

α_1 -Antitrypsin deficiency · 2: Genetic aspects of α_1 -antitrypsin deficiency: phenotypes and genetic modifiers of emphysema risk

D L DeMeo, E K Silverman

The genetic aspects of AAT deficiency and the variable manifestations of lung disease in PI Z individuals are reviewed. The role of modifying genetic factors which may interact with environmental factors (such as cigarette smoking) is discussed, and directions for future research are presented.

Thorax 2004;59:259–264. doi: 10.1136/thx.2003.006502

The susceptibility to develop chronic obstructive pulmonary disease (COPD) results from a combination of genetic and environmental factors. The most important environmental risk factor for COPD is cigarette smoking, but individuals vary in their susceptibility to the effects of cigarette smoke and only a minority of smokers will develop COPD. Severe α_1 -antitrypsin (AAT) deficiency is a proven genetic risk factor for COPD.¹ However, this genetic predisposition is present in only 1–2% of COPD patients, suggesting that most COPD in the general population probably represents a complex genetic disease with multiple genetic and environmental contributions. Even in individuals with severe AAT deficiency, the development and manifestations of COPD are highly variable, which suggests that modifier genes, environmental exposures, and gene \times environment interactions may be relevant to disease expression. Because of the potential role of modifier genes in COPD associated with AAT deficiency, AAT deficiency provides a useful paradigm for understanding genetic and environmental effects, and their interactions in more common forms of COPD.

This review will focus on the genetic aspects of AAT deficiency and the variable manifestations of lung disease in PI Z individuals. The risk of lung disease in heterozygous individuals will also be discussed, as will directions for future research.

COMPLEX TRAIT PARADIGM: LESSONS FROM OTHER MENDELIAN DISEASES

Most monogenic or “Mendelian” diseases—classically considered in a one gene-one disease framework—probably have some characteristics of complex diseases. Complex diseases like COPD are typically influenced by multiple genetic and environmental determinants. A classic example of a monogenic disease is sickle cell anaemia. Individuals with sickle cell anaemia are homozygous for a single base pair alteration in the β

globin locus which results in haemoglobin S. This mutant haemoglobin assumes a sickle shape when deoxygenated, causing an array of clinical consequences. However, affected individuals vary widely in disease severity. One known genetic modifier of sickle cell disease is hereditary persistence of fetal haemoglobin in which continued production of Hb F impairs sickling and limits disease severity.

In pulmonary medicine, cystic fibrosis and AAT deficiency—classic monogenic disorders that display marked variability in disease susceptibility—demonstrate elements of genetic complexity. In severe AAT deficiency the Z mutation leads to low serum protein levels, but PI Z individuals vary markedly in lung and liver disease development and severity. The altered AAT protein is the product of a single gene, but the disease phenotype is probably a result of many genes. Genetic modifiers of lung disease in AAT deficient individuals may also provide insight into COPD unrelated to AAT deficiency.

CHARACTERISATION OF THE GENE FOR AAT DEFICIENCY

The identification of the association between AAT deficiency and pulmonary emphysema was an important contribution to the protease-anti-protease hypothesis for emphysema. Eriksson and colleagues showed that AAT deficiency followed a Mendelian pattern of inheritance, supporting a major gene effect.² Determination of the effects of genetic alterations on protein structure has since provided an understanding of differential disease manifestations associated with deficient versus dysfunctional AAT protein types. The AAT protein is encoded by the protease inhibitor (PI) locus located on chromosome 14q32.1,^{3–7} and the gene for AAT has been cloned and sequenced.^{8,9} The PI gene is 12.2 kb in length with seven exons and six introns; the protein encoded includes 394 amino acids with the active site of the enzyme inhibitor at methionine 358. Leucocyte elastase, a neutrophil enzyme associated with elastin degradation and lung tissue injury and destruction, is bound to this active site and permanently inactivated.

PI variants

The PI locus is highly polymorphic with approximately 123 single nucleotide polymorphisms (SNPs) listed in public SNP databases (accessed by SNPper at <http://innateimmunity.net> on 30 July 2003).¹⁰ Differences in speed of migration of different protein variants on gel electrophoresis

See end of article for authors' affiliations

Correspondence to:
Dr D L DeMeo, Channing
Laboratory and Division of
Pulmonary and Critical
Care Medicine,
Department of Medicine,
Brigham and Women's
Hospital, 181 Longwood
Avenue, Boston, MA
02446, USA; dawn.
demeo@channing.harvard.
edu

have been used to identify the PI phenotype, and these differences in migration relate to variations in protein charge resulting from amino acid alterations.¹¹ The M allele results in a protein with a medium rate of migration; the Z form of the protein has the slowest rate of migration. Some individuals inherit null alleles that result in protein levels that are not detectable, but these alleles are not readily identified using isoelectric focusing of serum. Individuals with a Z pattern on serum isoelectric focusing are referred to as phenotype PI Z (encompassing both PI ZZ and PI Znull genotype variants).

