Alpha One Antitrypsin Deficiency: From Gene to Treatment

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Key Words

α 1-antitrypsin deficiency • Molecular biology • Genetics, population • Epidemiology, molecular • Biological therapy

Abstract

α 1-antitrypsin deficiency is a genetic disorder which contributes to the development of chronic obstructive pulmonary disease, bronchiectasis, liver cirrhosis and panniculitis. The discovery of α 1-antitrypsin and its function as an anti-protease led to the protease-antiprotease hypothesis, which goes some way to explaining the pathogenesis of emphy-

sema. This article will review the clinical features of α 1-antitrypsin deficiency, the genetic mutations known to cause it, and how they do so at a molecular level. Specific treatments for the disorder based on this knowledge will be reviewed, including α 1-antitrypsin replacement, gene therapy and possible future therapies, such as those based on stem cells.

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Introduction

α 1-antitrypsin deficiency (AATD) was first described in 1963 by Laurell and Eriksson [1], who reported an absence of the α 1-band on protein electrophoresis of serum taken from a patient at a local respiratory hospital. The observation that people with this deficiency develop early-onset emphysema [2] and chronic obstructive pulmonary disease (COPD) suggested a role for pathways involving α 1-antitrypsin (AAT) in the pathogenesis. The main function of AAT is to protect the tissues against neutrophil elastase (NE) [3], with a smaller role in defending against damage by other serine proteases, such as cathepsin G and proteinase 3. These enzymes induce emphysema and bronchial damage in animal models [4–6], giving rise to the protease-antiprotease hypothesis of disease in man. This theory suggests that

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when there is an imbalance of proteases (which digest elastin and other components of the extracellular matrix as well as damaging epithelial tissues) and protective antiproteases, excess damage occurs, manifesting as emphysema and COPD [7]. AATD can therefore be used as a disease model for COPD; thus research into the condition has led to and complemented much of the work in usual COPD. This has implicated other proteases in the pathogenesis of usual COPD, such as the matrix metalloproteinases [8], lending further support to the protease-antiprotease hypothesis, perhaps as part of an integrated cascade [9].

AAT is an acute-phase glycoprotein, synthesised in hepatocytes [10] and subsequently secreted into the plasma. It is also produced in smaller quantities by alveolar macrophages, circulating monocytes [11] and possibly lung epithelial cells [12, 13]. Figure 1 shows the structure of AAT, highlighting some of the features that classify it as part of the serpin family of proteins, whose structure is based on 3 β -sheets and a mobile reactive loop which acts as a binding site for the target protease [14, 15].

For AAT, the target protease is predominantly NE, though it can also bind irreversibly to proteinase 3 and cathepsin G. Point mutations in its mobile domain can lead to polymerisation of the protein within the liver [16], such that it is not secreted effectively into the plasma, resulting in low levels within the lung and vulnerability to NE-induced damage. Mutations like these underlie the most common forms of AATD, and ultimately are caused by genetic polymorphisms, thus providing a target for gene therapy that might cure the disease. However, amongst subjects with the same AAT genotype there is considerable variation in the clinical manifestations of the disease [17], even after taking into account environmental influences, such as smoking. This suggests that there are other genetic factors influencing disease susceptibility, meaning that AATD can also be a model for complex genetic diseases. This review will describe the genetic basis of AATD, clinical features of the disease and approaches to treatment derived from this knowledge.

The Genetics of AATD

The AAT protein is 394 amino acids in size, with the active site residue being methionine at position 358 (fig. 1). It is encoded by the protease inhibitor (Pi) locus on chromosome 14q [18–20]. The Pi locus (fig. 2) is 12.2

Fig. 1. The structure of AAT.

kb in length with 4 coding exons (II, III, IV, V), 3 non-coding exons (IA, IB, IC) and 6 introns; the region coding for the reactive loop is within exon V. It has been fully sequenced and cloned [21] and shows a co-dominant pattern of inheritance [2]. It is a highly pleomorphic gene with approximately 125 single nucleotide polymorphisms (SNPs) reported in public databases [22], a proportion of which have an effect on AAT level or function. Traditionally, each variant is identified by its speed of migration on gel electrophoresis, the most common forms being F (fast), M (medium), S (slow) and Z (very slow) [23]. Alteration in the speed of movement through a gel occurs because of variation in protein charge, due to changes in amino acid composition [24]. The PiM allele is the wild type, and is the most prevalent. The PiZ allele is a more common deficiency variant in northern Europeans, whilst the PiS deficiency variant is more common in south-west Europe [25]. A detailed review of their frequencies worldwide has been published previously [25].

