Trinity Biobank DNA collection at St. James’s Hospital Institute of Molecular Medicine, Dublin. The Trinity Biobank is a national buccal swab DNA collection selected at random from the electoral register.

Quantification of AAT
AAT levels were measured by radial immunodiffusion (RID) (Siemens) or by nephelometry (Dade-Behring BN II). It must be noted that discrepancies exist when comparing these two methods for serum AAT quantification. Nephelometric methods can overestimate AAT concentrations due to haemoglobin or lipid interference, while RID-based methods have been shown to overestimate AAT concentrations by as much as 35-40% [14] and are less precise than nephelometric methods with higher coefficients of variation [15]. Moreover, the lower sensitivity inherent to the RID method because of the high lower limit of detection (0.33 g/L) becomes a factor when testing ZZ individuals with AAT concentrations < 0.33 g/L.

Phenotyping
Qualitative detection and characterisation of AAT phenotypes was carried out using the Hydrasys electrophoresis platform (Sebia) and the Hydragel 18 A1AT Isofocusing kit (Sebia)[Figure 3.2A] [16]. This isoelectric focusing (IEF) method
on agarose gel has an added immunofixation step which utilises a specific antibody to AAT, rendering it superior to traditional IEF techniques.

**Genotyping**

Genotyping was performed on a LightCycler 480 (Roche) with specific primers and probes (Metabion) designed for the Z and S mutations as described in a previous publication [Figure 3.2B] [17].

**Data elaboration and statistical analysis**

The prevalence and numbers of genotypes in the Irish population was calculated by applying the Hardy-Weinberg principle. Frequencies of genotypes and phenotypes were calculated and a derivative parameter, notably the type and number of mutations in each database, defined (Z, S, etc.). Data was analysed by descriptive statistics, percentage distribution, chi-square tests and methods for calculating odds ratios (ORs), 95% confidence intervals (95% CIs), accuracy and other contingency parameters, as appropriate [18]. Statistical significance was assumed at two-tailed $p < 0.05$, unless stated otherwise.

**RESULTS**

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

![Figure 3.2: Methods employed for analysis of AAT mutations. (A) Typical isoelectric focusing gel used for identification of AAT phenotype. (B) Genotyping assay used to identify the Z mutation.](image)

![Figure 3.3: Analysis of AAT mutations in Ireland. (A) 1,100 DNA samples in the Biobank collection were genotyped for the S and Z mutations. (B) 3,000 Irish individuals were screened as part of the national targeted detection programme following ATS/ERS guidelines.](image)
In the Trinity Biobank collection, 113 MS heterozygotes, 46 MZ heterozygotes, 2 SS homozygotes, and 2 SZ compound heterozygotes were identified (Figure 3.3A). This data yields a frequency of 0.0218 for the Z allele and 0.0541 for the S allele in the Irish population. Assuming Hardy-Weinberg equilibrium and based on a population of 4.24 million inhabitants (Census of Ireland 2006, www.cso.ie) these allele frequencies yield 2,015 ZZ individuals, 10,001 SZ individuals and 12,409 SS individuals (Table 3.1). Thus, the estimated prevalence of severe AATD (ZZ homozygotes) in Ireland is 1/2,104. In addition to ZZ AATD, the estimated prevalence of intermediate AATD (SZ compound heterozygote) is 1/424, with this phenotype also at increased risk of lung and possibly liver disease, while the estimated prevalence of mild AATD (SS homozygote) is 1/341. Finally, in terms of carriers, the calculated Z and S allele frequencies yield 170,832 MZ heterozygotes and 423,947 MS heterozygotes with an estimated prevalence of 1/25 for MZ and 1/10 for MS.

**Prevalence of AATD in the Irish National Targeted Detection Programme**

A total of 3,000 individuals screened for AATD as part of the national targeted detection programme identified 430 MZ heterozygotes, 263 MS heterozygotes, 44 SZ compound heterozygotes, 42 ZZ homozygotes, and 14 SS homozygotes (Figure 3.3B), with allele frequencies of 0.0938 for the Z mutation and 0.0518 for the S mutation in the targeted population. A further 20 individuals with the I mutation [Arg39Cys], associated with a mild plasma deficiency [19] similar to the S

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Prevalence [% of total population, 95% CI]</th>
<th>Numbers in Ireland</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>1/10 [10.00%, 9.70 – 10.30%]</td>
<td>423,947</td>
</tr>
<tr>
<td>MZ</td>
<td>1/25 [4.03%, 3.97 – 4.09%]</td>
<td>170,832</td>
</tr>
<tr>
<td>SS</td>
<td>1/341 [0.29%, 0.20 – 0.40%]</td>
<td>12,409</td>
</tr>
<tr>
<td>SZ</td>
<td>1/424 [0.24%, 0.23 – 0.25%]</td>
<td>10,001</td>
</tr>
<tr>
<td>ZZ</td>
<td>1/2,104 [0.05%, 0.04 – 0.06%]</td>
<td>2,015</td>
</tr>
</tbody>
</table>

**TABLE 3.1: Estimated Prevalence of AAT Genotypes in Ireland**

Data from the Trinity Biobank presented as prevalence [% of total population, 95% confidence interval (CI)]. These figures are based on an Irish population of 4.24 million in the Republic of Ireland (www.cso.ie).

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>N</th>
<th>Mean AAT [g/L +/- SEM]</th>
<th>AAT range [g/L]</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV</td>
<td>1</td>
<td>1.28</td>
<td>n/a</td>
</tr>
<tr>
<td>MI</td>
<td>12</td>
<td>1.285 +/- 0.095</td>
<td>0.93 – 2.08</td>
</tr>
<tr>
<td>MS</td>
<td>263</td>
<td>1.199 +/- 0.021</td>
<td>0.499 – 3.32</td>
</tr>
<tr>
<td>MZ</td>
<td>430</td>
<td>0.871 +/- 0.014</td>
<td>0.442 – 4.08</td>
</tr>
<tr>
<td>IS</td>
<td>3</td>
<td>1.004 +/- 0.103</td>
<td>0.801 – 1.14</td>
</tr>
<tr>
<td>SS</td>
<td>14</td>
<td>0.842 +/- 0.046</td>
<td>0.556 – 1.20</td>
</tr>
<tr>
<td>iZ</td>
<td>5</td>
<td>0.605 +/- 0.076</td>
<td>0.333 – 0.801</td>
</tr>
<tr>
<td>iS</td>
<td>3</td>
<td>1.004 +/- 0.103</td>
<td>0.801 – 1.14</td>
</tr>
<tr>
<td>SS</td>
<td>14</td>
<td>0.842 +/- 0.046</td>
<td>0.556 – 1.20</td>
</tr>
<tr>
<td>SZ</td>
<td>44</td>
<td>0.564 +/- 0.021</td>
<td>0.23 – 0.98</td>
</tr>
<tr>
<td>ZZ</td>
<td>42</td>
<td>0.11 +/- 0.012</td>
<td>0.05 – 0.333</td>
</tr>
</tbody>
</table>

**TABLE 3.2: AATD Phenotypes and Concentrations in the INTDP.**

AATD phenotypes and AAT concentrations from 3,000 individuals screened listed according to increasing risk of disease. Data presented as mean AAT concentration +/- standard error of the mean (SEM).
mutation, were identified with an allele frequency of 0.0033, as well as two individuals with extremely rare mutations, V [20] and Zbristol [21]. Serum AAT levels among the various phenotypic groups illustrate the relationship between decreasing AAT levels and increasing risk of disease (Table 3.2).

**Comparison of Z and S allele frequencies**

The frequency of the S allele in a random sample from the general population was similar to that identified in the targeted population. However, the frequency of the Z allele was four-fold higher in the targeted population compared to the general population (Figure 3.4). The numbers of Z and S alleles in both populations are presented in Table 3.3. The risk of being registered with a specific condition in the INTDP database is 4.6 times higher in subjects carrying the Z mutation than in other carriers or non-carriers (OR = 4.64, 95% CI 3.41 - 6.19). This is most likely due to the increased risk associated with the Z mutation compared to the S mutation alone (OR = 4.48, 95% CI 2.88 - 5.92).

**Discussion**

Our study describes the prevalence of AATD in a randomly selected sample of 1,100 individuals from the Irish population and in a targeted population, specifically with regard to the two most common alleles associated with AATD, the Z and S mutations. In the general Irish population the Z mutation occurs at a frequency of 0.0218, while the S mutation occurs at a frequency of 0.0541. This means 1 in 25 Irish individuals are heterozygous for the Z allele and 1 in 10 are heterozygous for the S allele. More importantly from a clinical perspective, 1 in 2,104 Irish individuals are ZZ homozygotes, 1 in 424 are SZ compound heterozygotes, and 1 in 341 are SS homozygotes. In comparison, when we looked at the INTDP population the Z mutation occurred at a frequency of 0.0938 and the S mutation occurred at a frequency of 0.0518. This means 1 in 7 tested were ZZ and 1 in 11 tested were MS. Strikingly, 1 in 71 tested were ZZ, 1 in 68 were SZ and 1 in

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>INTDP</th>
<th>Biobank</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>S versus other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S alleles</td>
<td>311</td>
<td>119</td>
<td>430</td>
</tr>
<tr>
<td>Other (including Z)</td>
<td>5689</td>
<td>2081</td>
<td>7770</td>
</tr>
<tr>
<td>Total alleles</td>
<td>6000</td>
<td>2200</td>
<td>8200</td>
</tr>
<tr>
<td>Z versus other</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z alleles</td>
<td>563</td>
<td>48</td>
<td>611</td>
</tr>
<tr>
<td>Other (including S)</td>
<td>5437</td>
<td>2152</td>
<td>7589</td>
</tr>
<tr>
<td>Total alleles</td>
<td>6000</td>
<td>2200</td>
<td>8200</td>
</tr>
<tr>
<td>Z versus S</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Z alleles</td>
<td>563</td>
<td>48</td>
<td>611</td>
</tr>
<tr>
<td>S alleles</td>
<td>311</td>
<td>119</td>
<td>430</td>
</tr>
<tr>
<td>Total alleles</td>
<td>874</td>
<td>167</td>
<td>1041</td>
</tr>
</tbody>
</table>

Data analysed by chi-square test with Yates correction.
214 were SS. Taken as a whole, 27.1% of the targeted population contained at least one AAT mutation.

Comparing data from the two groups investigated, the allele frequency for Z is over four-fold higher in the targeted population compared to a sample of the general population. Targeted detection programmes produce a higher rate of AATD detection although the risk of missing asymptomatic individuals exists. However, the four-fold increase in Z frequency observed highlights the effectiveness of the targeted screening approach as advocated by ATS/ERS as the most cost-effective method of detection [12]. There is particular emphasis in the INTDP on aggressive family screening to identify asymptomatic relatives before significant lung damage has occurred. This increases the rate of detection of deficient alleles, and in our study 14% of INTDP individuals were screened because of a deficient first-degree relative. Nonetheless, the high frequency of the Z allele in the targeted group underlines the role of this mutation in the pathogenesis of lung and liver disease. In contrast to Z, the allele frequency for S was not significantly increased in the targeted population compared to the Biobank cohort. This finding was not surprising considering the lack of strong evidence for risk of disease due to the S mutation. The S allele is associated with a mild plasma deficiency of AAT as the S AAT protein is less polymerogenic than Z AAT [19], and the S allele is assumed clinically significant only when co-inherited with Z or other deficient alleles. For example, the SZ genotype is a significant risk factor for COPD [22-23]. Evidence also exists of abnormal liver function as characterised by elevated liver enzymes in newborn SZ individuals [2] and a possible risk for liver disease in later life [24-25]. However, a risk of COPD due to the MS genotype was not found [23].

A previous Irish study investigated the prevalence of AATD in 111 Irish coeliac patients compared to 250 blood donors and found gene frequencies of 0.008 for Z and 0.04 for S in the blood donor group [26]. However, the small sample size and the less accurate isoelectrofocusing method employed may account for the discrepancies with our findings. We have previously used the same phenotyping method and found the MZ phenotype was often difficult to correctly identify, compared to the more accurate and reliable Sebia method now used in our laboratory. Similar to the revised Irish data on AATD presented here, studies in other countries may have significantly underestimated the frequency of the Z and S mutations due to small sample size and/or methodological limitations.

Throughout Europe the frequency of the Z and S mutations varies widely between countries, geographic regions, and ethnic groups. Approximately 3 - 4% of northern Europeans carry the Z allele and 6% carry the S allele [27]. The highest frequency of the S allele is found in the Iberian Peninsula with a mean gene frequency of 0.0564, suggesting the mutation is likely to have arisen in the region. Placing our results in a European context, we observe that the frequency of 0.0541 for the S mutation in Ireland is among the highest in Europe, and similar to the Iberian Peninsula. The frequency of the Z variant is highest in northern and western European countries with a mean gene frequency of 0.014, peaking in southern Scandinavia with a gene frequency of > 0.02 [28]. Similar to the S allele, the frequency of 0.0218 for the Z allele in the Irish population is also among the highest in Europe. Interestingly, as the genotyping methods employed to study the Biobank cohort only identify Z and S alleles it is worth considering the gene frequencies described may still underestimate AATD in Ireland. Other rare SERPINA1 mutations could also be present in the Biobank cohort, for example, we identified 22 rare non-Z, non-S mutations in the INTDP cohort which would be missed by our genotyping method.

The high prevalence of AATD in Ireland is not without precedent. Ireland has the highest prevalence of cystic fibrosis [29-30] and haemochromatosis [31] in Europe, as well as high frequencies of other genetic diseases [32]. This can be partly explained by the geographical isolation of an island on the fringes of Western Europe, with the genetic background of the population remaining largely undisturbed by the demographic movements that prevailed on
mainland Europe. The Z mutation is thought to have arisen from a single origin 66 generations or 2,000 years ago [33-34], and its high frequency in southern Scandinavia suggests that the mutation arose in this area and was subsequently dispersed by migration patterns such as the Viking colonisation of north-western Europe between 800 and 1200 AD [35]. The relatively high frequency of the Z allele in the Irish population may represent a Viking genetic footprint resulting from significant settlement in Ireland in the period from 800 to 1200 AD when large towns and urban centres were established by Viking settlers including modern Dublin, Limerick and Cork [36]. The long history of emigration from Ireland would also suggest that populations of Irish descent in countries such as America, Canada, and Australia contain high frequencies of the Z mutation (and S mutation) and may benefit from screening for AATD.

The relatively high frequency of the S mutation could suggest that the tribes who first settled on Irish shores may have migrated from the Iberian Peninsula. The S mutation is older than the Z and is postulated to have arisen in the north of the Iberian Peninsula and subsequently spread throughout Europe during mass migration [37]. For example, one of the highest reported frequencies of the S allele in Europe is in the region of Galicia in north-western Spain [38] and in general high S frequencies are found all along the western Atlantic seaboard [28]. Other genetic similarities have been described that suggest a shared ancestral heritage among the populations on the Atlantic façade of Europe, stretching from northern Iberia to western Scandinavia and dating back to the end of the last Ice Age [39].

Another intriguing theory postulated to explain the high prevalence of AATD in European populations is the Z and S mutations confer a survival advantage on heterozygotes, of particular relevance in the pre-antibiotic era [40]. Polymers of Z AAT protein have been found in lung lavage and shown to act as neutrophil chemoattractants [41], and an enhanced inflammatory response has been demonstrated in MZ heterozygotes [42]. The proposed hypothesis suggests the Z and S alleles favour the generation of polymers at sites of inflammation and these polymers help focus and amplify the host inflammatory response to eradicate invading infectious organisms.