In white ethnic groups the most common alleles are the M variants with allele frequencies of greater than 0.95 and normal AAT levels. Many common subtypes of M alleles have been identified¹² but these are all associated with normal serum AAT levels. The S variant occurs at a frequency of 0.02–0.03 and is associated with mild reductions in serum AAT levels. The Z variant, with a frequency of 0.01–0.03 in white populations, is associated with a severe reduction in serum AAT levels. Worldwide distributions of the frequencies of the different alleles have been reviewed in the paper by Luisetti and Seersholm earlier in this series.¹³

The protein type observed in serum electrophoresis (for example, isoelectric focusing or earlier techniques such as crossed gel immunoelectrophoresis) is referred to as the PI phenotype. The use of the term phenotype is strictly correct in that the visualised protein pattern represents the observed expression of a particular genetic trait. However, “phenotype” typically refers to characteristics of disease that are further removed from genotypic expression than protein bands on a gel. The inheritance of a PI phenotype follows an autosomal co-dominant pattern. The PI Z phenotype, as identified by isoelectric focusing, has been associated with serum AAT protein levels less than 15% of PI MM levels and the development of early onset COPD.

AAT PROTEIN DEFICIENCY AND DYSFUNCTION

The pathogenesis of COPD associated with AAT deficiency is intimately tied to the inhibition of neutrophil elastase, an enzyme implicated in the development of emphysema.¹⁴

Certain alleles of the PI locus result in deficient levels of AAT. The polymorphic features of the PI locus may lead to AAT deficiency by abnormalities in gene expression, gene translation, and intracellular protein processing (see partial list in table 1). The molecular defect in the Z allele is a substitution of a lysine for a glutamic acid at position 342 due to a single base alteration in the gene. The low protein levels result from polymerisation of the protein within the hepatocyte endoplasmic reticulum, with subsequent reduction in serum levels due to intracellular accumulation.¹⁵ Polymers of AAT have been described in lung lavage fluid of PI Z individuals;¹⁶ this local polymerisation may also contribute to reduced proteolytic defences in the lung.

A number of rare null variants have been described which result from a broad range of molecular mechanisms. For example, the null variant QO_{isola di procida} is associated with deletion of most of the coding region, and QO_{granite falls} is associated with abnormalities in mRNA splicing due to a frameshift mutation that leads to no detectable mRNA.¹⁷ Other rare null variants cause absent serum protein levels due to intracellular degradation of protein. In addition to these null variants, other rare variant alleles exist with electrophoretic mobility similar to M or S alleles but with very low serum levels of AAT. These low expressing variants are associated with several different mechanisms of protein deficiency. For example, M_{heerlen} and M_{procida} have low serum AAT levels due to intracellular degradation of protein,^{18–20} whereas M_{malton} and S_{siyama} are associated with intracellular accumulation of protein.^{21–23}

With regard to quantitative serum levels of AAT, the PI genotype is the most important genetic determinant. Martin and colleagues studied serum AAT levels in 583 individuals from 114 pedigrees and found that the PI locus was the primary determinant of serum AAT levels.²⁴ Depending on the covariates included in the regression modelling, Silverman *et al* similarly concluded that PI type accounted for 72–92% of the variation in serum AAT levels within families of known PI Z individuals.²⁵ As discussed below, although most of the variation in PI level is due to the

Table 1 Selected PI variants, cellular defects, and disease association

PI allele	Type of mutation	Cellular defect	Disease association	Reference**
Normal alleles				
M (various subtypes)	Substitution (1 bp)	None	Normal	See text
Xchristchurch	Substitution (1 bp)	None	Normal	60
Deficiency alleles				
S	Substitution (1 bp)	IC degradation	Lung	See text
Z*	Substitution (1 bp)	IC accumulation	Lung, liver	See text
M _{malton}	Deletion (3 bp)	IC accumulation	Lung, liver	21
S _{siyama}	Substitution (1 bp)	IC accumulation	Lung	22, 23
M _{heerlen}	Substitution (1 bp)	IC degradation	Lung	18, 19
M _{procida}	Substitution (1 bp)	IC degradation	Lung	34
M _{mineral springs} *	Substitution (1 bp)	IC degradation	Lung	33
Null alleles				
QO _{granite falls}	Deletion (1 bp)	Stop codon; no mRNA	Lung	17
QO _{ludwigshafen}	Substitution (1 bp)	No protein	Lung, liver	61
QO _{hongkong 1}	Deletion (2 bp)	Truncated; IC accumulation	Lung	62
QO _{isola di procida}	Deletion (17 kbp)	Deletion of coding regions; no mRNA	Lung	20, 63
Dysfunctional alleles				
Pittsburgh	Substitution (1 bp)	Anti-thrombin 3 activity	Bleeding diathesis	29
M _{mineral springs} *	Substitution (1 bp)	Defective inhibition of neutrophil elastase	Lung	33
Z*	Substitution (1 bp)	Defective inhibition of neutrophil elastase	Lung, liver	See text

IC = intracellular, bp = base pair(s).

*Note that M_{mineral springs} and Z have dysfunctional characteristics described based on altered rates of association and inhibition of neutrophil elastase, as well as deficiency characteristics.

**Reference to one or two reports of disease association in the literature.

genotype at the PI locus, individuals with similar AAT protein levels vary widely in their susceptibility to develop lung disease, probably in part as a result of genetic modifiers.

