Alpha-1-antitrypsin deficiency, the serpinopathies and conformational disease

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ABSTRACT — Alpha-1-antitrypsin deficiency results from point mutations that distort the structure of the protein to allow a unique protein-protein interaction that we have termed loop-sheet polymerisation. Polymers of Z α₁-antitrypsin accumulate within hepatocytes to form inclusion bodies that are associated with juvenile cirrhosis and hepatocellular carcinoma. The lack of circulating protein predisposes the Z α₁-antitrypsin homozygote to emphysema. This process also occurs in other members of the serine proteinase inhibitor (serpin) superfamily, antithrombin, C1-inhibitor and α₁-antichymotrypsin, in association with thrombosis, angioedema and chronic obstructive pulmonary disease, respectively, and we have recently shown that it underlies a novel inclusion body dementia. The interaction provides a useful paradigm for other 'conformational diseases' such as Huntington's disease, Creutzfeldt-Jakob disease and the amyloidoses.

Clinical features

Alpha-1-antitrypsin deficiency was reported in an Alaskan girl who died 800 years ago, and it may have accounted for the premature death of Frederic Chopin in 1849. It was first described as a clinical entity in 1963 by Laurell and Eriksson who noted an absence of the alpha-1 band on serum protein electrophoresis. The major function of α₁-antitrypsin is to protect the tissues against the enzyme neutrophil elastase. Its role in protecting the lungs against proteolytic attack is underscored by the association of its deficiency in plasma with early-onset panlobular emphysema (Fig 1), asthma, bronchiectasis and Wegener's granulomatosis. Over 70 naturally occurring variants have been described and characterised by their migration on isoelectric focusing gels. The two most common deficiency variants, S and Z, result from point mutations in the α₁-antitrypsin gene and make the protein migrate more slowly than normal M α₁-antitrypsin. S α₁-antitrypsin (Glu→Val) is found in up to 28% of Southern Europeans; although it results in plasma α₁-antitrypsin levels 60% of those of the M allele, it is not associated with any pulmonary sequelae. The Z variant (Glu→Lys) results in a more severe deficiency; in the homozygote, it is characterised by plasma α₁-antitrypsin levels of 10% of those of the normal M allele, and in the MZ heterozygote by levels of 60% (50% and 10% from the M and Z alleles, respectively). The Z mutation results in the accumulation of α₁-antitrypsin as inclusions in the rough endoplasmic reticulum of the liver (Fig 2). These inclusions predispose the homozygote to juvenile hepatitis, cirrhosis and hepatocellular carcinoma.

Molecular pathology of α₁-antitrypsin deficiency

Alpha-1-antitrypsin is the archetypal member of the serine proteinase inhibitor (serpin) superfamily. This family includes members such as α₁-antichymotrypsin, C1 esterase inhibitor, antithrombin and plasminogen activator...
inhibitor-1, which play an important role in the control of proteinases involved in the inflammatory, complement, coagulation and fibrinolytic cascades. The family is characterised by more than 30% sequence homology with \( \alpha \)-antitrypsin and conservation of tertiary structure. Consequently, physiological and pathological processes that affect one member may be extrapolated to another. The structure of the serpins is based on three \( \beta \)-sheets (A-C) and nine \( \alpha \)-helices. This structure supports an exposed mobile reactive loop that presents a peptide sequence as a pseudosubstrate for the target proteinase. In the case of \( \alpha \)-antitrypsin, the loop presents the \( P_{1} P_{1}^{'} \) residues methionine-serine as a ‘bait’ for neutrophil elastase. After docking, the protease is inactivated by a ‘mousetrap’ action that swings it from the top to the bottom of the protein (Fig 3) in association with the insertion of an extra strand in \( \beta \)-sheet A. This six-stranded protein bound to its target enzyme is then recognised by hepatic receptors and cleared from the circulation.