Variants may also be classified by their effect on AAT level and function – normal, deficient, null (nil detectable) or dysfunctional. Deficient subjects are prone to lung or liver disease, whilst those carrying null alleles are prone only to lung disease. Serum deficiency or absence of AAT occurs because of alterations in gene expression or translation, or abnormal intracellular processing. Dysfunctional alleles code for an abnormal form of AAT, which, whilst present at a detectable level, does not func-

Fig. 2. The structure of the AAT gene.

Table 1. Effects of genetic variation within the Pi locus

Allele	Genetic defect	Cellular defect	Disease association
Z	1-bp substitution	intracellular aggregation	lung, liver
S	1-bp substitution	intracellular aggregation	lung
Mmalton	3-bp deletion	intracellular aggregation	lung, liver
Siiyama	1-bp substitution	intracellular aggregation	lung
Mprocida [42]	1-bp substitution	intracellular degradation	lung
Mheerlen [43, 44]	1-bp substitution	intracellular degradation	lung
QOgranite falls	1-bp deletion	unstable mRNA	lung
QOhong kong	2-bp deletion	truncated protein	lung
QOisola di procida	deletion exons II–IV	no mRNA	lung
Pimperial springs	1-bp substitution	poor inhibition of NE	lung
PiPittsburgh	1-bp substitution	antithrombin III activity	bleeding diathesis

tion normally (such as the F variant). The majority of clinical disease due to AAT is from deficiency and null alleles.

The PiZ mutation is an SNP which results in an amino acid substitution – from glutamic acid to lysine – at position 342 (Glu342Lys), shown in figure 1. This causes a conformational change in the reactive loop that opens up β -sheet A and allows the reactive loop of a second molecule to insert at this point [15]. This process extends to form AAT polymers, which form inclusion bodies in hepatocytes, rather than being secreted into the circulation, a process which has been reproduced in vitro under physiological conditions [16]. This process causes hepatocyte death and ultimately liver disease in AATD, though the precise mechanisms for this are not well understood. In addition, the expression of liver disease varies widely, from jaundice only at birth, through to cirrhosis, perhaps because of other genetic influences on this aspect of the clinical phenotype [26]. Polymerisation of AAT in this way also underlies the PiMmalton (52Phe deleted) [27] and Siiyama (Ser53Phe) [28–30] forms of

AATD, that are common in Sardinia and Japan, respectively. A slower rate of polymer formation occurs with the PiS allele (Glu264Val), because the structural change in β -sheet A is not as radical [31, 32], resulting in a milder serum deficiency but little evidence of clinical disease. If an individual has the genotype PiSZ, then their clinical phenotype for liver disease [32] and lung disease in smokers [33] is usually intermediate between that of PiZ and PiS subjects.

Many null-allelic variants have been described which result in an absence of AAT, and are denoted as QO rather than Pi. A deletion of a single base pair leads to the QOgranite falls genotype, resulting in a premature stop codon and unstable mRNA [34]. Deletion of 2 bp in exon IV characterises QOhong kong, which similarly has a premature stop codon, and hence a truncated protein [35]. This protein is retained in the endoplasmic reticulum of the liver accounting for the absence of the protein in the serum. Three other null alleles have been described, which also result in the formation of stop codons either due to substitution, deletion or addition of base(s) in cod-

ing regions [36–38]. A larger deletion, comprising 17 bp, including exons II–IV is seen in the QOisola di procida allele, and is so substantial that no detectable mRNA or protein is produced [39].