In summary, the findings of our study have significant consequences. We show that AATD is more prevalent than previously estimated in Ireland [28], with over 2,000 ZZ and 10,000 SZ individuals at significantly increased risk of developing lung and liver disease. A further 170,000 MZ heterozygotes are estimated in the Irish population and this group may also be at risk of developing COPD, particularly in individuals who smoke [43]. Moreover, we reaffirm the importance of the Z allele in the clinical disorders associated with AATD. The INTDP enriched the population of those carrying the Z but not the S allele, suggesting the Z allele is more important in the pathogenesis of those conditions targeted by the detection programme.

It is clear from the data presented here that the statement “AATD is not a rare disease but a disease that is rarely diagnosed” is particularly apt in the Irish setting [44]. The continuing lack of awareness and under-diagnosis of this condition is alarming considering the high numbers of individuals at risk due to deficient SERPINA1 mutations. The advantages of early and accurate diagnosis of AATD are manifold and include closer observation and management of affected individuals, especially regarding pulmonary and liver health; family member testing; aggressive smoking cessation efforts; consideration of occupational hazards and environmental exposures; and significant economic benefits arising from the reduced burden on healthcare providers [11, 45].

Conclusion
This study demonstrates that the Z and S allele frequencies in Ireland are among the highest in the world, with large numbers of individuals at risk of disease due to AATD in the Irish population. The vast majority of these individuals remain undetected. The importance of an early diagnosis of AATD cannot be over-emphasised as the resulting appropriate medical follow-up
and lifestyle changes can help prevent or at least postpone the development of the lung and liver disease associated with this condition.

List of abbreviations

REFERENCES


44. de Serres FJ: Alpha-1 antitrypsin deficiency is not a rare disease but a disease that is rarely diagnosed. *Environ Health Perspect* 2003, **111**:1851-1854.

4. Targeted Detection Screening Programme

TESTING

Guidelines from the World Health Organisation (WHO), American Thoracic Society (ATS), and European Respiratory Society (ERS) advocate targeted detection programmes for AATD in patients with COPD, non-responsive asthma, cryptogenic liver disease and in first-degree relatives of known AATD individuals (Table 4.1).

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

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 investigates serum samples from suspected AATD individuals (Figure 4.1) and employs the Hydragel 18 AAT isofocusing kit (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 4.2A). The procedure involves isoelectric focusing on agarose gel, performed on the semi-automatic HYDRASYS system, followed by immunofixation with AAT antiserum. The assay is carried out in two stages. Firstly, isoelectric focusing on agarose gel is used to separate the proteins in serum samples taken from suspected AATD individuals. Secondly, immunofixation with enzyme-labelled anti-AAT antiserum identifies the different variants of AAT. This method has been found to be highly specific, rapid and simple to perform (F. Zerimech et al, Clinical Chemistry and Laboratory Medicine 2008). This is the most accurate method of screening 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 4.2B). 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.

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

RESULTS TO DATE

So far over 6,500 individuals with COPD, asthma, liver disease and asymptomatic first-degree relatives of known AATD individuals have
have been detected including 34 SS, 959 MZ, 655 MS, 34 MI, 10 IZ, and 5 IS phenotypes. The percentage of deficiency alleles (approximately 29%) 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 analysis 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 undiagnosed 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 screenings 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 98 new ZZ individuals. In addition to newly diagnosed ZZ individuals, a further 50 ZZ individuals have been referred to Beaumont Hospital by other centres and physicians (Figure 4.4).

The current total of individuals tested in the targeted detection programme to date is almost 6000 from hospitals nationwide, with a further 665 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 4.2). This is because Beaumont Hospital was the sole participating centre in the first year of the programme. After this first year, the screening programme expanded to include the majority of Dublin hospitals. The largest participating centre after Beaumont Hospital is St. Vincent’s University Hospital, which accounts for 15% of all Dublin requests.

![FIGURE 4.4: All new ZZ cases diagnosed by or referred to Alpha One Foundation since 2004 (total = 146)](image)

<table>
<thead>
<tr>
<th>TABLE 4.2: Requests from Dublin area hospitals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nationwide Requests</td>
</tr>
<tr>
<td>Cork University Hospitals</td>
</tr>
<tr>
<td>Letterkenny General Hospital</td>
</tr>
<tr>
<td>Sligo General Hospital</td>
</tr>
<tr>
<td>Cavan General Hospital</td>
</tr>
<tr>
<td>Midland Regional Hospital Mullingar</td>
</tr>
<tr>
<td>Bon Secours Tralee</td>
</tr>
<tr>
<td>Our Lady of Lourdes Hospital Drogheda</td>
</tr>
<tr>
<td>Midland Regional Hospital Tullamore</td>
</tr>
<tr>
<td>Roscommon County Hospital</td>
</tr>
<tr>
<td>Waterford Regional Hospital</td>
</tr>
<tr>
<td>Mayo General Hospital</td>
</tr>
<tr>
<td>Mid-Western Regional Hospital Limerick</td>
</tr>
<tr>
<td>Monaghan General Hospital</td>
</tr>
<tr>
<td>Galway University Hospitals</td>
</tr>
<tr>
<td>Clane General Hospital</td>
</tr>
<tr>
<td>Bon Secours Galway</td>
</tr>
<tr>
<td>Louth County Hospital</td>
</tr>
<tr>
<td><strong>Total</strong></td>
</tr>
</tbody>
</table>

![TABLE 4.3: Requests from hospitals nationwide (excluding Dublin)](image)
The targeted detection screening programme has received 2598 requests nationwide (excluding Dublin). The largest participating centre is Cork University Hospital (CUH), and 28% of all test requests from outside the Dublin area are from CUH (Table 4.3). The second biggest participating centre outside of Dublin is Letterkenny General Hospital (22%), closely followed by Sligo General Hospital (18%).

In the past 12 months we have presented results from our screening programme to the respiratory teams in St. Vincent’s University Hospital, the Mater Misericordiae University Hospital, St. James’s Hospital, St. Columcille’s Hospital in Loughlinstown, University Hospital Galway, Limerick Regional Hospital, Cavan General Hospital, and Peamount Hospital. We presented to members of the Immunology, Biochemistry, and Clinical Chemistry Departments in Tallaght Hospital, Crumlin Hospital, the Mater Misericordiae University Hospital, University Hospital Galway, Limerick Regional Hospital, and Cavan General Hospital. In addition, we also presented at the Dublin North Regional GP meeting, the Sligo COPD patient support group, and to medical students in Queen’s University Belfast. The main aim of these presentations is to increase awareness of AATD amongst the respiratory and paramedical community and the general public. While the respiratory teams are dealing with the patient population 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, but many do not offer the further confirmatory test (called a phenotype).

As a result of our presentations, we have seen an increase in test requests (Figure 4.5). Furthermore, in an excellent example of integrated thinking several laboratories have adopted a “red flag” system for AAT testing. This innovative 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 electronic suggestion is included on the lab report which recommends a further test to investigate for AATD. The threshold of 1.13 g/L 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 would lead to earlier diagnosis of AATD cases. The ultimate goal would be to include this red flag system on AAT concentration reports in every hospital in Ireland.
**Report Details**

<table>
<thead>
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<th>Patient ID:</th>
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<tr>
<td>Gender:</td>
<td>Female</td>
</tr>
<tr>
<td>Date Of Birth:</td>
<td>Unknown</td>
</tr>
<tr>
<td>MRN:</td>
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<td>Laboratory Number:</td>
<td></td>
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<tr>
<td>Consultant:</td>
<td>Dr UK NEQAS</td>
</tr>
<tr>
<td>Hospital:</td>
<td></td>
</tr>
<tr>
<td>Date Sample Taken:</td>
<td>09/10/2007</td>
</tr>
<tr>
<td>Genotype:</td>
<td>MM</td>
</tr>
<tr>
<td>AAT Level:</td>
<td>1.28 g/L (Normal range 1.2 - 2.0 g/L)</td>
</tr>
<tr>
<td>AAT% of Normal:</td>
<td>77%</td>
</tr>
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</table>

**Comments:**

All data reported by Dr. Tomás Carroll and Geraldine O’Brien

**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.

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**Alpha-1 Antitrypsin (AAT) Deficiency**

AAT-deficient patients are at high risk of developing life-threatening lung and liver disease because they have reduced amounts of this key antiprotease. The normal AAT protein is 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 in 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 patients (1 normal, 1 deficient) have 50-80% 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 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 patients 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 Alpha-1 patients in the development of lung disease.

---

**Status**

<table>
<thead>
<tr>
<th>AAT Phenotype / AAT Genotype*</th>
<th>What Does It Mean?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>MM</td>
</tr>
<tr>
<td>Carrier</td>
<td>MS</td>
</tr>
<tr>
<td>Carrier</td>
<td>MZ</td>
</tr>
<tr>
<td>AAT Deficiency</td>
<td>SS/SZ/ZZ</td>
</tr>
</tbody>
</table>

* A phenotype is reported when testing is on serum – a genotype is reported when testing is on DNA.
FIGURE 4.7: Geographical distribution of severe AATD (ZZ and SZ individuals) as percent of total cases detected to date. A bias may exist in some counties depending on the volume of samples received by the Alpha One Foundation for testing.
FIGURE 4.8: Geographical distribution of severe AATD (ZZ and SZ individuals) detected to date per 10,000 population. A bias may exist in some counties depending on the volume of samples received by the Alpha One Foundation for testing.
5. Laboratory Diagnosis of Rare Alpha-1 Antitrypsin Mutations in the Irish Population

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

It is therefore not surprising that the majority of tests used to diagnose AATD are designed to detect the Z and S mutations. However, over 100 other rare mutations have been identified in the AAT gene and a number of these rare mutations have been discovered in the Irish population (Table 5.1). In addition, we have discovered 2 completely novel Null (Q0) mutations. Null mutations are extremely rare and cause a complete block in the production of AAT. 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.

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 novel and rare 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 MM. The technique of starch gel electrophoresis originally used to separate AAT variants is responsible for the nomenclature used to 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 were used to designate variants.

### Table 5.1: Rare AAT mutations identified to date in the national targeted detection programme.

<table>
<thead>
<tr>
<th>Mutation</th>
<th>Molecular Basis</th>
<th>Cases</th>
<th>Cellular Effect</th>
<th>Disease Association</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Arg39Cys</td>
<td>49</td>
<td>Intracellular accumulation</td>
<td>Lung &amp; liver</td>
</tr>
<tr>
<td>F</td>
<td>Arg223Cys</td>
<td>12</td>
<td>Reduced antiprotease activity</td>
<td>Lung</td>
</tr>
<tr>
<td>Mmalton</td>
<td>Phe51 or 52</td>
<td>4</td>
<td>Intracellular accumulation &amp; polymerisation</td>
<td>Lung &amp; liver</td>
</tr>
<tr>
<td>Zbristol</td>
<td>Thr85Met</td>
<td>1</td>
<td>Intracellular accumulation &amp; defective glycosylation</td>
<td>Lung &amp; liver</td>
</tr>
<tr>
<td>Q0beaumont</td>
<td>370Phe - ΔT- 373Stop</td>
<td>1</td>
<td>No AAT produced</td>
<td>Lung</td>
</tr>
<tr>
<td>Q0cork</td>
<td>cod180Thr - ΔAC - 190Stop</td>
<td>1</td>
<td>No AAT produced</td>
<td>Lung</td>
</tr>
</tbody>
</table>

**Table 5.2: Common AATD phenotypes in the Irish population and corresponding AAT concentrations in order of increasing risk of disease.**

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cases</th>
<th>Mean AAT (g/L +/- SEM)</th>
<th>AAT Range (g/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS</td>
<td>587</td>
<td>1.222 +/- 0.014</td>
<td>0.499 - 3.820</td>
</tr>
<tr>
<td>MZ</td>
<td>811</td>
<td>0.903 +/- 0.009</td>
<td>0.442 - 4.08</td>
</tr>
<tr>
<td>SS</td>
<td>32</td>
<td>0.879 +/- 0.039</td>
<td>0.442 - 1.54</td>
</tr>
<tr>
<td>SZ</td>
<td>80</td>
<td>0.629 +/- 0.016</td>
<td>0.35 - 1.17</td>
</tr>
<tr>
<td>ZZ</td>
<td>91</td>
<td>0.23 +/- 0.008</td>
<td>0.11 - 0.52</td>
</tr>
</tbody>
</table>
began to be used in addition to the letter of the closest anodal allele. 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, and encode AAT serum levels below a protective threshold of 0.57 g/L. The two most common mutations associated with disease in populations of European descent are the Z (Glu342Lys) and S (Glu264Val) mutations, and both are caused by a single amino acid replacement of glutamic acid at positions 342 and 264 of the mature protein, respectively. Combinations of Z and S are responsible for over 95% of AATD (Table 5.2). In general, AAT alleles can be classified according to plasma levels and function and are divided into four broad groups:

(a) Normal: Normal alleles are most commonly M subtypes (M1, M2, M3, or M4) which account for 95% of gene variants and are characterized by normal plasma levels in homozygotes (>1.1 g/L).

(b) Deficient: The Z allele is the most common deficiency variant, with plasma levels of ZZ homozygotes in the range of 0.10 – 0.52 g/L. The S variant is also common but is only clinically significant if inherited with Z or other severe mutations (Mmalton, Null etc.). For example, SZ individuals have AAT plasma levels in the range of 0.33 – 0.98 g/L.

(c) Null: The class of SERPINA1 mutations termed silent or “Null” cause a complete block in AAT production and while ultra rare, confer a particularly high risk of emphysema. As these mutations do not cause the AAT protein to polymerise they pose no risk of liver disease. Most frequent among this class are those mutations that introduce a premature stop codon, for example Q0cairo.

(d) Dysfunctional: Like Null variants, dysfunctional alleles are extremely rare. For example, the single amino acid change caused by the Pittsburgh mutation (Met358Arg) at the active site of the AAT molecule converts it from an elastase inhibitor to a thrombin inhibitor and was discovered in 1983 in a child who died from an episodic bleeding disorder.

RARE AAT PHENOTYPES DETECTED IN THE IRISH POPULATION

A number of rare SERPINA1 mutations including I, F, Zbristol, and Mmalton have been detected during our screening programme. The I mutation [Arg39Cys] is present at a relatively high frequency (0.0038) in Ireland. Forty-nine cases have been identified, including heterozygotes (MI) and compound heterozygotes (IS and IZ). The F mutation [Arg223Cys] was found in 12 cases. In addition, 2 novel Null mutations have been identified, Q0beaumont and Q0cork.

The effect of the I and F mutations on the AAT molecule has been described, but to date any mention of 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 (Table 5.3). The point mutation underlying this variant causes less disruption when 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 negligible 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

FIGURE 5.1: Rare AATD phenotypes detected in the Irish population on an isoelectric focusing (IEF) gel (Sebia). Reference MM, MS, and ZZ standards are included for clarity. The Mmalton variant depicted is from an individual homozygous for this mutation.
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 F [Arg223Cys] variant was first described by starch gel electrophoresis but the molecular basis for this variant was not identified until much later. The point mutation in this variant introduces a cysteine instead of an arginine, the same amino acid substitution that underlies the I variant. The normal AAT molecule has a single cysteine residue and the introduction of a second cysteine potentially favours the formation of disulphide bonds 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 5.2). In the disease context, the inhibitory activity of F AAT protein against neutrophil elastase (NE) is reduced. This would suggest that individuals who co-inherit the F allele with another severe deficiency allele such as Z or Null would have a 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 well described 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 found in high numbers on the island of Sardinia in the Mediterranean. Finally, the Zbristol mutation is extremely rare but is thought to cause defective glycosylation of the AAT protein.