The structure of the serpins is very much a dual-edged sword in that it is central to their role as effective anti-proteinases, but also renders them liable to undergo conformational change in association with disease. Point mutations can destabilise \( \beta \)-sheet A to allow incorporation of the loop of another serpin molecule. Sequential loop insertions result in chains of polymers that are retained within the cell of synthesis. This process is best characterised for the severe Z deficiency variant of \( \alpha \)-antitrypsin that results in protein retention in hepatocytes in association with cirrhosis. The Z mutation of \( \alpha \)-antitrypsin is at residue \( P_{1} \) (17 residues proximal to the \( P_{1} \) reactive centre) at the head of strand 5 of \( \beta \)-sheet A and the base of the mobile reactive loop (Fig 3). The mutation opens \( \beta \)-sheet A, thereby favouring the insertion of the reactive loop of a second \( \alpha \)-antitrypsin molecule to form a dimer (Fig 4(a)). This can then extend to form polymers (Fig 4(b) and (c)) that tangle in the endoplasmic reticulum of the liver to form inclusion bodies. Support for this comes from the demonstration that Z \( \alpha \)-antitrypsin forms chains of polymers when incubated under physiological conditions. The rate of formation is accelerated by raising the temperature to 41°C, and can be blocked by peptides that compete with the loop for annealing to \( \beta \)-sheet A. The role of polymerisation \textit{in vivo} was clarified by finding \( \alpha \)-antitrypsin polymers in inclusion bodies from the livers of Z \( \alpha \)-antitrypsin homozygotes.

Although many \( \alpha \)-antitrypsin deficiency variants have been described, only two other mutants of \( \alpha \)-antitrypsin have been associated with plasma deficiency and hepatic inclusions: \( \alpha \)-antitrypsin Siyama (\( ^{\text{53}} \text{Ser} \rightarrow \text{Phe} \)) and \( \alpha \)-antitrypsin Mmalton (\( ^{\text{52}} \text{Phe} \) deleted). Both mutants also stabilise \( \beta \)-sheet A to allow the formation of loop-sheet polymers \textit{in vivo} (Fig 4(c)). The temperature and concentration dependence of polymerisation, together with genetic factors, may account for the heterogeneity in liver disease amongst individuals who are homozygous for the Z mutation. As \( \alpha \)-antitrypsin is an acute-phase protein, its concentration will rise during episodes of inflammation. At these times, the formation of polymers is likely to overwhelm the degradative pathway, thereby exacerbating the formation of hepatic inclusions and the associated hepatocellular damage. There is anecdotal evidence to support this hypothesis from the prospective study of Sveger.

**Key Points**

**Alpha-1-antitrypsin deficiency results from point mutations that favour the formation of chains of polymers which are retained within hepatocytes as inclusion bodies.**

This process also underlies the deficiency of other members of the same superfamily: C1-inhibitor, antithrombin and \( \alpha \)-antichymotrypsin in association with angiodema, thrombosis and chronic obstructive pulmonary disease, respectively.

Polymerisation of the neuron-specific protein, neuroserpin, underlies a novel inclusion body dementia (familial encephalopathy with neuroserpin inclusion bodies).

This process is a useful paradigm for other conformational diseases: Alzheimer’s disease, Huntington’s disease, Creutzfeldt-Jakob disease and the amyloidoses.
Sweden in which 200,000 newborn babies were screened. Two of the 120 Z homozygotes who were identified and followed into late adolescence developed progressive jaundice during the course of the study, following appendicitis in one patient and severe pneumonia in the other.

Polymerisation also underlies the mild plasma deficiency of the S and I variants of α₁-antitrypsin. The point mutations responsible for these variants have less effect on β-sheet A than does the Z variant. Thus, the rates of polymer formation are much slower than that of Z α₁-antitrypsin; this leads to less retention of protein in hepatocytes, milder plasma deficiency and the lack of a clinical phenotype. However, if a mild, slowly polymerising I or S variant of α₁-antitrypsin is inherited together with a rapidly polymerising Z variant, the two can interact to form heteropolymers within hepatocytes, inclusions and cirrhosis.

The serpinopathies: serpin polymerisation in emphysema, thrombosis, angioedema and dementia

The single most important factor in the development of emphysema in patients with α₁-antitrypsin deficiency is smoking. The combination of antiprotease deficiency and cigarette smoke can have a devastating effect on lung function. We have shown that the antiproteinase screen within the lung can be further reduced in Z α₁-antitrypsin homozygotes by the spontaneous formation of loop-sheet polymers. This conformational transition inactivates the protein, thereby further reducing the already depleted levels of α₁-antitrypsin available to protect the lungs. The relationship of intrapulmonary Z α₁-antitrypsin polymers to smoking, infection and rate of decline in lung function in Z homozygotes requires further evaluation in prospective studies.