In addition to serum protein deficiency associated with the Z allele, it is likely that PI Z individuals have additional susceptibility due to dysfunction of Z type AAT as an inhibitor of neutrophil elastase. With regard to its antiprotease activity, AAT has the highest rate constant of association with neutrophil elastase.²⁶ Ogushi and colleagues compared the inhibitory capacity of AAT against neutrophil elastase as well as the rate constant for neutrophil elastase in 10 PI ZZ individuals and seven PI M1M1 individuals. They observed that AAT-elastase complexes of Z type were less stable than M1 type complexes, and that some of the elastase was liberated from Z complexes, allowing for continued protease activity. They also observed that, compared with M1 type AAT, Z type AAT required twice as long to inhibit neutrophil elastase. This suggested that PI Z individuals are susceptible to developing emphysema due to both deficient AAT levels and dysfunction in the protein.²⁷

In addition to inherited mechanisms of AAT dysfunction, acquired dysfunction is also probably important in the pathogenesis of COPD. Even in individuals with normal serum AAT levels, oxidative inactivation may contribute to the decline in lung function due to the rendering of AAT functionally deficient/dysfunctional.²⁸ Variations between individuals in "acquired" protein dysfunction may provide clues to the variable susceptibility to cigarette smoke observed in AAT deficient individuals and in the general population. Thus, susceptibility of PI Z individuals who smoke to develop COPD probably relates to a combination of reduced protein levels due to polymerisation of the Z protein and reduced secretion, decreased inhibitory function of each molecule, and inactivation of protease specific activity with inflammation (probably due to protein oxidation).

In addition to the dysfunctional aspects associated with the Z protein, the PI Pittsburgh mutation results in a dysfunctional AAT protein with activity similar to antithrombin. Lewis *et al* described the clinical phenotype in one patient (a bleeding diathesis) and deemed the variant antithrombin Pittsburgh.²⁹ However, it was noted that this variant had electrophoretic and antigenic features of AAT. Further investigation showed a single base pair substitution at amino acid position 358 of AAT resulting in a critical methionine to arginine substitution.³⁰ In characterising this dysfunctional protein, investigators have shown that recombinant Pittsburgh AAT protein, although bereft of anti-elastase properties, is a potent inhibitor of plasma kallikrein and activated factor XII.^{31 32} PI Pittsburgh is a dramatic example of protein dysfunction that may result secondary to mutations in the AAT gene. Another rare example of

protein dysfunction is observed in PI M_{mineral springs}. This variant is characterised by a single alteration in codon 67 resulting in the substitution of a glutamic acid for glycine.³³ PI M_{mineral springs} is associated with low secretion of protein and dysfunctional characteristics that limit the efficacy of neutrophil elastase inhibition; homozygotes are at increased risk for emphysema.³⁴

FROM GENE TO PROTEIN TO DISEASE

Adequate levels and normal function of the AAT protein provide a critical defence against proteolytic stress. Severely AAT deficient individuals (including PI ZZ, PI Znull, and PI null-null) are at the highest risk of developing COPD. PI null-null individuals, who lack any AAT, have rarely been reported.^{35 36} With the small number of null-null subjects identified, it is unclear whether they have an increased risk for lung disease compared with PI Z individuals. Similarly, it is not clear whether PI ZZ and PI Znull individuals have differential risks for lung disease.

The PI phenotype does not distinguish between PI ZZ and PI Znull individuals; only Z protein is observed. If PI Z patients are receiving AAT augmentation therapy, the PI phenotype will appear as PI MZ. If molecular genotyping of DNA is performed using an approach such as allele specific hybridisation, the S and Z alleles can be readily determined but molecular probes for null alleles are not widely available. Thus, PI Znull and PI MZ individuals will both appear to have one Z and one non-Z, non-S allele in molecular genotyping. The combination of a very low AAT serum protein level with genotyping can suggest a PI Znull rather than a PI MZ genotype (table 2), although this particular distinction is readily made by isoelectric focusing.

AAT DEFICIENCY AS A COMPLEX DISEASE: PI Z INDIVIDUALS AND THE HYPOTHESIS OF MODIFIER GENES

In the investigation of complex disease genetics, intermediate phenotypes are component characteristics of disease that provide a quantitative approach to describe disease variability. Although PI Z individuals frequently develop airflow obstruction or COPD at an early age, the characteristics and severity of the pulmonary disease are variable (box 1), suggesting the presence of other factors that modify disease expression. Modifier genes could have a role at any stage of the disease—from disease development to disease related mortality—and the understanding of the complexity of COPD will be enhanced by accurate and standardised phenotypic descriptions.

Historically, a key intermediate phenotype for COPD has been the level of forced expiratory volume in 1 second (FEV₁). However, initial disease descriptions relied upon radiographic and clinical findings suggestive of obstructive

Table 2 Selected phenotype and genotype correlations

Alleles inherited	Phenotype (e.g. isoelectric focusing)	Serum level (e.g. nephelometry)	Molecular genotype*** (e.g. allele specific hybridisation)
MM	M	Normal	Non-S, non-Z/non-S, non-Z
ZZ	Z*	Very low	ZZ
Znull	Z*	Very low	Z/non-S, non-Z
MZ	MZ	Intermediate	Z/non-S, non-Z
Mnull	M	Intermediate	Non-S, non-Z/non-S, non-Z
SZ	SZ**	Low	SZ

*Appears as PI MZ phenotype on AAT augmentation therapy.

**Appears as PI MSZ phenotype on AAT augmentation therapy, although multiple bands would make accurate phenotyping difficult.

***Current methods include using allele specific probes by hybridisation or introduction of a restriction enzyme digestion site that detects S or Z alleles.