The high prevalence of rare AAT mutations in Ireland highlights the importance of a comprehensive diagnostic work up of all patients with low AAT levels. A low AAT level (< 1.13 g/L) indicates the presence of a mutation in the AAT gene and therefore 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 and Prof. Maurizio Luisetti, and is the leading European laboratory in the identification of rare and novel AAT mutations. In addition, these rare mutations can be missed or incorrectly identified as M using some of the more readily available commercial genotyping assays which only detect Z and S. On account of our 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.

### TABLE 5.3: Rare AATD phenotypes in the Irish population and corresponding AAT concentrations in order of prevalence.

<table>
<thead>
<tr>
<th>Phenotype</th>
<th>Cases</th>
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6. The National Alpha-1 Antitrypsin Deficiency Patient Registry

The Alpha-1 registry is a confidential database that collects information on individuals with alpha-1 deficiency and alpha-1 carriers. It gathers information regarding alpha-1 level and genotype along with general health data and how alpha-1 is affecting the livelihood of an individual.

When patients attend the Alpha-1 clinic their permission is sought to allow their details to be entered into the Alpha-1 registry. Patients from 29 counties in Ireland have been included on the registry to date. This year we hope to increase patient numbers on the registry from other hospitals throughout the country.

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

**WHO PARTICIPATES IN THE ALPHA-1 REGISTRY?**
All patients with Alpha-1 Deficiency and Alpha-1 carriers treated in Ireland can participate in the registry. The more individuals that participate, the greater the quality of information available from the registry.

**WHAT IS THE REGISTRY?**
The Alpha-1 registry is a confidential database, containing information from individuals diagnosed with Alpha-1 Antitrypsin Deficiency (Alpha-1) and individuals identified as Alpha-1 carriers. The registry was established in 2007 by the Alpha One Foundation.

**WHAT IS THE FUNCTION OF THE REGISTRY?**
The registry’s function is to improve our understanding of Alpha-1 condition, promote the development and improvement of treatments and ultimately help to provide a cure for Alpha-1.

**WHO HAS ACCESS TO INFORMATION OF THE ALPHA-1 REGISTRY?**
The registry database is based in the Alpha One Foundation in Beaumont Hospital. The database is encrypted so it cannot be accessed by anyone unless they have an encryption key. The database is also password protected. Staff of the Alpha One Foundation have individual passwords and are the only ones who can access the database.

**WHAT HAPPENS IF YOU GIVE YOUR CONSENT TO BE INCLUDED ON THE PATIENT REGISTRY?**
Once you have given your consent a member of the Alpha One Foundation will have permission to look at your medical chart and transfer all relevant information to the secure registry. Not all information can be gathered from a patient’s chart so certain questions may be asked by the consent taker when you give consent. These questions cover patient’s family history for example if the patient’s 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 is no penalty or change to your care.

**CAN I WITHDRAW FROM THE REGISTRY?**
Any individual who is enrolled on the registry has the right to withdraw from the registry at any time. Any information on the individual already captured on the registry will be removed.

For any further question or queries relating to the Alpha-1 Registry please contact:
**Geraldine O’Brien**, Research Scientist, Alpha-1 Suite, Beaumont Hospital, Dublin 9. Telephone: (01) 809 3871  Email: alpha1@rcsi.ie

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

**UPDATE ON THE NATIONAL ALPHA-1 PATIENT REGISTRY**

The National Alpha-1 Patient Registry was established in 2007 to compile information on Alpha-1 (AATD) individuals throughout Ireland and to monitor disease progression. This database provides valuable clinical information and demographics on AATD individuals. This allows the Alpha One Foundation to analyse data from AATD individuals to facilitate clinical research and increase awareness of the disease.

**DEMOGRAPHIC CHARACTERISTICS**

There are currently 178 patients entered into the Alpha-1 Patient Registry comprising ZZ, SZ, MZ, SS and MS genotypes. The largest patient group on the registry with 50% is the ZZ genotype of which 58%, \( n = 52 \) are male and 42% \( n = 38 \) are female. The mean age at diagnosis for ZZ patients was 45.32 +/- 1.93 years for males and 41.49 +/- 2.13 years for females [Table 6.1].

<table>
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**TABLE 6.1: Demographic information of ZZ patients on the Alpha-1 Registry**

**REASON FOR DIAGNOSIS**

The initial reason for diagnosis of ZZ AATD individuals from the registry was collected. Pulmonary symptoms (60%) and family screening (30%) were the predominant reasons for a diagnosis of ZZ AATD. Other reasons for diagnosis include liver disease, elevated liver function tests and panniculitis (Figure 6.1).

**FIGURE 6.1:** Reason for Diagnosis in ZZ patients on the National Registry.

- Emphysema (45%)
- Family History (30%)
- Bronchiectasis (7%)
- Asthma (4%)
- COPD (4%)

- Panniculitis (1%)
- Elevated LFTs (2%)
- Incidental Pickup (3%)
- Liver Disease (4%)
HIGH RESOLUTION COMPUTERISED TOMOGRAPHY FINDINGS

High Resolution Computerised Tomography (HRCT) of the thorax is performed on ZZ AATD individuals to analyse the structure of their lungs and to confirm the presence of emphysema. HRCT data was available on 84 ZZ AATD individuals. The predominant HRCT findings were emphysema (42%), bronchiectasis (12%) and fibrosis (5%) (Figure 6.2). 28% of ZZ AATD individuals had normal HRCT scans. Of these, 50% were symptomatic while the remainder were diagnosed via family screening (Figure 6.3). The diagnosis of symptomatic ZZ AATD individuals with normal HRCT was primarily due to incidental pickup, elevated liver enzymes (LFTs), asthma and panniculitis. Family screened ZZ AATD individuals show a reduced level of lung damage compared to symptomatic patients ZZ AATD patients (Figure 6.3). However, a small percentage of family screened patients had emphysema (7%) and bronchiectasis (2%) on HRCT and this emphasises the need for early detection of asymptomatic AATD.

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.

ZZ AATD individuals identified by family screening have significantly increased FEV₁ (86.2 +/- 5.9%) compared to ZZ AATD individuals identified by targeted screening (55.87 +/- 3.79% p= 0.0008) (Figure 6.4). 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.

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.

In addition, our PFT data tells us that ZZ AATD individuals that smoked had significantly decreased lung function compared to never-smoking ZZ AATD individuals (mean FEV₁ of ever-smokers 54.42 +/- 3.9% v never-smokers 88.24 +/- 4.8%, p<0.0001) (Figure 6.5). The mean age of the ever-smoking cohort (50.9 +/- 1.4 years) was not significantly different to the mean age of the never-smoking cohort (50.0 +/- 2.9 years).

This 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). All AATD patients 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 single biggest 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.

REGISTRY DATA ACCORDING TO PROVINCE

The patient data from the registry was classified according to the four provinces of Ireland and according to phenotype (Figure 6.6). Leinster had the largest number of patients on the registry. This would be expected as it is the most populated province and the majority of Registry data is collected from patients attending Beaumont Hospital.
7. Research Studies and Clinical Trials

ALPHA-1 INTRAVENOUS AUGMENTATION THERAPY CLINICAL TRIAL

This study is being conducted in Beaumont Hospital by Professor McElvaney and his team. This is a placebo-controlled, double-blinded, multicentre phase III / IV study to compare the efficacy and safety of the drug Zemaira® in patients with Emphysema due to Alpha-1 proteinase inhibitor deficiency. The duration for each patient is 2 years.

So far there have been 24 patients recruited onto this study and they are all at various stages in the trial. The trial involves having weekly intravenous infusions of Zemaira®, an Alpha-1 proteinase inhibitor or a placebo which is a dummy treatment that looks like the real thing. As the study is double-blinded, neither the participating patients nor our study staff knows which therapy has been assigned to them. There is an equal chance of receiving either treatment. As of August 2011 we have 16 patients who have continued onto the extension phase of the study. This is where each patient receives Zemaira® for at least two years.

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

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

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

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

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

- Diagnosis of alpha-1 antitrypsin deficiency.
- Non smokers or ex-smokers who have stopped at least 6 months prior to screening.
- Age range of 18 – 65 years of age, male and female.
- Emphysema with an FEV1 of 35-70% predicted range.

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

Recruitment for this study is now closed.

AAT FOR INHALATION

Professor McElvaney is also running a clinical trial looking at the safety and efficacy of inhaled Alpha-1 antitrypsin (AAT) in Alpha-1 Antitrypsin Deficient patients with Emphysema compared to placebo.

“Kamada-AAT for inhalation” is a stable, liquid preparation of human AAT that can be aerosolised (inhaled). The inhalation will be performed with the eFlow® device (PARI, Germany). The human AAT protein is taken from human blood plasma sources collected in the USA and approved by US-FDA. The blood is processed and filtered according to strict standards to produce the human AAT protein used in “Kamada-AAT for inhalation”. Administration of “Kamada-AAT for inhalation” will reach the target organ directly and thus patients should require a much lower therapeutic dose of AAT. Self-administration by inhalation is also simpler and less stressful for the patient than IV infusion.

The study drug or the equivalent dose of placebo will be administered at a dose of 80mg, twice daily for a period of 50 weeks. Pulmonary function tests will be performed and blood samples will be taken 7 times during the study. You will be tested for HIV and hepatitis at screening and at the end of this study. Additionally, you will be given a physical examination at each study visit.
There are currently 12 patients on this study. Recruitment has stopped temporarily and should resume in October/November 2011.

For further information on the clinical trials please contact the Alpha 1 research nurse, Grace Mullins on 01-8093864 (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 Gerry McElvaney, Department of Medicine RCSI, Smurfit Building, Beaumont Hospital, Dublin 9, Ireland.

The purpose of this study is to obtain information about individuals (and their family members) that are carriers of the Z alpha-1 antitrypsin (AAT) gene. Acquisition of an abnormal alpha-1 gene from each parent leads to severe deficiency in alpha-1 protein levels which results 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 enrol 400 parents and siblings of 100 alpha-1 antitrypsin carriers (PI MZ) with diagnosed GOLD Stage II - IV COPD into this study. The inclusion criteria for PI MZ carriers are as follows:

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

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

- Any interstitial lung diseases
- PI types other than PI MM or PI MZ
- Non-biological siblings of the PI MZ 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 fulfil the above criteria and are interested in partaking in this clinical research study, please contact:

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

Tel: +353-1-809-3976
Mob: +353-86-776-3943
Email: kmolloy@rcsi.ie
GRANTS AWARDED

**Title:** Alpha-1 antitrypsin finds leukotriene attractive

**Funding body:** US Alpha-1 Foundation, 2 years from July 2011

**Principal Investigator:** Emer P. Reeves

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

**Abstract:** Emphysema in alpha-1 antitrypsin (AAT) deficient (AATD) patients typically starts at an earlier stage in life, compared to patients with chronic obstructive pulmonary disease without AATD. Key studies have demonstrated that neutrophil derived factors including degranulated proteases, play a crucial pathological role. There are several types of inflammatory chemoattractants for neutrophils and recent in vitro and in vivo research findings from our laboratory have demonstrated that serum AAT binds circulating neutrophils and coordinates both CXCR1 and soluble immune complex receptor mediated neutrophil migration. Now our attention is focused on a third major neutrophil stimulant, namely leukotriene B4 (LTB4) which activates neutrophils through BLT1 and BLT2 receptors. The aim of this project is to investigate AAT as an anti-inflammatory BLT antagonist. We propose that AAT modulates LTB4, BLT1,2 receptor engagement, thereby reducing cell degranulation and release of the potent monocyte activator azurocidin. By analyzing clinically stable AATD patients, homozygous for the Z allele, our preliminary data has shown that low serum levels of AAT, leads to an increase in neutrophil degranulation and release of azurocidin. Additionally, our in vitro results have shown that exogenous AAT inhibits degranulation of both ZZ-AATD and MM control cells. The pioneering significance of this proposal lies in its' potential to elucidate how AAT exerts this anti-inflammatory effect and the aim of this study is to investigate the ability of LTB4 to bind plasma AAT, thereby modulating BLT1,2 receptor engagement. We further hypothesize that increased levels of degranulated azurocidin from ZZ-AATD neutrophils can activate circulating monocyte cells and plays an integral role in the inflammatory response and pathophysiological cascade leading to lung disease in AATD. The scientific knowledge obtained within this study is fully translational and will corroborate scientific results to actual benefits of AAT augmentation therapy in AATD individuals. From our preliminary studies we have identified a major feature of AATD upon which augmentation therapy can positively impact and the proposed project will investigate the ability of infused AAT, to normalize the LTB4 response of ZZ neutrophils ex vivo.

**Funding body:** Medical Research Charities Group MRCG/2010

**Title:** Accelerated neutrophil apoptosis is associated with alpha-1 antitrypsin deficiency.

**Principal Investigator:** Prof NG McElvaney

**Co-applicant:** Emer P. Reeves

**Abstract:** Alpha 1-antitrypsin (AAT) is a secretory protease inhibitor produced primarily in the liver. The functional AAT molecule is found in abundance within human plasma, with normal concentrations in the range of 20-53µmol/L. Despite its name, AAT is the major physiological inhibitor of a range of serine proteases and within the lung it can protect the alveolar matrix from destruction by neutrophil elastase (NE) and thus maintains a protease-antiprotease balance. Recent data indicate alternative anti-inflammatory functions for AAT. AAT has been reported to inhibit neutrophil reactive oxygen species production, control lipopolysaccharide (LPS)-induced cytokine and chemokine release in monocytes and regulate IgE and IgG4 production by human B cells. Moreover and most relevant to this project, a novel anti-apoptotic role for AAT in lung alveolar and endothelial cells has been demonstrated.

AAT deficiency (AATD) is a lethal hereditary disorder characterized by low plasma levels of AAT and accumulation of the misfolded protein within hepatocytes and colangiocytes. The most common form of AATD is associated with allele Z, or homozygous ZZ. Serum levels of AAT in these patients are approximately 10-15% of
normal serum levels. Polymerised aggregates of AAT are implicated in liver cirrhosis and chronic hepatitis and loss of natural anti-protease screen results in early onset and pathogenesis of emphysema. Replacement (augmentation) therapy has become a standard treatment for lung disease associated with AATD and clinical studies have shown that augmentation therapy is associated with a reduction in frequency and severity of lung infections and a marked slow down in the course of lung deterioration.

Neutrophils are the primary effector cells responsible for the pathological manifestations of AATD lung disease and 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 defense and a safe turnover of these potentially harmful cells. Neutrophils have a half-life of 8-20 hours, following which they undergo spontaneously apoptosis. Accelerated neutrophil apoptosis has been previously linked with rheumatoid arthritis, renal failure and observed within sputum neutrophils of COPD patients. Increased accelerated macrophage apoptosis has been correlated with Streptococcus infections, raising the possibility that premature neutrophil apoptosis may contribute to microbial infection during AATD lung disease progression.

We hypothesized that AAT directly impacts upon the half life of the circulating neutrophil. The goal of this innovative study is to demonstrate that neutrophils of AATD individuals illustrate accelerated neutrophil apoptosis. This abnormal process may predispose patients to bacterial infections, which occur with increased frequency and severity in AATD patients. To date accelerated neutrophil apoptosis in chronic obstructive pulmonary disease (COPD) has received little attention, and the role of AAT within this process has not been studied. The specific aims of this study include a comparison of the rates of AATD and normal neutrophil apoptosis and to uncover the extracellular and/or intracellular mechanism by which AAT modulates neutrophil cell death.