Amongst the dysfunctional proteins described, those associated with poor inhibition of NE may be associated with lung disease. An example of this is Mmineral springs [40] caused by an SNP that leads to a single amino acid substitution (Gly67Glu) with intracellular aggregation of AAT; the latter characteristic means that it is also associated with serum deficiency. A different amino acid substitution at the active site (Met358Arg), known as PiPittsburgh, not only leads to reduced anti-NE activity, but also to an anticoagulant effect by way of inhibition of factor IXa, kallikrein and factor XIIIf [41]. Some of the polymorphisms discussed here are summarised in table 1.

Genetic and Environmental Interactions

AATD is a good example of a genetic disease where there is significant genotype-environment interaction. This is defined as a non-additive contribution of gene and environment to the clinical phenotype [45]; thus, together the 2 influences confer a different level of risk than that expected by addition alone. In AATD, for example, the PiZ phenotype confers a far greater risk of lung function abnormalities and rapidity of decline in smokers than in non-smokers [46, 47] because of the neutrophilic inflammation and consequent NE release, induced in the airways by cigarette smoke [7]. Similarly, in pulmonary arterial hypertension there may be an interaction between fenfluramine exposure and genotype that influences disease development [48]. Other respiratory diseases thought to have multiple genetic influences on phenotype include asthma [49] and sarcoidosis [50]. If these genes have additive or synergistic effects, these are referred to as epistatic interactions. In AATD there is some evidence that as yet unknown genes contribute to the clinical phenotype, as there is familial clustering of spirometric abnormalities in PiZ and PiMZ subjects [51, 52] and declining lung function in PiMZ individuals [53].

Genetic epidemiology studies in usual COPD have revealed many candidate genes [54], which may be contributing to disease in subjects who also have AATD. Case-control studies looking for these effects in AATD have not been widely published, but have implicated a polymorphism in the gene coding for endothelial nitric oxide

synthase (NOS)3 and an SNP in the glutathione S transferase P1 gene (GSTP1) [55]. The NOS3 SNP was shown to have a significant correlation with severity of lung disease, defined by FEV₁ [56]. However, the authors were unable to show any functional variation in NOS3 with this SNP, and concluded that it must lie in linkage disequilibrium with another gene that caused the association. The GSTP1 SNP is an A→G change at nucleotide +313, resulting in a single amino acid substitution (Ile105Val) [57] shown to increase the metabolism of carcinogenic aromatic epoxides [58]. Studies of the relationship of this variant to lung disease have also varied in their results. It would be expected that the 105Ile variant would be associated with higher levels of lung damage, since it is less active against oxidants, but in AATD this was not found to be the case [55]. Further family and case-control studies are underway and may begin to clarify reasons for phenotypic heterogeneity in AATD.

Clinical Features of AATD

AATD is associated with emphysema, chronic bronchitis, bronchiectasis, neonatal jaundice, liver cirrhosis, vasculitis and panniculitis [17]. The diagnosis of AATD will usually be made after investigation of these conditions, but is influenced by local and national practice, particularly with regard to neonatal and family screening. The PiZ allele is carried at a frequency of 0.5–4% in Europe, being more common in northern Europe [25]. Potential deficiency alleles (both PiZ and PiS) have been estimated to occur in 10% of cases of COPD in Caucasians in the USA [59]. Allele frequency studies may not be as useful as genotype studies, however, as PiMS heterozygotes are not thought to be at risk of disease [60] and PiMZ subjects only have a small risk of developing COPD relative to PiMM individuals and may never have clinically significant problems [61]. A more recent review of genotype frequencies has suggested that in Europe the PiZZ genotype occurs in approximately 1 in 25 individuals in the UK, with the PiMZ genotype occurring in 1 in 2,000 individuals [62]. Studies of these genotypes amongst patients with a diagnosis of COPD have shown a prevalence of 1–4.5% for PiZZ and up to 17.8% for PiMZ [60].

In a recent survey of over 5,000 American patients registered with AATD support groups, 81% exhibited symptoms of chest disease, including asthma, chronic bronchitis and emphysema [63]. This may reflect a selection bias, as those without symptoms are less likely to

be diagnosed [64]. In our UK AATD cohort, 65% of subjects have been diagnosed after investigation of chest symptoms, with the majority of the remainder being ascertained through family screening [unpubl. data]. Many of the symptoms of AATD are non-specific, with subjects experiencing cough, wheeze and chest infections which may contribute to the delay in diagnosis experienced by many patients, which is 5.6 years on average from onset of symptoms [65]. The classical presentation is similar to usual COPD, though often at a younger age, or with less smoke exposure [17] and in the UK, current national guidelines only suggest testing for the condition in these groups [66]. Nevertheless, many patients present at an older age, like usual COPD, and thus testing for AATD should be considered in all patients with COPD.