Box 1 Variable pulmonary characteristics in severe AAT deficiency

- Age of onset of obstructive lung disease
- Variability of pulmonary function
 - Spirometric measures of airflow obstruction
 - Reduced transfer factor
- Asthma related phenotypes
 - Bronchodilator responsiveness
 - Airway hyperresponsiveness (e.g. methacholine, histamine)
 - Atopy
- Other pulmonary phenotypes
 - Chronic bronchitis
 - Bronchiectasis
- Radiographic findings (measured on chest CT scan)
 - Basilar predominance versus diffuse emphysema
 - Emphysema severity
- Disease related morbidity
 - Exacerbation frequency
 - Hospitalisation
 - Exercise limitation
- Disease related mortality

lung disease; three of the five PI Z individuals first described by Laurell and Eriksson had clinical findings which suggested COPD.¹ In the 40 years since this observation, many series of PI Z individuals with COPD have been published.^{37–40} However, most were evaluated for AAT deficiency because of COPD, and ascertainment bias is an important aspect to consider when reviewing reports of the natural history of disease. Silverman *et al* evaluated 52 PI Z subjects and observed that 20 of 30 PI Z individuals not ascertained with COPD (non-index individuals) had FEV₁ >65% predicted. Index PI Z individuals, identified as AAT deficient because they had COPD, all had reduced FEV₁ levels. The variable natural history of lung disease in non-index individuals leads to the hypothesis that other modifying factors, potentially genetic, are relevant to disease expression.

Modifying genetic factors may act through gene × environment interactions, as suggested by the variable susceptibility of AAT deficient individuals to the effects of cigarette smoke. Overall, PI Z individuals who smoke develop obstructive lung disease at an earlier age than non-smoking PI Z individuals.^{38–40} However, there is variability in the development and age of onset of airflow obstruction associated with cigarette smoking, with some former and current PI Z smokers having normal lung function.⁴¹ Several studies have focused on non-smokers to assess whether factors other than cigarette smoking contributed to the development of chronic respiratory symptoms and lung disease in PI Z individuals. Black and Kueppers observed significant variability in pulmonary symptoms and pulmonary function among non-smoking PI Z individuals.⁴² They hypothesised that host factors were intrinsic to this variability. Piitulainen *et al* have also observed significant

variability among PI Z non-smokers, with reduced spirometric values correlated with wheezing, occupational exposures, and male sex.⁴³

The intersection of technology and genetic investigation has provided the opportunity to define new disease phenotypes. With the more frequent use of chest CT scanning in the assessment of emphysema, the understanding of the anatomical distribution of emphysema (as well as preclinical changes) will provide new intermediate phenotypes for genetic epidemiological investigation of AAT deficiency.

FAMILIAL AGGREGATION

The first step in demonstrating that genetic factors influence a complex trait is typically to show that the trait aggregates in families. Information on familial aggregation of disease characteristics in PI Z relatives is quite limited. In the St Louis AAT study Silverman and colleagues investigated quantitative phenotypes in 82 PI MZ first degree relatives of PI Z participants considered as having significant airflow obstruction (FEV₁ ≤65% predicted) or without significant airflow obstruction (FEV₁ ≥65% predicted). A trend for lower FEV₁ values among PI MZ relatives of PI Z individuals with significant airflow obstruction was found compared with PI MZ relatives of PI Z subjects without significant airflow obstruction (FEV₁ 93.1% v 101.1 % predicted).⁴⁴ When only the PI MZ parents were examined, despite similar smoking histories, FEV₁ also tended to be reduced in PI MZ parents of PI Z individuals with significant airflow obstruction compared with parents of PI Z individuals without significant airflow obstruction (FEV₁ 75.3% v 95.0% predicted). These results, together with segregation analysis on 44 AAT deficient pedigrees,⁴⁵ suggest the presence of other genetic factors contributing to the development of airflow limitation in AAT deficiency. However, compelling evidence for genetic modifiers of lung disease in severe AAT deficiency based on familial aggregation in PI Z siblings has not yet been provided.

GENETIC MODIFIERS

Despite the lack of clear evidence for familial aggregation of lung disease unrelated to AAT type in PI Z individuals, several types of evidence do suggest that genetic modifiers of AAT deficiency exist. As noted above, there is marked variability in the development and severity of lung disease in PI Z individuals. In order to identify genes that modify the expression of major gene disorders like AAT deficiency, one approach is to select candidate genes on the basis of known pathophysiology of the disease and to investigate genetic variants such as single base polymorphisms (SNPs) for association with disease or intermediate phenotypes of disease. Novoradovsky and colleagues investigated NOS3 polymorphisms as potential genetic factors relevant to variable disease expression in PI Z individuals.⁴⁶ In a case-control study they evaluated six NOS3 polymorphisms for association with airflow obstruction in 55 PI Z individuals with FEV₁ <35% predicted, 122 PI Z individuals with FEV₁ >35% predicted, and 93 control subjects. Two coding region polymorphisms were associated with severe airflow obstruction in PI Z individuals. These polymorphisms, although not obviously contributing to functional alterations in the NOS3 protein, may be located near other functional variants. Further studies are needed to clarify the role of NOS3 as a genetic modifier of lung disease in PI Z individuals.