Our preliminary data clearly show that AAT is a genuine membrane and secretory vesicle protein of neutrophils. Of extreme interest, our exciting studies have shown that AAT directly interacts with, and quantitatively regulates, the level of membrane bound CD16. We believe this to be an important result as neutrophil apoptosis is associated with a marked down regulation of CD16. Of major significance AATD individuals illustrate low levels of neutrophil membrane bound AAT and concomitantly reduced levels of CD16. Thus we hypothesise that AATD neutrophils undergo accelerated or premature apoptosis as a direct result of low levels of cell associated AAT.

This innovative study will focus on the clinical relevance of AAT augmentation therapy and with respect to assigning an AAT anti-apoptotic role, will challenge the hypothesis that infused AAT in AATD individuals effectively binds circulating neutrophils in vivo efficiently modulating cellular apoptosis. Technically we aim to combine a well developed clinical framework with quantitative signalling mechanisms specifically of the apoptosis pathway pre- and post- AAT augmentation therapy.

The long-term objective of this research is to develop the means to control lung disease associated with AATD. The potential ramifications of AAT as a modulator of neutrophil activity will add a new understanding to the role of AAT in health and disease.

POSTER PRESENTATIONS

Irish Thoracic Society Annual Scientific Meeting, Cork, Ireland, November 2010

Novel regulation of interleukin-8 CXCR1 binding and signalling by alpha-1 antitrypsin

Bergin DA, Reeves EP, O’Neill SJ and McElvaney NG

Dept of Medicine, Respiratory Research Division Royal College of Surgeons in Ireland, Education and Research Centre, Beaumont Hospital, Dublin 9, Ireland.
**Introduction:** Alpha-1 antitrypsin (AAT) deficiency (AATD) is an autosomal recessive disease that results in reduced serum levels of AAT and predisposes individuals to early onset emphysema. Key studies have demonstrated that neutrophil and neutrophil-derived factors play a crucial pathological role in AATD lung disease. AAT is a key serine protease inhibitor, but recent studies have identified AAT as having novel anti-inflammatory properties. The aim of this was to investigate whether AAT could act as a novel inhibitor of IL-8 induced neutrophil activation.

**Method:** Binding studies were carried out utilizing a modified ELISA protocol. Isolated healthy neutrophils were treated with varying concentrations of AAT and IL-8. Western blot analysis was employed to quantify Akt phosphorylation. Neutrophil G- and F-actin ratio was quantified using Western blot and the formation of F-actin leading edges was evaluated by immunofluorescence microscopy.

**Results:** Our results demonstrate a novel role for AAT in binding and modulation of IL-8 signalling. The binding between the two proteins was dependent on the glycosylation state of AAT. This AAT:IL-8 complex impairs IL-8 interaction with its cognate receptor CXCR1 and prevents further downstream signalling events.

**Conclusion:** This study has uncovered a novel role for AAT in the modulation of a key inflammatory signalling pathway.

Irish Thoracic Society Annual Scientific Meeting, Cork, Ireland, November 2010

Prize Winner - Second Prize in Poster Competition

miR-940 regulates alpha-1 antitrypsin mRNA expression in vitro

T Hassan, CM Greene, I Oglesby, S O’Neill, NG McElvaney

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

**Introduction:** The protease-antiprotease imbalance is the pathological mechanism of emphysema due to alpha-1 antitrypsin (AAT) deficiency while in liver disease, it is due to the accumulation of the abnormal ZZ protein. By enhancing our understanding of the mechanism of AAT expression, we may develop new therapies to treat AAT-related lung and liver disease. MicroRNAs (miRNAs) are short endogenous non-coding RNAs that function as post-transcriptional negative regulatory molecules of target gene expression. This aim of this study is to identify a key microRNA involved in the regulation of AAT expression.

**Method:** We interrogated a selection of miRNA target prediction databases to identify miRNAs that potentially regulate expression of AAT. We then determined AAT and miRNA expression in monocytic (THP-1), bronchial epithelial (16HBE143-) and liver (HepG2) cell lines by qRT-PCR and Taqman assay using the 2^-ddct calculation for relative expression. We then performed miRNA validation study by transfection of cells with pre-miR 940.

**Results:** The human AAT gene was analysed for putative miRNA binding sites using multiple established bioinformatic algorithms. Only miR-940 was predicted in two databases (Microcosm and TargetScan). miR-940 and AAT are reciprocally expressed in the three cell lines tested. In cells that were transfected with pre-miR 940, there were more than 2 and 3-fold reduction of AAT mRNA expression in HBE and HepG2 cells respectively. miR 940 expression also decreased AAT protein expression by HepG2 cells.

**Conclusion:** The miR-940:AAT ratio decreases with increasing AAT expression. Pre-miR 940 overexpression decreases AAT suggesting that miR-940 may indeed have a role in regulating AAT expression. As miRNAs represent a new class of drug targets, therapeutic modulation of AAT is possible by increasing or decreasing miRNA expression to treat both lung and liver disease aspects of the disease.
Alpha-1 antitrypsin orchestrates polymorphonuclear neutrophil survival through autocrine IL-6 production in individuals with alpha-1 antitrypsin deficiency.

**Author:** Hurley K, Bergin DA, Reeves EP, McElvaney NG.

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

**Introduction:** Alpha-1-antitrypsin (AAT) deficiency (AATD) is the most frequent form of genetically determined emphysema, with 2,000 Irish people predicted to have severe AATD. AAT opposes the destructive effects of neutrophil derived serine proteases on the lung but new evidence of its effects on innate immune cells is now emerging. Interleukin (IL)-6 is known to be involved in the resolution of inflammation and prolongs neutrophil survival. The aim of our study was to determine the rate of polymorphonuclear neutrophil (PMN) apoptosis in AATD individuals homozygous for the Z allele (ZZ-AATD) compared to healthy controls (MM).

**Method:** PMNs isolated from ZZ-AATD and MM individuals were incubated with and without AAT at physiological concentration (27.5µM). Kinetics of apoptosis were measured by caspase-3 cleavage by Western blotting, annexin V staining and CD16b expression by FACs analysis. Cytokine production was determined by cytokine array of cell supernatants and confirmed by ELISA. IL-6 mRNA expression was measured by RT-PCR.

**Results:** In ZZ-AATD PMNs the rate of apoptosis was greatly increased compared to MM control cells as measured by caspase-3 cleavage, annexin V staining and CD16b expression (p<0.05). IL-6 production was decreased at both the gene and protein level in the ZZ-AATD PMNs (p<0.05). In addition, treating ZZ-AATD and MM PMNs with AAT up-regulates the gene and protein expression of IL-6 (p<0.05).

**Conclusion:** We have identified a novel association of decreased autocrine production of IL-6 and accelerated PMN apoptosis in ZZ-AATD individuals. This proposed mechanism may highlights the important role of AAT in the resolution of inflammation through IL-6 production.

Altered polymorphonuclear cell apoptosis in individuals with alpha-1 antitrypsin deficiency is associated with endoplasmic reticulum stress

**Author:** Hurley K, Reeves E, Bergin DA, McElvaney OJ, McElvaney NG.

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

**Introduction:** 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 cause apoptosis. The aim of this project was to determine ER stress and the rate of neutrophil apoptosis in asymptomatic AATD individuals homozygous for the Z allele (ZZ-AATD) compared to healthy control cells (MM).

**Method:** Peripheral polymorphonuclear cells 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 annexin V staining by FACs analysis.

**Results:** 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. 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). The addition of AAT
greatly reduced caspase-3 cleavage and annexin V staining in ZZ-AATD neutrophils (p<0.05).

**Conclusion:** Our data shows that neutrophils undergo accelerated cell death in AATD possibly secondary to ER stress and that the addition of AAT to the ZZ neutrophil attenuates this apoptotic rate. We hypothesise that AAT augmentation therapy may normalise neutrophil apoptosis reducing inflammation and recurrent infections in patients with AATD.

**Irish Thoracic Society Annual Scientific Meeting, Cork, Ireland, November 2010**

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


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

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

**Aim:** The SZ level is regarded as the putative predictive threshold above which there is no increased risk for development of emphysema. This is the level aimed at by all augmentation study protocols to date. We evaluated our SZ population to see if the alpha one level associated with SZ phenotypes did give protection to the lung.

**Results:** We evaluated 20 SZ patients on the Irish National AAT registry. The mean age at diagnosis for SZ patients was [52.0 +/- 19.1] for males and [48.4 +/- 15.99] for females. The mean FEV1% is [94.45 +/- 20.29] of which all were smokers.

**Conclusion:** Our results in a relatively small population suggest that the SZ level of AAT does provide sufficient protection against protease induced hereditary emphysema and that augmentation protocols are correct in using protective threshold.

**American Thoracic Society Conference, Denver, Colorado, May 2011**

**Rare Alpha-1 Antitrypsin Mutations in the Irish Population**


**Respiratory Research, Department of Medicine, RCSI Education and Research Centre, Beaumont Hospital, Dublin, Ireland. *Department of Biochemistry and Clinical Genetics, University of Pavia, Italy.**

**Rationale:** 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. AAT deficiency 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.

**Methods:** 5,000 individuals were screened following ATS/ERS guidelines as part of the Irish national targeted detection programme. AAT levels were determined by nephelometry. AAT phenotyping was performed by isoelectric focussing. Patient DNA isolated from DBS samples was genotyped by PCR [Roche LightCycler]. Rare and novel mutations were identified by DNA sequencing of the SERPINA1 gene.

**Results:** A number of rare SERPINA1 mutations including I, V, F, X_christchurch, Z_malton, and M_malton were identified. The I mutation [Arg39Cys] was
present at a relatively high frequency (0.0038) in a targeted population, with over 40 cases identified. In addition, a new SERPINA1 mutation was identified.

**Conclusions:** 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, DNA sequencing of the SERPINA1 gene.

**American Thoracic Society Conference, Denver, Colorado, May 2011**

**The Irish National Alpha-1 Antitrypsin Deficiency Targeted Detection Programme**


**Rationale:** 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. AAT deficiency 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.

**Methods:** 5,000 individuals were 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 used to identify AATD patients and carriers.

**Results:** We identified 80 ZZ, 85 SZ, 27 SS, 850 MZ, 500 MS, 30 MI, 8 IZ and 3 IS individuals, yielding gene frequencies of 0.052 and 0.094 for S and Z respectively in a targeted population. Over 25% of the targeted population contained at least one deficient AAT allele.

**Conclusions:** 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 prove the maxim that AATD is not a rare disease but a disease that is rarely diagnosed.

**American Thoracic Society Conference, Denver, Colorado, May 2011**

**Evidence for altered neutrophil apoptosis in individuals with alpha-1 antitrypsin deficiency**


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

**Introduction:** Alpha-1 antitrypsin (AAT) deficiency (AATD) is the most frequent form of genetically determined emphysema, with 59,000 Americans estimated to have the most severe phenotype, the Z allele. AAT opposes the destructive effects of neutrophil serine proteases on the lung but its effect on innate immune cells in vivo is less well understood. New evidence suggests that AAT may play a role in regulation of alveolar and endothelial cell apoptosis (1).

**Aim:** The aim of this study was to investigate the effect of AAT on neutrophil apoptosis. Experiments were designed to determine the rate of neutrophil apoptosis in asymptomatic individuals with forced expiratory volume ≥ 80% homozygous for the Z allele (ZZ-AATD) compared to healthy control cells (MM).

**Methods:** Neutrophils were isolated from ZZ-AATD (n=3) and MM individuals (n=3) using dextran sedimentation, centrifugation and hypotonic lysis. Neutrophils (2 x 10⁶) were incubated at 37°C, 5% CO₂, at time intervals
between 0 and 22 hours, with and without AAT at physiological concentration (27.5µM). A neutrophil cytosolic fraction was obtained by cell lysis and ultra-centrifugation. Cleaved caspase-3, a marker of apoptosis and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) a cytosolic loading control were assessed by Western blotting. Cleaved caspase-3 activity as measured by densitometry was expressed as a percentage of GAPDH loading control. P values were calculated by 2-way Anova and student -t test using a statistical package.

**Results:** In ZZ-AATD neutrophils both the rate and the total amount of caspase-3 cleavage was greatly reduced compared to MM controls (p<0.0001). The onset of apoptosis only occurred in ZZ-AATD neutrophils at 6 hours but was seen in significant amounts in MM neutrophils at 4 hours. At 22 hours ZZ-AATD neutrophil apoptosis was only 50% of healthy controls. The addition of AAT delayed apoptosis in the ZZ-AATD (p=0.03) and MM neutrophil (p=0.02) at 6 hours. At 22 hours there was no statistical difference between the rate of apoptosis in the ZZ-AATD and MM neutrophil in physiological concentrations of AAT (p=0.81).

**Conclusions:** Our initial data shows that neutrophils undergo delayed cell death in AATD and that the addition of AAT equalises this rate of apoptosis with that of MM controls. We hypothesize that AAT augmentation therapy may normalise neutrophil apoptosis in ZZ-AATD patients leading to reduced inflammation and consequent lung disease.

**American Thoracic Society Conference, Denver, Colorado, May 2011**

**Title:** NADPH oxidase dysfunction in Alpha-1 antitrypsin deficient neutrophils.

**Authors:** Bergin DA, Reeves EP, O’Neill SJ and McElvaney NG.

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

**Rationale:** Alpha-1 antitrypsin (AAT) is a potent serine protease inhibitor that has novel anti-inflammatory properties. The importance of AAT in the modulation of inflammation is evident in the autosomal recessive disease AAT deficiency (AATD), where reduced serum levels of AAT result in severe lung disease. Neutrophils and neutrophil derived products play an important role in the development of this lung disease. One key neutrophil mechanism that is important during inflammation is the multi-enzyme component, the NADPH oxidase, which catalyses the generation of superoxide anion radical (O2·-). Our group has previously demonstrated that physiologic serum levels of AAT can inhibit NADPH oxidase activity. The aim of this study was two-fold. The first was to evaluate how AAT modulates neutrophil NADPH oxidase activity. The second was to determine if ZZ AATD neutrophil exhibits an altered state of activity compared to control neutrophils in relation to O2·- production.

**Methods:** Neutrophils were isolated from MM individuals and from healthy ZZ AATD patients. O2·- generation was quantified by measuring reduction of cytochrome c. Oxygen consumption by cells was measured using a Clark type II oxygen electrode. The translocation of two cytosolic components (p47phox and p67phox) of the NADPH oxidase to the membrane was evaluated by Western blot analysis. Neutrophil membranes were isolated by sonication and ultracentrifugation using sucrose gradients.

**Results:** Results demonstrate that AAT inhibits NADPH oxidase activity in a dose dependant manner; by reducing the physiological levels of AAT surrounding the cell, the rate and levels of O2·- generation increases. Furthermore upstream of O2·- generation, AAT can inhibit oxygen consumption and the translocation of p47phox and p67phox to the neutrophil membrane. In the setting of AATD, we observed that the rate and level of O2·- production was higher in ZZ AATD neutrophils compared to MM control cells.

**Conclusion:** Our results demonstrate and reaffirm the important role AAT plays in neutrophil homeostasis. Furthermore we show that ZZ AATD neutrophils are in a higher state of activity compared to MM neutrophils, which may indicate an intrinsic abnormality in the ZZ AATD neutrophil.

Title: Alpha-1 antitrypsin is a novel regulator of interleukin-8 signalling in neutrophils.

Authors: Bergin DA, Reeves EP, O’Neill SJ and McElvaney NG.