Lung function tests usually show features typical of usual COPD, such as airflow obstruction with or without low gas transfer, and increased lung volumes. However, it is not uncommon for changes more usually associated with asthma, such as reversibility after bronchodilators, to be seen. In the National Heart, Lung, and Blood Institute AATD registry, reversibility of at least 12% in FEV₁ was present in 28% of subjects at their baseline assessment [67]. Normal lung function may also be seen, most often, but not exclusively, in asymptomatic subjects. Amongst our patients homozygous for the Z allele (PiZZ), 9.1% have normal lung function at their baseline assessment; 59% of these were diagnosed through family screening and 12.2% were tested because of respiratory symptoms [unpubl. data].

Smoke exposure is likely to be the most important determinant of lung function, with differences detectable between smokers and non-smokers even at an age of 18 years [68], and a significant relationship between smoking and lung function decline in adults [69]. This is consistent with our own data where 29.9% of British PiZZ subjects who had never smoked had normal spirometry at baseline, compared to only 5.3% of those who had smoked [unpubl. data]. Other determinants of more rapid decline of lung function include male sex, low body mass index, frequent exacerbations and the severity of upper zone emphysema [70].

High-resolution computed tomography (HRCT) scanning is now widely used in the assessment of usual COPD and COPD due to AATD, with many advances having been made in recent years in the interpretation of the data generated from such images [71, 72]. Scan images can be used to diagnose emphysema and bronchiectasis, their type and distribution, as well as making objective mea-

asures of severity based on the density of the CT image [71]. Our own work has shown that emphysema distribution in AATD relates to lung function [73], though it is not yet known if this is also true in usual COPD. In contrast to usual COPD, most patients with AATD have lower zone-predominant emphysema on HRCT. This is not universal, however, and upper zone-dominant emphysema, when diagnosed using both visual features and quantitative density measures, is present in 12.1% of PiZZ subjects in our registry [unpubl. data]. Specific tests for AATD include measuring the serum level of AAT, with a level of less than 11 μ M considered pathophysiologically important, and genotyping. Genotyping has the advantage that it will pick up null genes, rare deficiency alleles and forms of AATD where the level of the protein is normal, but it does not function as it should, such as the F variant.

Approaches to Treatment

At present, treatment for AATD depends on the individual presentation: for most subjects this will mean interventions targeted at COPD. Treatment approaches targeting the molecular basis of disease, rather than its consequences, have the potential to treat all aspects of the condition, and are the subject of much current research. Such approaches include AAT replacement, blocking the polymerisation process within hepatocytes and gene therapy. Alternatively, liver transplantation, by replacing the organ in which most AAT is made, returns AAT levels to normal. If this is performed prior to the development of lung disease, it offers a cure, but can only be used in those with end-stage liver disease, at present, due to the shortage of suitable organs and the implications of subsequent life-long immunosuppression.

AAT Replacement

Since serum deficiency of AAT, and hence reduced protection against NE in the lung, underlies the lung disease seen in AATD, a logical approach is to treat it by trying to raise the circulating level of AAT. In non-smokers with the PiSZ phenotype, epidemiological studies have shown little or no increase in the risk of lung disease compared to controls [33], thus their level of AAT (typically 11–14 μ mol/l) was taken to be the minimum needed to protect the lung from NE-mediated damage.