In addition to studying candidate genes, positional cloning represents a second approach to identifying genes that may modify the development of pulmonary disease in PI Z individuals. At present there are both European and North American initiatives to identify modifier genes in AAT deficiency through genome-wide linkage-based approaches

using PI ZZ siblings. Because large numbers of patients with the PI ZZ genotype are necessary to have adequate power with this approach, collaborative research efforts are important to elucidating AAT as a complex human disease.

Although linkage studies have not yet been reported in PI ZZ individuals, linkage studies have been performed for spirometric phenotypes in severe early onset COPD pedigrees (without AAT deficiency) and in families from the general population.⁴⁷⁻⁵⁰ In the Boston Early Onset COPD study, significant linkage on chromosome 2 was observed to FEV₁/FVC as a quantitative phenotype, and several regions of suggestive linkage have also been shown to COPD related phenotypes.⁴⁸⁻⁵¹ The recent replication of the chromosome 2 linkage to FEV₁/FVC in pedigrees from the general population in Utah increases the likelihood that a genetic determinant of airflow obstruction is located in that genomic region, which could also influence the development of airflow obstruction in PI Z individuals.⁵⁰

HETEROZYGOUS GENOTYPES AND RISK FOR DISEASE

PI MZ individuals have intermediate serum protein levels of approximately 60% of PI MM individuals; PI MS individuals have levels approximately 80% of PI MM individuals. However, PI MZ and PI MS individuals may have AAT serum protein levels that overlap with PI MM individuals. PI SZ heterozygotes have serum AAT levels approximately 35% of PI MM individuals. The risk of COPD in heterozygous individuals has been the topic of longstanding controversy. However, understanding the risk associated with these heterozygous states is important from a public health perspective, as recent reports suggest (from analysis of databases from 58 countries) the potential existence of at least 116 million carriers of deficiency alleles (PI MZ and MS) and 3.4 million deficiency allele combinations (PI SZ, SS and ZZ) worldwide.⁵²

PI MZ and PI SZ individuals

Studies performed to date have both supported and refuted an increased risk of COPD in PI MZ individuals, with case-control studies typically finding some increased risk for COPD in PI MZ subjects and population based surveys often finding similar pulmonary function levels in PI MZ and PI M subjects. One case-control study showed that, in 526 patients with COPD, there was an odds ratio of 5 for COPD in PI MZ patients compared with controls.⁵³ In a population based study of 9187 individuals Dahl and colleagues observed faster rates of decline in FEV₁ and increased rates of airflow obstruction in PI MZ versus PI M individuals in the Copenhagen Heart Study.⁵⁴ In a study of 1551 PI MZ individuals versus 14 484 general population controls (PI type unknown), Seersholm *et al* observed that PI MZ individuals listed on the Danish AAT Register had a higher risk of a diagnosis of obstructive lung disease on discharge from hospital, although this increased risk was predominantly amongst relatives of PI Z individuals.⁵⁵ In the Lung Health Study, Sandford and colleagues found a higher proportion of PI MZ individuals among those with a rapid decline in pulmonary function than in those with a slow decline in pulmonary function; this association was stronger in individuals with a family history of COPD.⁵⁶ In addition to supporting some increased risk for COPD among PI MZ individuals, the studies of Seersholm and Dahl suggest the presence of susceptibility factors that are present in families due to shared genes and/or shared environments. However, in a recent longitudinal study of 2016 adults in Tucson, Arizona, the frequency of PI MZ did not differ in those with or without physician diagnosed emphysema or chronic bronchitis, nor was there a statistically significant difference

between PI types for changes in mean FEV₁ slope over time. It is therefore still uncertain whether *all* PI MZ individuals are at a slightly increased risk for COPD or if a subset of PI MZ individuals are at substantially increased risk due to other genetic and/or environmental susceptibility factors.

The risk of obstructive lung disease in PI SZ individuals has also been investigated. In an evaluation of 59 PI SZ individuals from the NHLBI AAT Deficiency Registry, many ascertained with a prior diagnosis of COPD, Turino and colleagues found that, among lifelong non-smokers, PI SZ individuals had lower rates of airflow obstruction than PI Z individuals. Among the smokers (former and current), rates of airflow obstruction were similar for PI SZ and PI Z individuals, suggesting an increased risk for COPD among PI SZ individuals who smoke.⁵⁷ In the Danish AAT Deficiency Register, 66 non-index PI SZ cases identified through family studies had FEV₁ values in the normal range (mean FEV₁ 94.7% predicted).⁵⁸ An investigation of 25 PI SZ individuals by Hutchinson and colleagues found a reduced FEV₁ in 11 of 14 patients identified through a pulmonary clinic compared with one of 11 individuals identified through family studies.⁵⁹ With progress in understanding genetic modifiers in PI Z individuals, the risk and variability in PI MZ and PI SZ individuals may also be clarified by genetic epidemiological methods.

CONCLUSION

Susceptibility to COPD probably requires the intersection of appropriate environmental exposures and several (if not many) genetic determinants. The variability in the development of COPD among PI Z subjects is probably a function of modifier gene influences as well as gene \times gene and gene \times environment interactions. Positional cloning and candidate gene approaches performed in large cohorts of carefully phenotyped PI Z individuals (as well as potentially in PI MZ individuals) will provide new directions in AAT deficiency research; hopefully, this research will elucidate the mechanisms for the variable expression of lung disease in patients with severe AAT deficiency. In addition, new insights into COPD from modifier gene studies in AAT deficiency may also lead to improved understanding of disease susceptibility in patients with non-AAT deficiency related COPD.