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

Rationale: Alpha-1 antitrypsin (AAT) deficiency (AATD) is an autosomal recessive disease that results in reduced serum levels of AAT and predisposes individuals to early onset emphysema. Key studies have demonstrated that excessive infiltration of neutrophils into the lung plays a crucial pathological role in AATD lung disease. While AAT is primarily a serine protease inhibitor, it also possesses novel anti-inflammatory properties. A key molecule involved in excessive neutrophil migration is the chemokine, IL-8, and the aim of this study was to investigate whether AAT could act as a novel inhibitor of IL-8 induced neutrophil activation.

Methods: Binding studies were carried out utilizing a modified ELISA protocol. Isolated healthy neutrophils were treated with varying concentrations of AAT and IL-8. Western blot analysis was employed to quantify Akt phosphorylation. Neutrophil G- and F-actin ratio was quantified using Western blot and the formation of F-actin at the cell leading edge was evaluated by immunofluorescence microscopy. We quantified the effect of AAT augmentation therapy on IL-8 induced chemotaxis of ZZ AATD neutrophils in the presence of 50 % (v/v) autologous serum utilizing a neuroprobe chemotaxis chamber.

Results: Our results demonstrate a novel role for AAT in binding and modulating IL-8 signalling. IL-8 bound with greater affinity to serum purified AAT than to recombinant non-glycosylated AAT (P<0.001). Serum purified AAT illustrated the ability to bind to IL-8 and impair its interaction with its cognate receptor, CXCR1 (P<0.002). As a result, this further impacted on downstream signalling events and inhibited Akt phosphorylation (P<0.05) and the formation of F-actin. In the presence of respective ZZ and MM sera, results also revealed that ZZ AATD neutrophils on day 2 post augmentation therapy illustrated a chemotactic index towards IL-8 that was similar to MM neutrophils and this was also significantly reduced compared to ZZ AATD neutrophils on day 0 pre-therapy and day 7 post-therapy (P=0.02 and P=0.003 respectively).

Conclusion: This study has uncovered a novel role for AAT in the modulation of a key inflammatory signalling pathway through CXCR1 receptor. Furthermore it demonstrates that aside from its anti-protease function, serum AAT is a novel modulator of IL-8 signalling activity.


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


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

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

Methods: The most common AAT phenotype associated with lung disease is ZZ. A chart review of AATD patients on the National Alpha-1 Registry was performed on ZZ patients (n=70). Our registry collects data on pulmonary function tests, GOLD guidelines, initial reason for screening, complications, and smoking history.
**Results:** We found that ZZ individuals identified as a result of family screening have significantly increased FEV1 (78.5 +/- 6.9%) compared to ZZ patients identified by targeted symptomatic screening (55.0 +/- 4.8%, p=0.0062). ZZ patients with a history of smoking had significantly decreased lung function (FEV1, 50.42 +/- 3.9%) compared to never-smoking ZZ individuals (FEV1, 90.55 +/- 4.9%, p < 0.0001).

**Conclusions:** 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.

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**American Thoracic Society Conference, Denver, Colorado, May 2011**

**Title:** Neutrophil derived CD16b: Alpha-1 antitrypsin complex; a novel biomarker of inflammation in Cystic Fibrosis.

**Authors:** Bergin DA, Reeves EP, Fitzgerald S, Vega-Carrascal I, Hayes E, Keenan J, Clynes M, Low TB, O'Neil SJ and McElvaney NG.

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

**Rationale:** People with cystic fibrosis (PWCF) are predisposed to a continuous cycle of infections and a disproportionate neutrophil dominated response. As a result PWCF incur numerous pulmonary exacerbations, which results in progressive decline of lung function. Pulmonary exacerbations in PWCF are currently defined by a variety of clinical criteria. The use of inflammatory biomarkers represents a potential diagnostic tool to help indicate an increased state of inflammation in PWCF and provide an alternative to the clinical criteria defining exacerbations in PWCF. Recent work by our group has shown that chemokines such as IL-8 induce rapid shedding of a CD16b-alpha-1 antitrypsin (AAT) complex from the neutrophil membrane. The aim of this work was to evaluate the presence of this neutrophil derived soluble complex in plasma and its use as a biomarker of the inflammatory status of PWCF.

**Methods:** Membranes from neutrophils of PWCF during an exacerbation and post-antibiotic treatment and normal healthy controls were analyzed by 2D-PAGE using CyDye™ labeling. Levels of CD16b and AAT were quantified by flow cytometry and Western blot analysis of neutrophil membranes and supernatants. Plasma was obtained from PWCF, individuals with chronic obstructive pulmonary disease, AAT deficient patients and healthy controls. Levels of IL-8 and CD16b-AAT complex were quantified utilizing a modified ELISA protocol employing a capture antibody against CD16b and a detection antibody against AAT.

**Results:** Decreased levels of CD16b-AAT complex were detected on CF neutrophil membranes compared to CF cells post-antibiotic treatment and healthy controls (P<0.05). The CD16b-AAT complex is rapidly released from neutrophils upon stimulation with low levels of IL-8. Quantification of IL-8 in plasma resulted in detection of significantly higher levels in PWCF compared to the patient groups and healthy controls (P<0.0001). A similar result was observed with regards to the CD16b-AAT complex, as significantly higher levels were detected in plasma of PWCF (P<0.0001). In plasma samples collected from PWCF pre and post exacerbation, levels of IL-8 and CD16b-AAT complex decreased in individuals following successful antibiotic treatment (P=0.03).

**Conclusion:** Levels of the novel neutrophil derived CD16b-AAT complex in plasma correlates with the inflammatory status of PWCF.


*Is there a Difference in Health Related Quality of Life between Family Screened Alpha*
One Antitrypsin Deficiency Individuals and Symptomatically Screened Individuals?

**Authors:** C. O’Connor, Z. Moore, N.G. McElvaney.  
*Respiratory Research, Department of Medicine, RCSI Education and Research Centre, Beaumont Hospital, Dublin, Ireland.*

**Rationale:** Alpha-1 Antitrypsin Deficiency (AATD) is an autosomal co-dominant genetic disorder characterized by insufficient secretion or production of serum alpha-1 antitrypsin. The condition is associated with a substantially increased risk for the development of chronic obstructive pulmonary disease (COPD) by the third or fourth decades of life and is also associated with risks for development of hepatic disease.

**Aim:** The two methods of diagnosis are symptomatic screening and family screening. The American Thoracic Society and European Respiratory Society Guidelines recommend family screening for all first-degree relatives of known AATD patients.

**Method:** The objective of this quantitative, cross-sectional study was to determine differences in Health Related Quality of Life (HRQoL) between family screening AATD patients and symptomatically screened individuals attending an Alpha-1 Clinic. HRQoL was measured using St George’s Respiratory Questionnaire, other variables measured were lung function measurements, respiratory exacerbations, vaccinations, and smoking history. Results identified a statistically significant difference in HRQoL ‘symptom’ score (mean difference -21.05; 95% CI 37.31 to -4.78; p = 0.013) between the two groups with those symptomatically screened displaying poorer HRQoL.

**Conclusion:** In conclusion, family screened AATD individuals have better HRQoL compared to symptomatically screened individuals. This is the first study investigating HRQoL within the Irish AATD population.

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**PUBLISHED RESEARCH**


**The role of proteases, endoplasmic reticulum stress and SERPINA1 heterozygosity in lung disease and α-1 anti-trypsin deficiency.**

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**Abstract:** The serine proteinase inhibitor α-1 anti-trypsin (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.

**Pulmonary Medicine 2011;2011:826160. Epub 2011 Mar 31.**

**Quantification and evaluation of the role of antielastin autoantibodies in the emphysematous lung.**

Low TB, Greene CM, O’Neill SJ, McElvaney NG.  
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Abstract: Chronic obstructive pulmonary disease (COPD) may be an autoimmune disease. Smoking causes an imbalance of proteases and antiproteases in the lung resulting in the generation of elastin peptides that can potentially act as autoantigens. Similar to COPD, Z alpha-1 antitrypsin deficiency (Z-A1ATD) and cystic fibrosis (CF) are associated with impaired pulmonary antiprotease defences leading to unopposed protease activity. Here, we show that there is a trend towards higher bronchoalveolar lavage fluid (BALF) antielastin antibody levels in COPD and Z-A1ATD and significantly lower levels in CF compared to control BALF; the lower levels in CF are due to the degradation of these antibodies by neutrophil elastase. We also provide evidence that these autoantibodies have the potential to induce T cell proliferation in the emphysematous lung. This study highlights that antielastin antibodies are tissue specific, can be detected at elevated levels in COPD and Z-A1ATD BALF despite their being no differences in their levels in plasma compared to controls, and suggests a therapeutic role for agents targeting these autoantibodies in the lungs.


Z α-1 antitrypsin deficiency and the endoplasmic reticulum stress response.

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Abstract: The serine proteinase inhibitor α-1 antitrypsin (AAT) is produced principally by the liver at the rate of 2 g/d. It is secreted into the circulation and provides an antiprotease protective screen throughout the body but most importantly in the lung, where it can neutralise the activity of the serine protease neutrophil elastase. Mutations leading to deficiency in AAT are associated with liver and lung disease. The most notable is the Z AAT mutation, which encodes a misfolded variant of the AAT protein in which the glutamic acid at position 342 is replaced by a lysine. More than 95% of all individuals with AAT deficiency carry at least one Z allele. ZAAT protein is not secreted effectively and accumulates intracellularly in the endoplasmic reticulum (ER) of hepatocytes and other AAT-producing cells. This results in a loss of function associated with decreased circulating and intrapulmonary levels of AAT. However, the misfolded protein acquires a toxic gain of function that impacts on the ER. A major function of the ER is to ensure correct protein folding. ZAAT interferes with this function and promotes ER stress responses and inflammation. Here the signalling pathways activated during ER stress in response to accumulation of ZAAT are described and therapeutic strategies that can potentially relieve ER stress are discussed.


Measurement of the unfolded protein response (UPR) in monocytes.

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Abstract: In mammalian cells, the primary function of the endoplasmic reticulum (ER) is to synthesize and assemble membrane and secreted proteins. As the main site of protein folding and posttranslational modification in the cell, the ER operates a highly conserved quality control system to ensure only correctly assembled proteins exit the ER and misfolded and unfolded proteins are retained for disposal. Any disruption in the equilibrium of the ER engages a multifaceted intracellular signaling pathway termed the unfolded protein response (UPR) to restore normal conditions in the cell. A variety of pathological conditions can induce activation of the UPR, including neurodegenerative disorders such as Parkinson’s disease, metabolic disorders such as atherosclerosis, and conformational disorders such as cystic fibrosis. Conformational disorders are characterized by mutations that
modify the final structure of a protein and any cells that express abnormal protein risk functional impairment. The monocyte is an important and long-lived immune cell and acts as a key immunological orchestrator, dictating the intensity and duration of the host immune response. Monocytes expressing misfolded or unfolded protein may exhibit UPR activation and this can compromise the host immune system. Here, we describe in detail methods and protocols for the examination of UPR activation in peripheral blood monocytes. This guide should provide new investigators to the field with a broad understanding of the tools required to investigate the UPR in the monocyte.


Pulmonary proteases in the cystic fibrosis lung induce interleukin 8 expression from bronchial epithelial cells via a heme/meprin/epidermal growth factor receptor/Toll-like receptor pathway.

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Abstract: A high intrapulmonary protease burden is characteristic of cystic fibrosis (CF), and the resulting dysregulation of the protease/anti-protease balance has serious implications for inflammation in the CF lung. Because of this inflammation, micro-bleeds can occur releasing hemoglobin into the lung. The aim of this study was to investigate the effect of the protease-rich environment of the CF lung on human hemoglobin and to assess the proinflammatory effect of heme on CF bronchial epithelium. Here, we show that the Pseudomonas proteases (Pseudomonas elastase and alkaline protease) and the neutrophil proteases (neutrophil elastase (NE) and proteinase-3) are capable of almost complete degradation of hemoglobin in vitro but that NE is the predominant protease that cleaves hemoglobin in vivo in CF bronchoalveolar lavage fluid. One of the effects of this is the release of heme, and in this study we show that heme stimulates IL-8 and IL-10 protein production from ΔF508 CFBE41o(-) bronchial epithelial cells. In addition, heme-induced IL-8 expression utilizes a novel pathway involving meprin, EGF receptor, and MyD88. Meprin levels are elevated in CF cell lines and bronchial brushings, thus adding to the proinflammatory milieu. Interestingly, α(1)-antitrypsin, in addition to its ability to neutralize NE and protease-3, can also bind heme and neutralize heme-induced IL-8 from CFBE41o(-) cells. This study illustrates the proinflammatory effects of micro-bleeds in the CF lung, the process by which this occurs, and a potential therapeutic intervention.


Protein misfolding and obstructive lung disease.

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Abstract: The endoplasmic reticulum has evolved a number of mechanisms to manage the accumulation of incorrectly folded proteins. This results in loss of function of these proteins, but occasionally, in conditions such as α-1 antitrypsin (A1AT) deficiency, the misfolded protein can acquire a toxic gain of function promoting exaggerated ER stress responses and inflammation. Mutations leading to deficiency in a second serine proteinase inhibitor, α-1 antichymotrypsin (ACT), can induce potentially similar consequences. A1AT and ACT deficiencies are associated with chronic obstructive lung disease. Until recently, it was thought that the lung diseases associated with these conditions were entirely due to loss of antiprotease protection in the lung (i.e., loss of function), whereas gain of function was the major cause of the liver disease associated with A1AT deficiency. This paradigm is being increasingly challenged because ER stress is...
being recognized in bronchial epithelial cells and inflammatory cells normally resident in the lung, giving rise to an inflammatory phenotype that adds to the proteolytic burden associated with these conditions. In this article, we describe the cellular mechanisms that are activated to cope with an increasing burden of misfolded proteins within the ER in A1AT and ACT deficiency, show how these events are linked to inflammation, and outline the therapeutic strategies that can potentially interfere with production of misfolded proteins.


α-1 Antitrypsin regulates human neutrophil chemotaxis induced by soluble immune complexes and IL-8.


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

Abstract: Hereditary deficiency of the protein α-1 antitrypsin (AAT) causes a chronic lung disease in humans that is characterized by excessive mobilization of neutrophils into the lung. However, the reason for the increased neutrophil burden has not been fully elucidated. In this study we have demonstrated using human neutrophils that serum AAT coordinates both CXCR1- and soluble immune complex (sIC) receptor-mediated chemotaxis by divergent pathways. We demonstrated that glycosylated AAT can bind to IL-8 (a ligand for CXCR1) and that AAT-IL-8 complex formation prevented IL-8 interaction with CXCR1. Second, AAT modulated neutrophil chemotaxis in response to sIC by controlling membrane expression of the glycosylphosphatidylinositol-anchored (GPI-anchored) Fc receptor FcγRIIIb. This process was mediated through inhibition of ADAM-17 enzymatic activity. Neutrophils isolated from clinically stable AAT-deficient patients were characterized by low membrane expression of FcγRIIIb and increased chemotaxis in response to IL-8 and sIC. Treatment of AAT-deficient individuals with AAT augmentation therapy resulted in increased AAT binding to IL-8, increased AAT binding to the neutrophil membrane, decreased FcγRIIIb release from the neutrophil membrane, and normalization of chemotaxis. These results provide new insight into the mechanism underlying the effect of AAT augmentation therapy in the pulmonary disease associated with AAT deficiency.
8. Alpha-1 Antitrypsin Deficiency – A Guide for the Recently Diagnosed Individual

THE DIAGNOSIS OF ALPHA-1
Alpha-1 Antitrypsin Deficiency, also known as AAT Deficiency or Alpha-1, is a medical diagnosis that should lead to open discussions with your doctor and family. Many Alphas, as individuals diagnosed with Alpha-1 are known, live full and productive lives. The following information is designed to help you learn about this inherited disorder so that you and your family can take the necessary preventative measures to stay as healthy as possible.