An early study showed that it was possible to obtain AAT from pooled plasma and administer it by weekly

Table 2. Status of selected rAAT production systems

Host	Protein location	Yield	Stage of testing	References
<i>E. coli</i>	intracellular	up to 38 mg/l	animals	[94–97]
Yeasts	intracellular or secreted	up to 1,230 mg/l	animals	[98–100]
Insect cells	secreted	not reported	in vitro	[90]
Transgenic rice	secreted	4.6–5.7 mg/l	animals	[101, 102]
Transgenic mice	milk	0.5–7 mg/ml	in vitro	[103]
	urine	up to 65 mg/l		[104]
Transgenic rabbits	milk	4 g/l	in vitro	[105]
Transgenic sheep	milk	up to 35 g/l	phase I ¹	[91–93]
Transgenic goats	milk	20 g/l	in vitro	[106]

¹ Further trials abandoned.

intravenous infusion, resulting in a serum nadir level of AAT (sufficient to protect the lung) associated with an increase in anti-elastase activity in the lower respiratory tract, as measured in bronchoalveolar lavage fluid [74]. Although this study was small, it was enough for the product to gain a license for treatment of patients with the PiZ or null phenotypes [75] in the USA, Canada and parts of Europe. Observational work in the USA has since supported the use of AAT replacement, showing a slower decline in lung function in those with an FEV₁ of 35–49% predicted when receiving AAT augmentation [76]. Similar work in Europe has concurred [77, 78], whilst a Canadian study reported a beneficial effect but failed to show the association with baseline FEV₁ [79]. The only randomised study to date, carried out in Denmark and Holland, showed a trend towards a reduction in emphysema progression, quantified by CT scanning, but did not reach statistical significance [80]. However, it did indicate that a larger trial would be sufficiently powered to detect a significant difference over 2 years and such a study has been completed recently (results are awaited).

Inhaled AAT may be a more efficacious method of administration, since only a small proportion of intravenous AAT reaches the lung. Initial studies of aerosolised forms of plasma-derived and recombinant AAT have shown that twice daily administration can raise the concentration in the epithelial lining fluid to normal [81] and some can reach the interstitium in animal models [82], though at much lower concentrations relative to the epithelium [83]. This is because of minimal protein movement across the epithelial barrier [84]. Thus, although this route may not replenish the interstitium, AAT activity in the epithelial lining fluid would be of benefit in re-

ducing NE-induced airway inflammation, thus indirectly protecting the interstitium by reducing neutrophil traffic. A murine model of COPD has supported the use of inhaled AAT in reducing emphysema severity [85], lending more credence to this therapeutic approach. Further work in this area is indicated to clarify any potential benefit for patients.

Recombinant AAT

Although AAT replacement therapy shows promise in slowing the progression of lung disease, new ways of obtaining the product must be found as the plasma-derived supply is variable in purity and activity [86], and is likely to be insufficient to meet demand. The human Pi gene has been expressed in a variety of hosts, but no recombinant therapeutic product has been licensed yet. Such a product would need to be safe, clinically efficacious and cost-effective.

Expression in *Escherichia coli* has been the most widely used system, but is problematic because the recombinant AAT (rAAT) is not glycosylated, which affects the folding of the protein, such that it aggregates more easily, has reduced activity and a shorter half-life in the blood after infusion [87]. Conjugation with polyethylene glycol prolongs the half-life of *E. coli*-derived rAAT, and may be a useful strategy for the future [88]. Yeasts have advantages over bacteria for the production of therapeutic proteins, as they do not produce endotoxins and can provide some post-translational modifications [87]. Problems still arise, however, because the glycosylation process in yeasts differs from that of humans, such that elongation of carbohydrates occurs, resulting in structures of high mannose content – a process known as hypermannosylation [89]. This could lead to immune responses in hu-

Fig. 3. Gene therapy in AATD.

mans because of non-human glycan residues, resulting in inactivation at best and allergic reactions at worst. Active rAAT has also been expressed in insect cells hosting baculovirus expression vector systems [90], again because of possible benefits in post-translational modification of the protein [87], but concerns regarding non-human glycans remain. Sources of rAAT from transgenic animals might overcome this problem, and are feasible for large-scale production [91, 92]. However, the first trial of inhaled rAAT from sheep milk reported a systemic antibody response, worse on repeated exposure, to the small amounts of sheep AAT and α 1-antichymotrypsin in the product [93]. The current status of rAAT production systems is shown in table 2.