ACKNOWLEDGEMENTS

The authors are supported by NIH K08 HL 072918 (DLD) and NIH R01 HL 68926 (EKS).

Authors' affiliations

D L DeMeo, E K Silverman, Channing Laboratory and Division of Pulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston, MA 02446, USA

REFERENCES

- 1 **Laurell CB**, Eriksson S. The electrophoretic alpha 1-globulin pattern of serum in alpha 1-antitrypsin deficiency. *Scand J Clin Lab Invest* 1963;**15**:132-40.
- 2 **Eriksson S**. Studies in alpha 1-antitrypsin deficiency. *Acta Med Scand Suppl* 1965;**432**:1-85.
- 3 **Darlington GJ**, Astrin KH, Muirhead SP, *et al*. Assignment of human alpha 1-antitrypsin to chromosome 14 by somatic cell hybrid analysis. *Proc Natl Acad Sci USA* 1982;**79**:870-3.
- 4 **Cox DW**, Markovic VD, Teshima IE. Genes for immunoglobulin heavy chains and for alpha 1-antitrypsin are localized to specific regions of chromosome 14q. *Nature* 1982;**297**:428-30.
- 5 **Turleau C**, de Grouchy J, Chavin-Colin F, *et al*. Two patients with interstitial del (14q), one with features of Holt-Oram syndrome. Exclusion mapping of PI (alpha-1-antitrypsin). *Ann Genet* 1984;**27**:237-40.
- 6 **Schroeder WT**, Miller MF, Woo SL, *et al*. Chromosomal localization of the human alpha 1-antitrypsin gene (PI) to 14q31-32. *Am J Hum Genet* 1985;**37**:868-72.
- 7 **Yamamoto Y**, Sawa R, Okamoto N, *et al*. Deletion 14q(q24.3 to q32.1) syndrome: significance of peculiar facial appearance in its diagnosis, and deletion mapping of PI(alpha 1-antitrypsin). *Hum Genet* 1986;**74**:190-2.