UNDERSTANDING ALPHA-1
Alpha-1 is an inherited disorder. Alpha-1 may result in the development of serious lung and/or liver disease. Approximately 2,000 individuals in Ireland are estimated to have the severe form of Alpha-1 (ZZ), and a further 200,000 have a mild form of the disease and may be at risk of lung and liver disease especially if they smoke.

Large amounts of the alpha-1 antitrypsin (AAT) protein are made in the liver and released into the blood. The proteins are made from normal genes, which are an inherited component of every cell that direct specific biological functions. In Alpha-1 individuals, altered genes make abnormally-shaped AAT proteins. The abnormal protein is retained in the liver and cannot be fully released into the blood, creating a deficiency in your body.

Alpha-1 is:
• A genetic disorder inherited from your parents.
• A reduced amount or absence of the AAT protein in the blood.
• A cause of chronic obstructive pulmonary disease, even in people who have never smoked.
• A leading cause of genetic liver disease in infants and children.
• Often misdiagnosed.

Alpha-1 can cause:
• Lung disorders, including asthma-like symptoms, chronic bronchitis, emphysema, or bronchiectasis. These problems, usually seen in adults, are caused by the AAT protein deficiency and are commonly grouped under the term COPD, which means chronic obstructive pulmonary disease. Normal AAT protein levels protect the lung tissue from the destructive effects of white blood cells circulating in that organ. When levels of the protein are low or absent, lung deterioration can occur.
• Liver disorders, including elevated liver enzymes, neonatal hepatitis, chronic liver disease, cirrhosis, and liver cancer. These problems are due to the accumulation of the abnormal protein in the liver cells. Although less common, Alpha-1 related liver disease can be seen at all ages beginning in infancy.
• Panniculitis, a skin disease created by an excess of white blood cell products which can cause painful lumps under or on the surface of the skin.

GENETICS OF ALPHA-1
In each of us, the normal AAT protein is made from a pair of genes obtained from your parents. Each parent donates one alpha-1 gene. The normal protein is made from the M gene. For unknown reasons, there are close to 100 altered or abnormal variants of the M gene, also called alleles, but only a few can cause serious lung, liver or skin disorders. Two important alleles are the S and Z gene variants. An individual with different gene variant pairs, such as the MZ genes, is called a heterozygote. Someone with similar gene variant pairs, such as the ZZ combination, is called a homozygote. Individuals with one normal and one variant alpha-1 gene are called carriers. Alphas can have parents who are both carriers, one parent who is a carrier and one who is severely deficient, or two parents who are severely deficient. Since half of an individual’s genes are inherited from each parent, the diagram below shows four possible genetic combinations for children of two Alpha-1 carriers, meaning each parent has one normal M and one altered gene, in this case the Z allele. It also illustrates how carriers who may be unaware of their risk might have a child with the most severe form of the disease, the ZZ combination.
**Risks Associated with Common Genetic Variants**

**Normal (MM):**
Does not have the disorder and does not carry any altered alpha-1 genes.

**Carrier (MS):**
It is unclear whether there is a risk for developing disease symptoms but this individual does have an altered alpha-1 gene. Most studies do not indicate an increased risk for disease.

**Carrier (MZ):**
An MZ carrier has a mild AAT protein deficiency and has a slightly increased risk of developing disease.

**Alpha-1 (SZ) or (ZZ) or (Null Null):**
Moderate (SZ) to severe (ZZ, Null/Null) AAT deficiency. This individual will develop Alpha-1-related disease and has two altered alpha-1 genes.

**Testing for Alpha-1:**
The diagnosis of Alpha-1 is usually determined by three rather sophisticated laboratory tests that require a small sample of blood. The tests:

- Measure the amount of the AAT protein in your blood
- Characterize the phenotype of the protein in the blood, and
- Determine the patient's alpha-1 genotype.

**Alpha-1 Blood Levels**
Most commercial medical laboratories can measure AAT protein blood levels. Clinically-significant deficiency exists when the blood levels are less than 1.13 grams per litre (g/L). Patients with such low levels should be phenotyped or genotyped to determine the type of AAT genes they have.

**Alpha-1 Phenotype**
This test characterizes the type of alpha-1 genes (for example, MM, MZ, SZ, or ZZ) that circulates in your blood by visualizing the movement of the protein in a special electric field. Close to 100 types of alpha-1 gene variants producing AAT protein have been identified. The SZ and ZZ phenotypes are the most common genetic variants associated with the lowest blood levels (the most serious deficiency) of the AAT protein.

**Alpha-1 Genotype**
The genotype is determined through a test that identifies the actual genetic variants producing the AAT protein. This test detects the S and Z alleles with great accuracy.

Most medical laboratories report AAT blood levels and phenotypes; specialist laboratories must determine genotypes. Genotyping is often used to identify rare or new AAT mutations. Your physician can suggest the best testing strategy for you and your family members.

**Lung Symptoms of AAT Deficiency:**
If you have respiratory symptoms, you and your physician may observe:

- Shortness of breath while at rest or with exercise
- Wheezing, persistent cough
- Recurrent lung infections
- Persistent sputum (or phlegm) production
- History of suspected allergies and/or asthma
- Sinus infections

**Possible Liver Symptoms:**
- Increased liver enzymes detected via a blood test ordered by a physician
• Jaundice or yellowing of the eyes and skin
• Enlarged liver and/or spleen, which may be noted by the patient, parent of child, or physician
• Ascites or fluid collection in the abdomen
• Cirrhosis, an excessive accumulation of scar tissue in the liver noted by a physician
• Vomiting of blood
• Persistent itching
• Noticeable change in energy level or becoming easily fatigued
• Blackish, purplish, dark or pale-collared stools
• Poor appetite

Even alphas may not have signs or symptoms:
Even without symptoms, you should follow the recommendations in the next sections. If you practice a healthy lifestyle and obtain appropriate medical care, you may remain healthy. However, this does not mean that you will not have symptoms in the future, so monitoring of your medical condition by a healthcare provider is suggested.

WHAT DOES HAVING ALPHA-1 MEAN TO ME?
The following sections deal with issues that are potentially major concerns for Alpha’s.

Having Alpha-1 may require:
Changes in lifestyle
If you or your child has Alpha-1, it may be necessary to make lifestyle changes to stay healthy, including:
• Quitting smoking and keeping the Alpha adult or child away from second-hand smoke
• Avoiding exposure to dust and fumes
• Exercising regularly
• Eating well
• Drinking alcohol with caution, if at all. The consumption of alcoholic beverages can cause damage to the liver in normal people. Many authorities recommend low, infrequent or no alcohol consumption for ZZ patients, and patients with any indication of liver damage should avoid alcohol completely.
• Review labels of over-the-counter medications, vitamins or herbal supplements carefully. Avoid products with acetaminophen and alcohol, both of which can injure the liver.

Environmental awareness
Environmental recommendations include avoiding pollutants that irritate the lungs, as well as avoiding liver toxins. Individuals should assess their home and work environments carefully, and consult with an occupational medicine specialist if indicated. Examples of such environmental irritants are:
• Cigarette smoke, from personal smoking or second-hand tobacco smoke
• Industrial and occupational pollutants such as dust, flower and tree pollen, ash, volatile compounds, fumes and other allergens
• Air pollution
• Wood-burning stoves
• Fumes from cleaning solvents such as bleach, ammonia or household and industrial cleaners
• Paints and/or toxic agents
• Precautions should also be taken when handling chemicals and other materials, as those may be absorbed through the skin.
• The liver detoxifies poisonous chemicals that enter the body. If the liver is damaged, the detoxification process is altered.

Increased doctor visits
Alphas should seek expert medical treatment and may need to visit their physician more often.

Various treatments
Alpha’s have various treatment options, depending upon their symptoms. The most common treatments are:
• Behavioural and lifestyle modification
• Medication therapy for lung problems
• Surgical therapy for lung disease
• Procedural treatments for the complications of liver disease
• Organ transplantation
WHAT DO I DO NOW?

Individuals with Alpha-1 should NEVER smoke. Evidence shows that smoking tobacco products significantly increases the risk and severity of emphysema in Alphas and may decrease their lifespan by as much as ten years or more. Exercise and nutritional programs also contribute to maintaining a healthier body. You must aim to achieve and maintain a healthy lifestyle by adopting the following recommendations:

Smoking cessation
If you smoke, it is extremely important that you quit. This is necessary because smoking destroys the small amount of AAT protein in the lungs of those affected by the disorder and also attracts white blood cells that release harmful enzymes. These damaging enzymes speed the development of lung disease. If you are an Alpha, your lungs do not have the normal defences against white blood cells. If your child has been diagnosed with Alpha-1, it is important to protect them from exposure to second-hand smoke. Educate your children on the dangers of smoking and the importance of avoiding second-hand smoke.

Avoid pollutants and infection
You should avoid occupational and environmental pollutants that can be inhaled, including pollen, dust, or organic fumes, and second-hand tobacco smoke. These substances can irritate your lungs and cause or worsen lung problems. Chemicals can also be absorbed through the skin and thus damage the liver. Avoid air pollution and aerosolised sprays at all times. It is also important to realize that you may encounter pollutants and infections, both at home and at work.

In the workplace
Avoid exposure to inorganic or organic dust, (coal, hay, etc.) or irritating gases (chlorine, isocyanates, etc.). Seek the healthiest possible work environment. Demand clean indoor air, with proper ventilation and filtration systems, and avoid second-hand tobacco smoke. Wear protective clothing such as rubber gloves when in contact with chemicals or other agents, many of which can be absorbed through the skin. Read labels closely. As previously suggested, consult with an occupational medicine specialist if you have any concerns about the specific effects of work-related exposures to your lungs and liver.

In the home
You should avoid:

- Household chemicals
- Respiratory irritants from wood-burning stoves, dust and pollen, and second-hand smoke
- Chlorine and ammonia, which are found in common household cleaning products
- Pesticides
- Pet dander

Since bacterial and viral infections are harmful to the lungs, avoid contact with sick or infectious people whenever possible. Hand washing with soap is the single most effective way to avoid both contracting and spreading infectious diseases. Carry a hand disinfectant gel with you for times when hand washing is not possible.

Develop an exercise program
Routine exercise improves mental outlook, stamina and physical well-being. Exercise is essential to all Alpha’s. It is important to exercise those muscles in the chest and upper body that are related to breathing as well as the large muscles of the legs.

Walking programs
Walking programs, strolling, swimming, and/or biking may improve your lung function and endurance.

Pulmonary rehabilitation
Pulmonary Rehabilitation is highly recommended for Alphas with all stages of lung disease or pulmonary problems. It includes exercise, breathing retraining, education, smoking cessation, and nutritional counselling and may help you achieve your fullest level of activity. As with all exercise programs, this
should be discussed with and recommended by your healthcare professional.

Personal exercise plan
You may want to have a personally tailored exercise program that is carefully monitored by your healthcare provider and/or exercise specialist. Start exercising slowly and build the intensity of your program over time as your tolerance increases.

Develop a nutrition program
Proper eating habits may help to preserve lung and liver function; therefore you should establish or maintain good eating habits. Maintaining an ideal body weight, whether or not you have lung and/or liver disease, is important. Additionally, some scientific research indicates that people with lung disorders need to consume more calories than “lung-healthy” people. If you have lung and/or liver problems, consider working with a nutritionist or registered dietician to set up an individualised nutrition program.

Reduce stressors:
Alphas report benefits with stress reduction techniques, including many relaxation exercises. These relaxation techniques may also contribute to a positive outlook on life and may prevent depression. Here are a few options to consider:

- Yoga
- Meditation
- Breathing exercises
- Muscle relaxation
- Biofeedback
- Visualization
- Hypnotherapy
- Positive think.

WHAT ARE THE CURRENT TREATMENTS FOR ALPHA-1?
You may benefit from lifestyle modification. However, if you have lung and/or liver disease, seek expert medical care to treat your condition(s).

Vaccinations:
- It is important to get annual influenza vaccination. The use of these prophylactic or preventative vaccinations is of the utmost importance.
- The pneumococcal vaccination may help prevent pneumonia. Consider repeating the vaccination every 5-10 years.
- Discuss getting the vaccines for Hepatitis A and B with your doctor.

Aggressive treatment of lung infections:
It is important to notify your doctor immediately when you suspect a lung infection. Because the lungs contain more white blood cells when you have an infection (and hence, more destructive enzymes), it may be necessary to take antibiotics to fight the infection.

These are some symptoms to watch for:
- Fever (with or without chills)
- Increased shortness of breath
- Increased coughing
- Changes in colour or thickness of sputum

Additional preventative measures:
Wash your hands frequently with soap to prevent the passage of viral or bacterial infections.

THERAPIES FOR ALPHA-1 LUNG DISEASE
Antibiotics
Bacterial infections in the lung can lead to a dramatic influx of white blood cells into the organ’s tissue and airways, which may be one of the major causes of lung destruction in Alpha-1. To minimize this risk, many physicians advocate aggressive antibiotic treatment at the first signs of a lung infection. Even though ‘pulmonary exacerbations’ may not be caused by bacterial infection or may be due to a virus, which would not be expected to benefit from antibiotics, the benefits of this aggressive approach may, in some patients, outweigh the risks of antibiotic overuse. These risks include encouraging the growth of antibiotic-resistant bacteria, overgrowth of yeast and other agents that can lead to disease, and allergic reactions.
Bronchodilators
Some of the symptoms of Alpha-1 are similar to common lung diseases such as asthma and COPD. Medications called bronchodilators, usually administered via inhalers, may be useful in relieving lung symptoms. Sometimes, different types of bronchodilators are combined to achieve maximum benefit. These medications allow better airflow in and out of the lungs by relaxing the smooth muscle that surrounds the airways.

Corticosteroids
Based on your healthcare provider’s recommendation, the use of corticosteroids (or simply, steroids) can be an appropriate treatment for lung symptoms in some individuals. Steroids help reduce inflammation within and around the airways, and can be administered by inhalation, in pill form, or intravenously (into a vein). Steroids administered by mouth or vein are usually reserved to treat severe lung problems.

Supplemental oxygen
Supplemental oxygen can be important and life-saving therapy for individuals with low blood oxygen levels. Some people, however, need supplemental oxygen primarily during exercise or with sleep. For some, it is especially important when travelling by air or at high altitudes. Ask your healthcare provider about your need for this treatment.

Augmentation therapy
Augmentation therapy is the process of receiving AAT protein that has been purified from the blood of human donors with normal alpha-1 genes. Augmentation therapy is usually given intravenously once a week. Clinical trials are currently taking place to see if this therapy is beneficial to patients.

Surgery options
Your doctor may evaluate the need for surgery recommended for patients with severe Alpha-1: lung transplantation.

Lung transplantation
Lung transplantation for one or both lungs is an option for some Alphas with severe lung disease. Lifelong drugs to suppress the immune system are required afterward. As with all surgery, the outcomes and quality of life after such procedures depend on a number of issues specific to each person. Please consult with your healthcare professional about these options.

THERAPIES FOR ALPHA-1 LIVER DISEASE

Augmentation therapy
There is no proven role for augmentation therapy to treat the liver manifestations of Alpha-1.