Therapies Targeting AAT Polymerisation and Secretion

The basic mechanism underlying both lung and liver disease in the most common forms of AATD is the accumulation of AAT in hepatocytes, with resultant serum deficiency. Polymerisation underlies accumulation in the PiZ form of AATD, and is initiated by protein misfolding, such that the Z form of AAT (Z-AAT) folds more slowly than the wild type [107], resulting in persistence of an intermediate form that is prone to polymer formation. If the processes underlying this can be altered, it may have clinically beneficial effects.

Chemical chaperones are a group of molecules that guide folding and may reverse cellular mislocalisation of proteins. Compounds known to have such activity include glycerol, trimethylamine N-oxide and 4-phenylbutyric acid. Of these, 4-phenylbutyric acid has been shown to mediate an increase in secretion of Z-AAT in cell culture and murine models [108]. Trimethylamine N-oxide has been shown to stabilise native AAT [109], but did not prevent Z-AAT polymerisation in a cell culture model [108]. A more recent study has found a complex array of chaperones involved in the secretion of Z-AAT in vitro [110], although the significance is not yet known. Changes in intracellular degradation of Z-AAT were observed after the administration of drugs that interacted with these chaperones [110], suggesting more potential routes for drug development.

Precise inhibition of polymerisation of Z-AAT can also be achieved by annealing peptides to the reactive loop of β -sheet A. Initial studies used molecules 11–13 residues in length [16], but found that they were not specific for Z-AAT and hence could not be used for drug design. The same group of researchers has now assessed smaller peptides and shown them to be more specific, whilst still effective in blocking the process [111–113], thus allowing AAT to be released from the cells. Drugs developed from such molecules could help prevent liver disease due to accumulation in AATD. Unfortunately, if a peptide is bound to the molecule it also prevents AAT

having any anti-elastase activity, hence it must be able to dissociate if it is also to provide protection to the lung. Parfrey et al. [113] have shown that some 6-mer peptides dissociate from Z-AAT under physiological conditions, with the dissociated material having some antiprotease activity, so further research of this approach may lead to useful therapies in the future.

Gene Therapy

This is the replacement of defective or absent genes within a cell such that the treated cell functions normally, and it is the most direct rationale for a genetic disorder such as AATD. Studies of gene therapy in animal models have used retroviral [114], adenoviral [115–119], adeno-associated viral [120–122] and liposomal [123, 124] vectors to transfect cells. Recombinant adeno-associated viral vectors have been the most successful delivery system thus far, as they are capable of achieving therapeutic levels of AAT [120, 121] and are less likely to induce an inflammatory response than adenoviral vectors. They also have a specific site for incorporation into the human genome (ASV site), thus giving the genetic material carried by the vector the potential for long-term expression.

A variety of routes of administration have been attempted for gene therapy. Initially, liver-directed therapy was attempted [114, 119], including portal vein injection of vector [115, 121], but this was impractical for use in humans. Airway instillation of vector suggested that inhaled or nebulised treatment might be a viable alternative [116, 124], but the most successful method so far has been intramuscular injection [120, 122]. This system (fig. 3) is now entering phase II trials at the University of Florida [125], but may be hampered by the large doses of vector needed to achieve a therapeutic level of AAT. It should

also be noted that such approaches, whilst potentially protecting the lung and other tissues, will not influence the liver disease.

Stem Cells

Research using stem cells has also shown some potential for treatment of AATD, though such approaches will require further development before clinical use. Stem cells can differentiate into liver cells capable of expressing AAT [126–128], thus, transplanting them into deficient patients might facilitate normal production of AAT. This would not prevent accumulation of polymers in the liver, however, so it would not necessarily affect liver manifestations of the disease. Alternatively, an approach targeting the lung might be used, as it is also possible to differentiate human embryonic stem cells into alveolar epithelial type II cells [129].

Conclusion

Knowledge of the genetics underlying common forms of AATD has generated several potential types of treatment. Although none are fully developed for human use, recombinant sources of AAT, chemical chaperones and gene therapy show promise for the future. Advances in genetic resources, such as the human genome project and HapMap [130, 131], and technology, including genome-wide scans, are making further research into genes which may be modifying the clinical phenotype in AATD more practical. Research in both the USA and Europe in this field could also lead to new targets for treatment, with potential for use in usual COPD as well as AATD.

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