- 8 **Lai EC**, Kao FT, Law ML, *et al.* Assignment of the alpha 1-antitrypsin gene and a sequence-related gene to human chromosome 14 by molecular hybridization. *Am J Hum Genet* 1983;**35**:385-92.
- 9 **Long GL**, Chandra T, Woo SL, *et al.* Complete sequence of the cDNA for human alpha 1-antitrypsin and the gene for the S variant. *Biochemistry* 1984;**23**:4828-37.
- 10 **Riva A**, Kohane IS. SNPper: retrieval and analysis of human SNPs. *Bioinformatics* 2002;**18**:1681-5.
- 11 **Fagerhol MK**, Laurell CB. The polymorphism of "prealbumins" and alpha-1-antitrypsin in human sera. *Clin Chim Acta* 1967;**16**:199-203.
- 12 **Frants RR**, Eriksson AW. Alpha1-antitrypsin: common subtypes of Pi M. *Hum Hered* 1976;**26**:435-40.
- 13 **Luisetti M**, Seersholm N. α_1 -Antitrypsin deficiency. 1: Epidemiology of α_1 -antitrypsin deficiency. *Thorax* 2004;**59**:164-9.
- 14 **Travis J**, Salvesen GS. Human plasma proteinase inhibitors. *Annu Rev Biochem* 1983;**52**:655-709.
- 15 **Mahadeva R**, Lomas DA. Genetics and respiratory disease. 2. Alpha 1-antitrypsin deficiency, cirrhosis and emphysema. *Thorax* 1998;**53**:501-5.
- 16 **Elliott PR**, Bilton D, Lomas DA. Lung polymers in Z alpha1-antitrypsin deficiency-related emphysema. *Am J Respir Cell Mol Biol* 1998;**18**:670-4.
- 17 **Nukiwa T**, Takahashi H, Brantly M, *et al.* Alpha 1-antitrypsin null/Granite Falls, a nonexpressing alpha 1-antitrypsin gene associated with a frameshift to stop mutation in a coding exon. *J Biol Chem* 1987;**262**:1199-2004.
- 18 **Hofker MH**, Nukiwa T, van Paassen HM, *et al.* A Pro \rightarrow Leu substitution in codon 369 of the alpha-1-antitrypsin deficiency variant PI MHeerlen. *Hum Genet* 1989;**81**:264-8.
- 19 **Kalsheker N**, Hayes K, Weidinger S, *et al.* What is Pi (proteinase inhibitor) null or PiQO? : a problem highlighted by the alpha 1 antitrypsin Mheerlen mutation. *J Med Genet* 1992;**29**:27-9.
- 20 **Takahashi H**, Nukiwa T, Satoh K, *et al.* Characterization of the gene and protein of the alpha 1-antitrypsin "deficiency" allele Mprocidia. *J Biol Chem* 1988;**263**:15528-34.
- 21 **Curiel DT**, Holmes MD, Okayama H, *et al.* Molecular basis of the liver and lung disease associated with the alpha 1-antitrypsin deficiency allele Mmalton. *J Biol Chem* 1989;**264**:13938-45.
- 22 **Seyama K**, Nukiwa T, Takabe K, *et al.* Siiyama (serine 53 (TCC) to phenylalanine 53 (TTC)). A new alpha 1-antitrypsin-deficient variant with mutation on a predicted conserved residue of the serpin backbone. *J Biol Chem* 1991;**266**:12627-32.
- 23 **Seyama K**, Nukiwa T, Souma S, *et al.* Alpha 1-antitrypsin-deficient variant Siiyama (Ser53[TCC] to Phe53[TTC]) is prevalent in Japan. Status of alpha 1-antitrypsin deficiency in Japan. *Am J Respir Crit Care Med* 1995;**152**:2119-26.
- 24 **Martin NG**, Clark P, Ofulue AF, *et al.* Does the Pi polymorphism alone control alpha-1-antitrypsin expression? *Am J Hum Genet* 1987;**40**:267-77.
- 25 **Silverman EK**, Province MA, Campbell EJ, *et al.* Family study of alpha 1-antitrypsin deficiency: effects of cigarette smoking, measured genotype, and their interaction on pulmonary function and biochemical traits. *Genet Epidemiol* 1992;**9**:317-31.
- 26 **Beatty K**, Bieth J, Travis J. Kinetics of association of serine proteinases with native and oxidized alpha-1-proteinase inhibitor and alpha-1-antichymotrypsin. *J Biol Chem* 1980;**255**:3931-4.
- 27 **Ogushi F**, Fells GA, Hubbard RC, *et al.* Z-type alpha 1-antitrypsin is less competent than M1-type alpha 1-antitrypsin as an inhibitor of neutrophil elastase. *J Clin Invest* 1987;**80**:1366-74.
- 28 **Carrell RW**. Alpha 1-antitrypsin: molecular pathology, leukocytes, and tissue damage. *J Clin Invest* 1986;**78**:1427-31.
- 29 **Lewis JH**, Iammarino RM, Spero JA, *et al.* Antithrombin Pittsburgh: an alpha 1-antitrypsin variant causing hemorrhagic disease. *Blood* 1978;**51**:129-37.
- 30 **Owen MC**, Brennan SO, Lewis JH, *et al.* Mutation of antitrypsin to antithrombin. alpha 1-antitrypsin Pittsburgh (358 Met leads to Arg), a fatal bleeding disorder. *N Engl J Med* 1983;**309**:694-8.
- 31 **Scott CF**, Carrell RW, Glaser CB, *et al.* Alpha-1-antitrypsin-Pittsburgh. A potent inhibitor of human plasma factor XIa, kallikrein, and factor XII. *J Clin Invest* 1986;**77**:631-4.
- 32 **Schapiro M**, Ramus MA, Jallat S, *et al.* Recombinant alpha 1-antitrypsin Pittsburgh (Met 358 \rightarrow Arg) is a potent inhibitor of plasma kallikrein and activated factor XII fragment. *J Clin Invest* 1986;**77**:635-7.
- 33 **Curiel DT**, Vogelmeier C, Hubbard RC, *et al.* Molecular basis of alpha 1-antitrypsin deficiency and emphysema associated with the alpha 1-antitrypsin Mmineral springs allele. *Mol Cell Biol* 1990;**10**:47-56.
- 34 **Crystal RG**. The alpha 1-antitrypsin gene and its deficiency states. *Trends Genet* 1989;**5**:411-7.
- 35 **Cox DW**, Levison H. Emphysema of early onset associated with a complete deficiency of alpha-1-antitrypsin (null homozygotes). *Am Rev Respir Dis* 1988;**137**:371-5.
- 36 **Garver RI Jr**, Mornex JF, Nukiwa T, *et al.* Alpha 1-antitrypsin deficiency and emphysema caused by homozygous inheritance of non-expressing alpha 1-antitrypsin genes. *N Engl J Med* 1986;**314**:762-6.
- 37 **Brantly ML**, Paul LD, Miller BH, *et al.* Clinical features and history of the destructive lung disease associated with alpha-1-antitrypsin deficiency of adults with pulmonary symptoms. *Am Rev Respir Dis* 1988;**138**:327-36.