The phenomenon of loop-sheet polymerisation is not restricted to α₁-antitrypsin, and has now been reported in other serpin variants to cause disease. Mutants of C1-inhibitor, antithrombin and α₁-antichymotrypsin can also destabilise the serpin architecture to form inactive polymers that are associated with angioedema, thrombosis and chronic obstructive pulmonary disease, respectively. The process is most striking in a recently described inclusion body dementia, familial encephalopathy with neuroserpin inclusion bodies, that results from polymerisation of the neuron-specific serpin, neuroserpin. The dementia has been described in two Caucasian families in the USA. In one family, 95% of affected individuals presented with dementia between the ages of 45 and 56; in the other, symptoms began at an earlier age, with epilepsy and progressive decline in cognitive function in the second and third decades of life. Both were characterised by eosinophilic neuronal inclusion bodies in the deeper layers of the cerebral cortex and substantia nigra. The inclusions were PAS-positive and diastase-resistant, but were distinctly different from any previously described entity, including Lewy bodies, Pick bodies and Lafora bodies. The inclusion bodies strikingly resembled those of Z α₁-antitrypsin in the hepatocytes of homozygotes with cirrhosis. Biochemical analysis revealed that the inclusions were formed of neuroserpin, and that affected individuals carried point mutations that would stabilise the protein to form polymers. Indeed, one of the mutations was in the same...
Position as the Siiyama variant which causes hepatic inclusions and profound plasma deficiency of α₁-antitrypsin. Structural analysis showed that the mutant neuroserpin had formed intraneuronal polymers identical to those of Z α₁-antitrypsin. Thus, therapies that attenuate serpin polymerisation may be useful in a whole range of diseases.

**Treatment of α₁-antitrypsin deficiency**

**Alpha-1-antitrypsin replacement therapy**

This new understanding of the structural basis of α₁-antitrypsin deficiency provides a platform for rational drug design to block polymerisation *in vivo* and so attenuate the associated liver disease. Any therapy that improves secretion from the liver will raise its circulating levels of α₁-antitrypsin and enhance the antiproteinase protection within the lung. Until this becomes possible, prevention of emphysema is better than cure, and there is good evidence that many Z α₁-antitrypsin homozygotes would develop only mild lung disease if they abstained from smoking. The genetic deficiency in the antielastase screen may be rectified biochemically by intravenous infusions of α₁-antitrypsin. This therapy appears to slow the rate of decline in lung function in patients with an FEV₁ of 35–49% predicted, but this has yet to be proven in randomised controlled trials. The only controlled trial that has assessed α₁-antitrypsin replacement therapy showed that although infusions of α₁-antitrypsin may slow down the progression of emphysema as assessed by high-resolution computed tomography, they have no effect on decline in FEV₁. Alpha-1-antitrypsin replacement therapy (Prolastin®, Bayer) is currently not available in the UK.

**Other treatments**

Other treatments at an early stage of development include gene therapy and retinoic acid. Vectors carrying the α₁-antitrypsin gene have been targeted to liver and lung, but there is insufficient gene expression for this to be clinically useful. The effects of retinoic acid on alveolar regeneration in the rat look promising, but have yet to be demonstrated in man.

In the meantime, patients with emphysema related to α₁-antitrypsin deficiency should receive conventional therapy, with advice on...
smoking cessation, trials of bronchodilators and inhaled corticosteroids. Where appropriate, they should be assessed for long-term oxygen therapy and single lung transplantation. The role of lung volume reduction surgery in this group is unclear.

Other conformational diseases

Conformational diseases arise when a constituent protein undergoes a change in shape or size with resultant self-aggregation and tissue deposition (for review, see Ref 56). Huntington's disease and spinocerebellar ataxia are caused by mutations that result in proteins with multiple glutamine repeats that are able to form intermolecular β-strand linkages57. Alzheimer's disease is associated with the cleavage of a neurotoxic peptide from the amyloid precursor protein58 and the formation of fibrils that are deposited in the tissues as amyloid plaques. These plaques, like all amyloid plaques, have marked β-strand linkage. Similarly, it is proposed that Creutzfeldt-Jakob disease (CJD) results from transition from a helical structure to a primarily β-sheet structure in the prion protein59. This can occur spontaneously in association with point mutations to give familial CJD, or may be propagated in normal prion proteins following the introduction of abnormally transformed prions. These may be introduced following inoculation by infected pituitary extracts or by bovine spongiform encephalopathy which is believed to be able to cross the species barrier (variant CJD). The common feature of all these conditions is a protein that undergoes a conformational switch to a new species that can form intermolecular β-strand linkages and be deposited in cells or tissues. All these conformational diseases lack the structural data currently available for serpin polymerisation which results from a β-strand linkage. Thus, serpin polymerisation offers a useful paradigm for the development of β-strand blockers to prevent the protein-protein linkage and so attenuate the associated disease. The lessons that have been learnt from the serpins may be applicable to the other conformational diseases60 that currently provide such an enormous challenge to medical science.