General treatment of liver complications
It is important for parents, caregivers, or significant others to be aware and advised of any indication of possible complications related to liver disease. Once liver injury is identified in an Alpha, the first course of action is to evaluate your lifestyle for ingestion of potential liver toxins, such as alcohol, large doses of certain vitamins, and some medications. As with the paediatric population, careful follow-up of abnormal liver function is needed. Liver disease is treated symptomatically and preventatively. In adults, symptoms such as vomiting blood may occur suddenly with no prior indication of illness. Substances in some medications may be harmful to your liver. These include both prescription and over-the-counter formulas with acetaminophen or alcohol, and nutritional supplements such as vitamins, herbs, and protein drinks. Make a list of any medicines you use and review it with your healthcare provider. Often, the liver injury can prove to be mild and temporary.

Medical/surgical procedures
There are several treatment options that may become necessary to improve the symptoms of advanced liver disease. These include:

- Large volume paracentesis (LVP), which is the removal of fluid from the abdomen.
- Banding or sclerotherapy of veins in the oesophagus to reduce bleeding from distended or swollen veins. Banding involves using rubber bands to stop blood flow. Sclerotherapy involves the injection of
a chemical irritant which causes blood flow to shift to nearby healthy blood vessels from the diseased vein.

- Portal vein decompression, which reduces the pressure in the blood vessels entering the liver from the digestive organs. This is a major surgery utilizing shunts to reroute blood flow to the liver and reduce the pressure in the blood vessel.

The latter two options help control the increased risk for bleeding in those with advanced liver disease.

**Liver transplantation**

Liver transplantation can dramatically improve the symptoms of advanced liver disease caused by Alpha-1. Because the organ recipient will begin to produce the protein of the donor liver, alpha-1 protein levels should be normal after the transplant. The option of living-donor liver transplantation is available at some transplant facilities. If you decide to explore this option, check with the transplant centre first to be certain that this choice is available to you.

**OTHER ISSUES OF CONCERN FOR ALPHAS**

Listed below are some issues that you may face after diagnosis. These are merely a starting point for discussion with your physician, genetic counsellor, family or pastoral care advisor.

**Psychosocial/family support**

**Q:** What do I tell family members?

**A:** We recommend that you inform blood relatives of the test result because of the genetic nature of the disorder.

**Q:** Should I urge family members to be tested?

**A:** After consulting with your doctor, it is reasonable to encourage your blood relatives to seek advice about getting tested. Because of the genetic nature of Alpha-1, your blood relatives could be carriers or have the disorder themselves and early detection is vital to help prevent disease. Genetic counselling is provided by the Alpha One Foundation for family members.

**Health insurance**

**Q:** Will the Alpha-1 diagnosis affect my health insurance?

**A:** Under the terms of the Disability Act 2005 (Part 4, Section 2) it is illegal to use (process) the results of genetic testing for Insurance, Life Assurance or Mortgage purposes [www.irishstatutebook.ie/2005/en/act/pub/0014/sec0042.html#sec42].

This also applies in the case of employment, health insurance and occupational pension. In other words, **genetic discrimination is illegal in Ireland**. What will be considered when a person is looking for a pension or health insurance will be the usual criteria:

- Health history (symptomatic)
- Smoking status
- Usual family history questions

**Employment**

**Q:** Can I continue to work?

**A:** The answer to this question usually depends upon two conditions:

- The present state of your health, and
- The possibility of unwanted airborne exposures such as dust and fumes at work, or other hazardous chemicals that might be in contact with your skin.

Work is good for your psychological and emotional well-being. If, after discussion with your doctor, you are physically able to do so and can avoid occupational hazards, you should continue to work.
<table>
<thead>
<tr>
<th>Glossary of Terms</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Allele</strong></td>
<td>Another general name for the altered form of a gene. For example, the Z allele is an altered form of the M gene. There are many different alpha-1 gene alleles.</td>
</tr>
<tr>
<td><strong>Alpha-1 Antitrypsin Protein</strong></td>
<td>The alpha-1 antitrypsin (AAT) protein is primarily made in the liver, which releases it into the bloodstream. The alpha-1 protein has many functions, one of which is to protect delicate tissue in the body from being destroyed by neutrophil elastase, a tissue-digesting enzyme most commonly found in circulating white blood cells. These enzymes are released into tissue when the white blood cells fight infection.</td>
</tr>
<tr>
<td><strong>Alpha-1 Antitrypsin Deficiency (Alpha-1)</strong></td>
<td>A genetic condition caused by the inability of the liver to produce the AAT protein, which creates a deficiency throughout the body. People with Alpha-1 might develop liver problems or lung diseases such as emphysema, or a skin disorder known as panniculitis. Others do not have any symptoms or illness.</td>
</tr>
<tr>
<td><strong>Ascites</strong></td>
<td>Fluid collection in the abdomen.</td>
</tr>
<tr>
<td><strong>Antibiotics</strong></td>
<td>Antibiotics are drugs that can kill or stop the growth of bacteria. Sometimes the term is used to describe drugs that can treat any infections such as those caused by bacteria, fungus, tuberculosis, and even viruses.</td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
<td>A condition of the lungs characterized by widespread narrowing of the airways due to spasm of the smooth muscle, swelling of the mucous membrane lining the respiratory tract, and the presence of mucus in the inner spaces of the airway branches leading to the lungs.</td>
</tr>
<tr>
<td><strong>Augmentation Therapy</strong></td>
<td>Intravenous administration of the alpha-1 antitrypsin protein purified from human blood and given in sufficient amounts to protect the lungs from damage.</td>
</tr>
<tr>
<td><strong>Bilirubin</strong></td>
<td>Bilirubin is a by-product of red blood cell breakdown that is normally formed in the liver. It creates the yellow tinge of normal serum, the yellow-green hue of bile, the brown colour in stools, and the yellow colour of urine. When the liver is not functioning normally, the bilirubin level can rise, which causes jaundice, a yellowing of the eyes and skin.</td>
</tr>
<tr>
<td><strong>Biopsy</strong></td>
<td>The term biopsy is used to describe both a procedure to remove tissue from an organ or a piece of tissue that is being examined under a microscope.</td>
</tr>
<tr>
<td><strong>Bronchiectasis</strong></td>
<td>Chronic dilation or widening of the bronchial tubes within the lung signals bronchiectasis. It is often caused by inflammatory diseases or obstruction and leads to chronic lung infection.</td>
</tr>
<tr>
<td>Condition</td>
<td>Description</td>
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</tr>
<tr>
<td>Cholestasis</td>
<td>A backup of bile in the liver; may result in jaundice, dark urine, pale stools, and itching.</td>
</tr>
<tr>
<td>Chronic Bronchitis</td>
<td>A lung disease characterized by inability to move air in and out of the lung combined with the production of sputum on most days of the year. This is one of the diseases caused by cigarette smoking.</td>
</tr>
<tr>
<td>Chronic Obstructive Pulmonary Disease (COPD)</td>
<td>COPD is a broad category of lung problems including emphysema, chronic bronchitis, bronchiectasis, and chronic asthma in adults. A main component of all these diseases is the obstruction of inhalation and exhalation. COPD is the fourth leading cause of death worldwide.</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>Cirrhosis is extensive scarring and hardening of the liver. This condition is most often associated with advanced liver disease.</td>
</tr>
<tr>
<td>Corticosteroids</td>
<td>A class of drugs modelled after hormones released by the body's adrenal glands. They are the most potent anti-inflammation drugs currently available and can be lifesaving to people with severe COPD and asthma, but they’re also known for having serious side effects.</td>
</tr>
<tr>
<td>Emphysema</td>
<td>A lung disease that involves damage to the alveoli or air sacs in the lungs. In emphysema, the damaged air sacs do not deflate normally so breathing is harder. Lungs with emphysema may be slow to expel used-up air and unable to fill with enough fresh air to ensure an adequate oxygen supply to the body. In Alphas, the lungs actually become hyper inflated or enlarged, and the emphysema occurs mainly in the lower lungs since that is where most of the AAT-deficient blood flows. Smoking-related emphysema is usually in the upper lungs. An Alpha who smokes or has smoked may have emphysema throughout their lungs.</td>
</tr>
<tr>
<td>Oesophageal Varices</td>
<td>Enlarged veins in the oesophagus resulting from the increased pressure in the portal vein through which blood flows into the liver. This commonly occurs in cirrhosis.</td>
</tr>
<tr>
<td>Fibrosis of the Liver</td>
<td>The presence of scar tissue made of collagen within the framework of the liver tissue. When the liver is badly scarred, the organ will not function properly.</td>
</tr>
<tr>
<td>Genes</td>
<td>Genes are sections of DNA that determine specific human characteristics; 25,000 genes exist. Each parent gives you one gene that can alone, or in combination, result in certain characteristics. Genes also hold the instructions for making proteins, each of which has a different function in the body.</td>
</tr>
</tbody>
</table>

**Fibrosis of the Liver**

The presence of scar tissue made of collagen within the framework of the liver tissue. When the liver is badly scarred, the organ will not function properly.
<table>
<thead>
<tr>
<th>Term</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td>The human genome is a very long complex combination of gene sequences. The genotype is a description of the variation of the sequence of a particular gene. The specific change in an individual's alpha-1 gene sequence, known as a genotype, determines their specific characteristics, which is their phenotype.</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>Inflammation of the liver which can be caused by viruses, abnormalities of the immune system and medications, as well as Alpha-1.</td>
</tr>
<tr>
<td>Hepatomegaly</td>
<td>Enlargement of the liver.</td>
</tr>
<tr>
<td>Hepatosplenomegaly</td>
<td>Enlargement of the liver and the spleen.</td>
</tr>
<tr>
<td>Heterozygote/Homozygote</td>
<td>Every cell of the body is composed of genes and every gene is actually a pair of alleles, one from the father and one from the mother. If your mother and father each give you the same allele, this gene is called a homozygote. If your mother and father each give you a different allele, this gene is called a heterozygote. Heterozygotes most often have one normal allele (M) and one abnormal allele (Z), a combination known as MZ. Alphas that are homozygotes have two abnormal genes, such as ZZ.</td>
</tr>
<tr>
<td>Influenza</td>
<td>Commonly known as the flu, influenza is an acute, contagious viral infection, commonly occurring in epidemics. It is characterized by inflammation of the respiratory tract and by the sudden onset of fever, chills, muscular pain, headache and severe fatigue.</td>
</tr>
<tr>
<td>Jaundice</td>
<td>A condition characterized by a yellowish tint of the skin, white portion of the eye, tissue lining of the mouth, and body fluids due to excess bilirubin in the blood.</td>
</tr>
<tr>
<td>Liver Enzymes</td>
<td>Proteins [specifically enzymes] found in high concentration in the liver and lower amounts in the blood and body tissue. The enzymes are released into the blood when liver cells are injured. Doctors can measure the amount of enzyme released from cells and estimate the extent of liver damage using the AST, ALT, alkaline phosphatase, and GGT-P tests.</td>
</tr>
<tr>
<td>Panniculitis</td>
<td>Panniculitis is an inflammation within the layers of fat beneath the skin which causes the skin to harden and form extremely painful lumps, patches, or lesions. It is likely that the damage is initiated by destructive action of unrestrained neutrophils. In some patients, damage from panniculitis may occur after an incident of trauma to the affected area. It occurs in children as well as adults, and has been linked to the ZZ and MZ phenotypes and possibly other alleles as well.</td>
</tr>
<tr>
<td>Term</td>
<td>Definition</td>
</tr>
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<td>---------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Phenotype</td>
<td>The specific characteristic or type of AAT protein circulating in your blood; it is genetically determined by the alpha-1 genes received from your mother and father at birth. Other environmental factors may affect these characteristics.</td>
</tr>
<tr>
<td>Phlegm</td>
<td>Thick, sticky, stringy mucus secreted by the mucous membrane of the respiratory tract, for example during a cold or other respiratory infection.</td>
</tr>
<tr>
<td>Pneumonia</td>
<td>An acute or chronic disease marked by inflammation of the lungs and caused by viruses, bacteria, or other microorganisms and sometimes by physical and chemical irritants.</td>
</tr>
<tr>
<td>Portal Hypertension</td>
<td>Blood flows from veins in the stomach, intestines, spleen and pancreas and goes into the liver through the portal vein. When the liver is diseased and unable to function properly, this blood flow is impaired, and pressure builds in the portal vein, which can cause a number of problems. This condition is known as portal hypertension.</td>
</tr>
<tr>
<td>Pruritus</td>
<td>Medical term for itching.</td>
</tr>
<tr>
<td>Sclerotherapy</td>
<td>A procedure that may be used in the treatment of bleeding from varices in the oesophagus. Intravenous medication is injected directly into the enlarged veins to stop the bleeding.</td>
</tr>
<tr>
<td>Spleen</td>
<td>An organ that is part of the lymphatic system in the human body. It functions as the body’s defence mechanism, is involved in the formation and destruction of certain blood cells, and acts as a blood reservoir. Blood from the spleen goes into the liver.</td>
</tr>
<tr>
<td>Splenomegaly</td>
<td>Splenomegaly, an enlarged spleen, occurs when the spleen has a disease or when portal hypertension develops due to liver disease.</td>
</tr>
<tr>
<td>Sputum</td>
<td>Matter coughed up and usually expelled from the mouth, especially mucus or pus that is expectorated (ejected or spit) in diseases of the air passages.</td>
</tr>
</tbody>
</table>
RESOURCES FOR FURTHER INFORMATION

Alpha One Foundation (Ireland)
www.alpha1.ie
Tel: 353 1 8093871
Fax: 353 1 8093561
Email: alpha1@rcsi.ie
Facebook: Alpha One Foundation Ireland
Charity Code: CHY14812

Quit Campaign
www.quit.ie
QUIT is a HSE health education campaign aimed at encouraging smokers to quit.

National Smokers Quitline
If you would like advice or support on Quitting, you can call the National Smokers’ Quitline on 1850201203

COPD Support
www.copdsupport.ie
Detailed Irish website on COPD.

Asthma Society
www.asthmasociety.ie
Website for the Asthma Society of Ireland

Alpha-1 Foundation (US)
www.alphaone.org
Link to the US Alpha-1 patient website.

UK Alpha-1 Awareness
www.alpha1awareness.org.uk/welcome.htm
Patient website maintained by the UK Alpha-1 Awareness Group.

Alpha1 Kids (US)
www.alpha1kids.org/index.php
Website for parents of newly diagnosed children with Alpha-1.

REFERENCE
Adapted from the Alpha-1 Foundation (USA) publication Alpha-1 Antitrypsin Deficiency: A Guide for the Recently Diagnosed Individual.
9. Alpha-1 Clinic

The Alpha-1 Clinic commenced in Beaumont Hospital in March 2010. Every Wednesday afternoon 10 patients are seen in the clinic, 6 of which are return visits and 4 are new referrals.

Over the last 18 months we have seen over 550 Alpha-1 patients, 225 of which were new referrals to the clinic. Upon diagnosis Alpha-1 patients can be fast-tracked to the Alpha-1 Clinic in Beaumont Hospital for medical assessment, follow-up care and genetic counselling. We welcome referrals from all other hospitals and GP practices around the country. At the clinic Alpha-1 individuals can also be assessed for clinical trial enrolment by the clinical research nurse. During their medical assessment a number of investigations may be ordered to investigate their respiratory and hepatic (liver) status.

PULMONARY FUNCTION TESTS
Pulmonary function tests are a group of tests that measure how well the lungs take in and release air and how well they move gases such as oxygen from the atmosphere into the body’s circulation.

How the Test is Performed?
In a spirometry test, you breathe into a mouthpiece that is connected to an instrument called a spirometer. The spirometer records the amount and the rate of air that you breathe in and out over a period of time.