- 38 **Janus ED**, Phillips NT, Carrell RW. Smoking, lung function, and alpha 1-antitrypsin deficiency. *Lancet* 1985;**1**:152-4.
- 39 **Larsson C**. Natural history and life expectancy in severe alpha1-antitrypsin deficiency. *Pi Z. Acta Med Scand* 1978;**204**:345-51.
- 40 **Tobin MJ**, Cook PJ, Hutchison DC. Alpha 1 antitrypsin deficiency: the clinical and physiological features of pulmonary emphysema in subjects homozygous for Pi type Z. A survey by the British Thoracic Association. *Br J Dis Chest* 1983;**77**:14-27.
- 41 **Silverman EK**, Pierce JA, Province MA, *et al.* Variability of pulmonary function in alpha-1-antitrypsin deficiency: clinical correlates. *Ann Intern Med* 1989;**111**:982-91.
- 42 **Black LF**, Kueppers F. Alpha 1-antitrypsin deficiency in nonsmokers. *Am Rev Respir Dis* 1978;**117**:421-8.
- 43 **Pitulainen E**, Tornling G, Eriksson S. Effect of age and occupational exposure to airway irritants on lung function in non-smoking individuals with alpha 1-antitrypsin deficiency (PiZZ). *Thorax* 1997;**52**:244-8.
- 44 **Silverman EK**, Province MA, Rao DC, *et al.* A family study of the variability of pulmonary function in alpha 1-antitrypsin deficiency. Quantitative phenotypes. *Am Rev Respir Dis* 1990;**142**:1015-21.
- 45 **Silverman EK**, Province MA, Campbell EJ, *et al.* Variability of pulmonary function in alpha-1-antitrypsin deficiency: residual family resemblance beyond the effect of the Pi locus. *Hum Hered* 1990;**40**:340-55.
- 46 **Novoradovsky A**, Brantly ML, Wacławski MA, *et al.* Endothelial nitric oxide synthase as a potential susceptibility gene in the pathogenesis of emphysema in alpha 1-antitrypsin deficiency. *Am J Respir Cell Mol Biol* 1999;**20**:441-7.
- 47 **Silverman EK**, Mosley JD, Palmer LJ, *et al.* Genome-wide linkage analysis of severe, early-onset chronic obstructive pulmonary disease: airflow obstruction and chronic bronchitis phenotypes. *Hum Mol Genet* 2002;**11**:623-32.
- 48 **Silverman EK**, Palmer LJ, Mosley JD, *et al.* Genome-wide linkage analysis of quantitative spirometric phenotypes in severe early-onset chronic obstructive pulmonary disease. *Am J Hum Genet* 2002;**70**:1229-39.
- 49 **Joost O**, Wilk JB, Cupples LA, *et al.* Genetic loci influencing lung function: a genome-wide scan in the Framingham Study. *Am J Respir Crit Care Med* 2002;**165**:795-9.
- 50 **Malhotra A**, Peiffer AP, Ruyjin DT, *et al.* Further evidence for the role of genes on chromosome 2 and chromosome 5 in the inheritance of pulmonary function. *Am J Respir Crit Care Med* 2003;**168**:556-61.
- 51 **Palmer LJ**, Celedon JC, Chapman HA, *et al.* Genome-wide linkage analysis of bronchodilator responsiveness and post-bronchodilator spirometric phenotypes in chronic obstructive pulmonary disease. *Hum Mol Genet* 2003;**12**:1199-210.
- 52 **de Serres FJ**. Worldwide racial and ethnic distribution of alpha1-antitrypsin deficiency: summary of an analysis of published genetic epidemiologic surveys. *Chest* 2002;**122**:1818-29.
- 53 **Barmann K**, Foke-Achterrath M, Koch G, *et al.* Heterozygosity in the Pi-system as a pathogenetic cofactor in chronic obstructive pulmonary disease (COPD). *Eur J Respir Dis* 1985;**66**:284-96.
- 54 **Dahl M**, Tybjaerg-Hansen A, Lange P, *et al.* Change in lung function and morbidity from chronic obstructive pulmonary disease in alpha 1-antitrypsin MZ heterozygotes: a longitudinal study of the general population. *Ann Intern Med* 2002;**136**:270-9.
- 55 **Seersholm N**, Wilcke JT, Kok-Jensen A, *et al.* Risk of hospital admission for obstructive pulmonary disease in alpha1-antitrypsin heterozygotes of phenotype PiMZ. *Am J Respir Crit Care Med* 2000;**161**:81-4.
- 56 **Sandford AJ**, Chagani T, Weir TD, *et al.* Susceptibility genes for rapid decline of lung function in the Lung Health Study. *Am J Respir Crit Care Med* 2001;**163**:469-73.
- 57 **Turino GM**, Barker AF, Brantly ML, *et al.* Clinical features of individuals with PiSZ phenotype of alpha 1-antitrypsin deficiency. Alpha 1-antitrypsin Deficiency Registry Study Group. *Am J Respir Crit Care Med* 1996;**154**:1718-25.
- 58 **Seersholm N**, Kok-Jensen A. Intermediate alpha 1-antitrypsin deficiency PiSZ: a risk factor for pulmonary emphysema? *Respir Med* 1998;**92**:241-5.
- 59 **Hutchison DC**, Tobin MJ, Cook PJ. Alpha 1 antitrypsin deficiency: clinical and physiological features in heterozygotes of Pi type SZ. A survey by the British Thoracic Association. *Br J Dis Chest* 1983;**77**:28-34.
- 60 **Brennan SO**, Carrell RW. Alpha 1-antitrypsin Christchurch, 363 Glu \rightarrow Lys: mutation at the P'5 position does not affect inhibitory activity. *Biochim Biophys Acta* 1986;**873**:13-9.
- 61 **Frazier GC**, Siewertsen MA, Hofker MH, *et al.* A null deficiency allele of alpha 1-antitrypsin, QOluudwigshafen, with altered tertiary structure. *J Clin Invest* 1990;**86**:1878-84.
- 62 **Sifers RN**, Brashears-Macatee S, Kidd VJ, *et al.* A frameshift mutation results in a truncated alpha 1-antitrypsin that is retained within the rough endoplasmic reticulum. *J Biol Chem* 1988;**263**:7330-5.
- 63 **Takahashi H**, Crystal RG. Alpha 1-antitrypsin Null(isola di procidia): an alpha 1-antitrypsin deficiency allele caused by deletion of all alpha 1-antitrypsin coding exons. *Am J Hum Genet* 1990;**47**:403-13.