References

1. Kiernan V. Warm hearts in a cold land. New Scientist 4 March 1995:10.
2. Kuzemko JA. Chopin's illnesses. J R Soc Med 1994;87:769–72.
3. Kuba AK, Young M. The long suffering of Frederic Chopin. Chest 1997;113:210–6.
4. Laurell C-B, Eriksson S. The electrophoretic α₂-globulin pattern of serum in α₁-antitrypsin deficiency. Scand J Clin Lab Invest 1963;15:132–40.
5. Beatty K, Bieth J, Travis J. Kinetics of association of serine proteinases with native and oxidized α₁-proteinase inhibitor and α₁-antichymotrypsin. J Biol Chem 1980;255:3931–4.
6. Carrell RW, Jeppsson J-O, Laurell C-B, Brennan SO, et al. Structure and variation of human α₁-antitrypsin. Nature 1982;298:329–34.
7. Eriksson S. Studies in α₁-antitrypsin deficiency. Acta Med Scand 1965;432(Suppl):1–85.
8. Mahadeva R, Lomas DA. Alpha-1-antitrypsin deficiency, cirrhosis and emphysema. Thorax 1998;53:501–5.
9. Col P, Pappas J, Moran D, Lieberman J. Variants of α₁-antitrypsin in Puerto Rican children with asthma. Chest 1993;103:812–5.
10. King MA, Stone JA, Diaz FT, Mueller CF, et al. α₁-antitrypsin deficiency: evaluation of bronchiectasis with CT. Radiology 1996;199:137–41.
11. Griffith ME, Lovegrove JU, Gaskin G, Whitehouse DB, Pusey CD. C-antineutrophil cytoplasmic antibody positivity in vasculitis patients is associated with the Z allele of alpha-1-antitrypsin, and P-antineutrophil cytoplasmic antibody positivity with the S allele. Nephrol Dial Transplant 1996;11:438–43.
12. Brantly M, Nukina T, Crystal RG. Molecular basis of alpha-1-antitrypsin deficiency. Am J Med 1998;84(Suppl 6A):13–31.
13. Owen MC, Carrell RW, Brennan SO. The abnormality of the S variant of human α₁-antitrypsin. Biochim Biophys Acta 1976;453:257–61.
14. Jeppsson J-O. Amino acid substitution Glu→Lys in α₁-antitrypsin Piz. FEBS Lett 1976;65:195–7.
15. Yoshida A, Lieberman J, Gadulis L, Ewing C. Molecular abnormality of human alpha-1-antitrypsin variant (Pi ZZ) associated with plasma activity deficiency. Proc Natl Acad Sci USA 1976;73:1324–8.
16. Sverger T. Liver disease in alpha-1-antitrypsin deficiency detected by screening of 200,000 infants. N Engl J Med 1976;294:1316–21.
17. Sverger T. The natural history of liver disease in α₁-antitrypsin deficient children. Acta Paediatr Scand 1986;77:847–51.
18. Eriksson S, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha-1-antitrypsin deficiency. N Engl J Med 1986;314:736–9.
19. Potempa J, Korzus E, Travis J. The serpin superfAMILY of proteinase inhibitors: structure, function, and regulation. J Biol Chem 1994;269:15957–60.
20. Huber R, Carrell RW. Implications of the three-dimensional structure of α₁-antitrypsin for structure and function of serpins. Biochemistry 1992;28:9515–66.
21. Whitstock JC, Skinner R, Lesk AM. An atlas of serpin conformations. Trends Biochem Sci 1998;23:63–7.
22. Johnson D, Travis J. Structural evidence for methionine at the reactive site of human α₁-proteinase inhibitor. J Biol Chem 1978;253:7142–4.
23. Stratigos E, Gettins PGW. Major proteinase movement upon stable proteinase-complex formation. Proc Natl Acad Sci USA 1997;4:453–8.
24. Wilczynska M, Fa M, Karolín J, Ohlsson P-J, et al. Structural insights into serpin-protease complexes reveal the inhibitory mechanism of serpins. Nat Struct Biol 1997;4:354–7.
25. Mast AE, Englund JJ, Pizzo SV, Salvesen G. Analysis of the plasma eleve reaction kinetics and conformational stabilities of native, proteinase-complexed, and reactive site cleaved serpins: comparison of α₁-proteinase inhibitor, α₂-antichymotrypsin, antithrombin III, α₂-antiplasmin, angiotensinogen, and ovalbumin. Biochemistry 1991;30:1723–30.
26. Lomas DA, Evans DL, Stone SR, Chang W-SW, Carrell RW. Effect of the Z mutation on the physical and inhibitory properties of α₁-antitrypsin. Biochemistry 1993;32:500–8.
27. Elliott PR, Lomas DA, Carrell RW, Abrahams J-P. Inhibitory conformation of the reactive loop of α₁-antitrypsin. Nat Struct Biol 1996;3:676–81.
28. Elliott PR, Abrahams J-P, Lomas DA. Wild-type α₁-antitrypsin is in the canonical inhibitory conformation. Review. J Mol Biol 1998;275:419–25.
29. Skinner R, Chang W-SW, Jin L, Pei X, et al. Implications for function and therapy of a 2.9 A structure of binary-complexed antithrombin. J Mol Biol 1998;283:9–14.
30. Seyama K, Nukiwa T, Souma S, Shimizu K, Kina S. α₁-antitrypsin-deficient variant Siiyama (Ser[5][TCC] to Pro[5][TTC]) is prevalent in Japan. Status of α₁-antitrypsin deficiency in Japan. Am Rev Respir Dis 1995;152:2119–26.
31. Roberta EA, Cox DW, Medline A, Wanless IR. Occurrence of alpha-1-antitrypsin deficiency in 155 patients with alcoholic liver disease. Am J Clin Pathol 1984;82:424–7.
Lomas DA, Finch JT, Seyama K, Nukiwa T, Carrell RW. Alpha 1-antitrypsin Siyama (Ser53→Phe). Further evidence for intracellular loop-sheet polymerisation. J Biol Chem 1993;268:15333–5.