For some of the test measurements, you can breathe normally and quietly. Other tests require forced inhalation or exhalation after a deep breath.

Lung volume measurement can be done in two ways:

- The most accurate way is to sit in a sealed, clear box that looks like a telephone booth (body plethysmograph) while breathing in and out into a mouthpiece. Changes in pressure inside the box help determine the lung volume.

- Lung volume can also be measured when you breathe nitrogen or helium gas through a tube for a certain period of time. The concentration of the gas in a chamber attached to the tube is measured to estimate the lung volume.

To measure diffusion capacity, you breathe a harmless gas for a very short time, often one breath. The concentration of the gas in the air you breathe out is measured. The difference in the amount of gas inhaled and exhaled measures how effectively gas travels from the lungs into the blood.

Results
Normal values are based upon your age, height, ethnicity, and sex. Normal results are expressed as a percentage. A value is usually considered abnormal if it is less than 80% of your predicted value. Normal value ranges may vary slightly among different laboratories. Talk to your doctor about the meaning of your specific test results (Manson et al, 2005).

CT SCAN OF THORAX
CT (Computerized Tomography) of Thorax (chest/lungs) scanning — sometimes called CAT scanning—is a non-invasive medical test that helps physicians diagnose and treat lung conditions. CT scanning combines special x-ray equipment with sophisticated computers to produce multiple images or pictures of the inside of the body. These cross-sectional images of the lungs can then be examined on a computer monitor, printed or transferred to a CD. CT scans of the lungs provide greater clarity and reveal more details of the lungs than regular x-ray exams.
ULTRASOUND OF THE ABDOMEN
Ultrasound imaging, also called ultrasound scanning, involves exposing part of the body (abdomen/liver) to high-frequency sound waves to produce pictures of the inside of the body. Because ultrasound images are captured in real-time, they can show the structure and movement of the body’s internal organs (abdomen/liver), as well as blood flowing through blood vessels. Ultrasound imaging is a non-invasive medical test that helps physicians assess, diagnose and treat medical conditions.

GENETIC COUNSELLING
Genetic counselling can inform individuals about their condition and how they inherited Alpha-1 and which family members may be affected. This is aimed at individuals who are diagnosed with Alpha-1 and also family members who would like to discuss the condition and those who may be concerned that they might be affected. Many people want to know of their own chance of inheriting or passing on a genetic condition in their family. All aspects of the reasons for testing are discussed with the individual. The following provides some information in relation to genetics.

Genes and Chromosomes
Our bodies are made up of millions of cells. Most of those cells contain a complete set of genes. Genes act like a set of instructions, controlling our growth and how our bodies work. They are also responsible for many of our characteristics, such as our eye colour, blood type or height. We have thousands of genes. We each inherit two copies of most genes, one copy from our mother and one copy from our father. That is why we often have similar characteristics to both of them.

Genes are located on small thread-like structures called chromosomes. Usually we have 46 chromosomes in most cells. One set of 23 chromosomes we inherit from our mother and one set of 23 chromosomes we inherit from our father. So we have two sets of 23 chromosomes, or 23 pairs.

Sometimes, there is a change (mutation) in one copy of a gene or chromosome which stops it from working properly. This change can cause a genetic condition because the gene is not communicating the correct instructions to the body. Some examples of genetic conditions include alpha-1 antitrypsin deficiency, cystic fibrosis and muscular dystrophy (Eurogentest, 2011).

What is a genetic test?
Most genetic tests examine DNA, the chemical in our cells that gives our bodies instructions about how to grow, develop and function. DNA is a string of coded messages organised into specific instructions called genes. Humans have 30,000 different genes, arranged on a number of thread-like structures, called chromosomes. We inherit our chromosomes from our parents, 23 from our mother and 23 from our father, so we have two sets of 23 chromosomes, or 23 ‘pairs’. If you think of genetics as the book of life, then our DNA are the letters, the genes are words, and the chromosomes are the chapters.

A genetic test can help identify if there is a change in a particular gene or chromosome. It is usually a blood. There are a number of reasons why a person might take a genetic test. Some of the reasons are listed below:

- Your doctor thinks you may have a genetic condition and wants to confirm the diagnosis.
- There is a genetic condition common in your family.
• You want to know if you are at high risk of developing the condition during your lifetime.
• You or your partner have a genetic condition that might be passed on to your children.

Benefits and Risks of Genetic Testing

The decision about whether to take a genetic test can be a difficult one. Taking a genetic test is your choice. Therefore it is important that you have discussed and understood all the information that you have been given to help you make your own decision. It is also important that you have the opportunity to discuss with the doctor any questions or worries that you may have.

Genetic testing can bring great benefits, but there are also a number of possible risks and limitations. It is important to understand the benefits and risks before making a decision (Eurogentest, 2011).

<table>
<thead>
<tr>
<th>GENETIC GLOSSARY</th>
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<tbody>
<tr>
<td><strong>Autosomal</strong></td>
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<tr>
<td>Involving the autosomes.</td>
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<tr>
<td><strong>Autosomal recessive genetic conditions</strong></td>
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<tr>
<td>These are conditions whereby a person has to inherit two changed copies (mutation) of the gene (a changed copy from each parent) to be affected by the condition. A person who has only one copy of the changed gene will be an unaffected carrier.</td>
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<tr>
<td><strong>Autosomes</strong></td>
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<tr>
<td>We have 23 pairs of chromosomes. Pairs 1 to 22 are called autosomes and look the same in men and women. Pair 23 is different in men and women and is called the sex chromosomes.</td>
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<tr>
<td><strong>Carrier</strong></td>
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<tr>
<td>A person who is generally not affected with the condition (at that moment), but carries one fault copy of a gene. In the case of recessive conditions, the person will not usually be affected; in the case of dominant conditions, the person may become affected at a later stage.</td>
</tr>
<tr>
<td><strong>Cell</strong></td>
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<tr>
<td>The human body is made up of millions of cells, which act like building blocks. Cells in different parts of the body look different and do different things. Every cell (except for eggs in women and sperm in men) contains two copies of each gene</td>
</tr>
<tr>
<td><strong>Chromosomes</strong></td>
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<tr>
<td>Thread-like structures which can be seen under the microscope and contain the genes. The usually number of chromosomes in humans is 46. One set of 23 chromosomes we inherit from our mother and one set of 23 chromosomes we inherit from our father.</td>
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<tr>
<td><strong>DNA</strong></td>
</tr>
<tr>
<td>A chemical substance which makes up the genes, and which contains the information needed for the body to work.</td>
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<tr>
<td><strong>Family tree</strong></td>
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<td>A diagram to show the people in your family who do and do not have the genetic condition, and how they are related to you and to each other.</td>
</tr>
<tr>
<td><strong>Gene</strong></td>
</tr>
<tr>
<td>Information needed for the body to work, stored in a chemical form [DNA] on chromosomes.</td>
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<td>Term</td>
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<td>----------------------</td>
</tr>
<tr>
<td>Genetic</td>
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<td>Genetic condition</td>
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<td>Genetic counselling</td>
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<td>Genetic counsellor</td>
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<td>Genetic test</td>
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<td>Hereditary condition</td>
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<td>Mutation</td>
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<td>Negative result</td>
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<td>Positive result</td>
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<td>Predictive testing</td>
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</table>

**REFERENCES**


EuroGentest: Free-access website providing information about genetic testing and links to support groups across Europe. Web: [www.eurogentest.org](http://www.eurogentest.org)

Orphanet: Free-access website providing information on rare diseases and orphan drugs, and links to support groups across Europe. Web: [www.orpha.net](http://www.orpha.net)
10. Recent Events

**Recent Events**

**Alpha-1 Patient Conference, October 2010 in Marino Institute of Education**

**John Walsh, President of the Alpha-1 Foundation in the United States speaking in Beaumont Hospital, November 2010**

**JOHN WALSH VISIT**

In November 2010 the Foundation was delighted to welcome John Walsh CEO and president of the Alpha-1 Foundation in the United States. John Walsh was diagnosed with Alpha-1 Antitrypsin Deficiency (Alpha-1) in 1989. Learning there was no organized effort to promote research and find a cure for the disorder in the United States, he decided to dedicate his life’s work to this end. In 1995 he co-founded the Alpha-1 Foundation, a not-for-profit corporation dedicated to providing the leadership and resources to increase research, improve health, promote worldwide detection, and find a cure for Alpha-1.

John is committed to serving the Alpha-1 Community in all his endeavours. He is a continuous advocate for Alpha-1. He was a member of the Alpha-1 Antitrypsin Deficiency Task Force that published the American Thoracic Society / European Respiratory Society Statement: Standards for the Diagnosis and Management of Individuals with Alpha-1 Antitrypsin Deficiency.

John visited the Alpha One Foundation in November 2010 and gave a presentation on Alpha-1 Antitrypsin Deficiency in Beaumont Hospital. He also attended our annual Chopin Recital in the Mansion House. Frederic Chopin suffered from chronic respiratory disease, probably alpha-1 antitrypsin deficiency, during his short but very productive life. The Alpha One Foundation celebrates Chopin’s life and draws attention to respiratory research especially research into Alpha-1 Antitrypsin Deficiency at this annual event. The Lord Mayor of Dublin Councillor Gerry Breen kindly invited us to use his residence for the occasion. We gratefully acknowledge his generosity.

**ALPHA-1 FOUNDATION ANNUAL PATIENT CONFERENCE**

Our annual patient conference took place in Marino Institute of Education in October 2010. This meeting is a unique opportunity for Alpha-1 patients and family members to meet and discuss issues in relation to their condition. Speakers included Prof N.G. McElvaney, Citizen Information Services and Health Promotion Unit, Beaumont Hospital.
Staff members of the research laboratory in the Education and Research Centre, Royal College of Surgeons in Ireland, Beaumont Hospital

Prof N.G. McElvaney, Orla Keane, John Walsh and Josephine McGuirk attending the Chopin Recital in the Mansion, November 2010

CHOPIN RECITAL

IRELAND AM APPEARANCE

Prof N. G. McElvaney and Josephine McGuirk spoke about detection and treatment of Alpha-1 Antitrypsin Deficiency on Ireland AM on TV3. Josephine spoke of her experience of being on a clinical trial and living with the Alpha-1.

RUN FOR ALPHA-1

The staff at the respiratory research laboratory in the education and research centre in the RCSI Building in Beaumont Hospital took part in a ‘RUN FOR ALPHA-1’ in St Anne’s Park, Raheny on the 9th April and raised over €300.
11. Alpha Support Group

**CHRISTMAS CARDS**

Alpha Support group have been very active again this year. For Christmas 2011 the group sold over 1,500 Christmas cards in aid of the Alpha One Foundation. This was a tremendous success and the group will be selling Christmas cards again this year. The Support Group welcomes anyone who wishes to organize or get involved in fund raising from the smallest event to larger ones. The group hopes to establish coffee mornings throughout different areas of the country during 2012.

**PUB QUIZ**

Harry English, family and friends from Gortishall, Ballyporeen in Co. Tipperary organised a pub quiz which raised over €3,000 for the Alpha One Foundation in March 2011.

**SLIM-A-THON**

As part of their New Year’s resolution a group of 20 ladies participated in a Slim-a-thon and Exercise-a-thon from January until Easter. After each exercise session or weight in they left a small donation which amounted to €3,000 in aid of the Alpha One Foundation.
MINI-MARATHON

The ladies were out again participating in the Flora Women’s Mini-Marathon in June 2011, this year over 40,000 women took part in this event to help raise an estimated €14million for charities. This is a great way to have a fun day out and also get involved in fund raising.

MOLECULAR MEDICINE IRELAND SEMINAR

Molecular Medicine Ireland hosted a most successful seminar on Friday 24 June on the topic of a research ethics system for the 21st century. Orla Keane, an Alpha-1 patient participated in a clinical trial for replacement therapy for alpha one antitrypsin deficiency, described how she had weighed up the risks of participating in the study. ‘As life is a risk, I decided to participate’, she said. She was very glad that she had as the new therapy had increased her quality of life. ‘I’m back on my bike after a 15 year gap’, she commented.
12. Social Media

Social networking and media have become more visible in everyday life with icons for Facebook and Twitter appearing everywhere. Social media is a very important tool for charities as they can directly communicate with patients and inform the general population. With this in mind the medical research charities group (MRCG) organised a social media workshop for research charities which was attended by members of the Alpha One Foundation.

The Alpha One Patients Support group had previously set up their own Facebook page and the success of this along with the workshop spurred the creation of the Alpha One Foundation Ireland’s Facebook page (www.facebook.com/pages/Alpha-One-Foundation-Ireland/182604975133732). As Facebook is used by 1.5 million people in Ireland it enables us to reach our target audience of Alpha One patients and professionals as well as raising awareness of Alpha-1 and our work. Although it has only been up and running for a short period of time we have got a good response from Alpha-1 patients and other Alpha-1 groups. We are also in contact with similar charities and we can follow the HSE Quit campaign of which we are a member. One advantage of the Facebook page is that as members check their own pages regularly they can be easily informed about any upcoming Alpha-1 events. The other advantage is that patients can have direct access to us and other patients.

Our Alpha-1 webpage (www.alpha1.ie) is linked to our Facebook page allowing greater visibility. The webpage provides more detailed information about Alpha-1, The Alpha One Foundation, Research and Patient Testing.

As we embrace the new era of social networking we are also conscious that not all our patients will have access to Facebook or our website and we can still be contacted through more traditional and trusted means of communication... phone, mail and email.
13. Acknowledgements

We would like to thank the following:

• John Walsh CEO and Angela McBride of the Alpha-1 Foundation in the United States
• Pat O’Brien, Eric Mahon, Helen Moore and Dr. Bill Tormey in the Beaumont Hospital Biochemistry Department for their continued support
• Professor Maurizio Luisetti and Dr. Ilaria Ferrarotti, Centre for Diagnosis of Inherited Alpha-1 Antitrypsin Deficiency, University of Pavia, Italy
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• Dr. Joseph McPartlin, Trinity Biobank, Institute of Molecular Medicine, St James’s Hospital, Dublin
• Larry Warren, former CEO of the Alpha One Foundation (Ireland)
• Professor Dermot Kenny and the RCSI Clinical Research Centre
• Health Research Board (HRB)
• Medical Research Charities Group (MRCG)
• Programme for Research in Third Level Institutes (PRTLI)

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

• Mercy University Hospital Cork,
• Midwestern Regional Hospital, Limerick
• Mullingar Regional Hospital,
• Our Lady of Lourdes Hospital Drogheda,
• Peamount 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.

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

• Bon Secours Hospital Tralee,
• Bon Secours Hospital Dublin,
• Cavan General Hospital,
• Cork University Hospital,
• Galway University Hospital,
• James Connolly Memorial Hospital Blanchardstown,
• Letterkenny General Hospital,
• Mayo General Hospital,
• 1 in 25 Irish individuals are carriers of Alpha-1.

• There are 2,000 Alpha-1 individuals in Ireland and almost 200,000 carriers.

• Less than 10% of Alpha-1 individuals have been diagnosed in Ireland.

• All individuals with chronic obstructive pulmonary disease (COPD), poorly controlled asthma, and cryptogenic liver disease should be tested for Alpha-1 as per WHO guidelines.

• All first degree relatives of known Alpha-1 individuals should be offered testing.

• Smoking cessation and the avoidance of harmful environmental and occupational pollutants are paramount for the prevention and management of lung disease in Alpha-1.

• Carrier individuals who smoke are at increased risk of developing lung disease.