Lomas DA, Elliott PR, Siddar SK, Foreman RC, et al. Alpha 1-antitrypsin Mmalton (53Phe deleted) forms loop-sheet polymers in vitro: evidence for the C sheet mechanism of polymerisation. J Biol Chem 1995;270:16864–70.

Dafforn TR, Mahadeva R, Elliott PR, Sivasothy P, Lomas DA. A kinetic description of the polymerisation of α1-antitrypsin. J Biol Chem 1999;274:9548–55.

Wu Y, Whitman I, Molmenti E, Moore K, et al. A lag in intracellular degradation of mutant α1-antitrypsin correlates with liver disease phenotype in homozygous PiZZ α1-antitrypsin deficiency. Proc Natl Acad Sci USA 1994;91:9014–8.

Teckman JH, Perlmuter DF. The endoplasmic reticulum degradation pathway for mutant secretory proteins α1-antitrypsin Z and S is distinct from that for an unassembled membrane protein. J Biol Chem 1996;271:13215–20.

Sveger A, Elliott PR, Stein PE, Bilston D, Carrell RW, Lomas DA. Structural explanation for the dysfunction of S α1-antitrypsin. Nat Struct Biol 1996;3:910–1.

Mahadeva R, Chang W-SW, Dafforn T, Oakley DJ, et al. Heteropolymerisation of S, I and Z α1-antitrypsin and liver cirrhosis. J Clin Invest 1999;103:999–1006.

Pituilainen E, Eriksson S. Decline in FEV1 related to smoking status in individuals with severe α1-antitrypsin deficiency. Eur Respir J 1999;13:247–51.

Larsson C. Natural history and life expectancy in severe α1-antitrypsin deficiency. PIZ. Acta Med Scand 1978;204:345–51.

Elliott PR, Bilton D, Lomas DA. Lung polymers in Z α1-antitrypsin related emphysema. Am J Respir Cell Mol Biol 1998;18:670–4.

Aulak KS, Eldering E, Hack CE, Lubbers YPT, et al. A hinge region mutation in C1-inhibitor (Ala336→Thr) results in nonsubstrate-like behavior and in polymerization of the molecule. J Biol Chem 1993;268:18088–94.

Eldering E, Verpy E, Roem D, Meo T, Tosi M. COOH-terminal substitutions in the serpin C1 inhibitor that cause loop overinsertion and subsequent multimerization. J Biol Chem 1995;270:2579–87.

Bruce D, Perry DJ, Borg J-Y, Carrell RW, Wardell MR. Thromboembolic disease due to thermalabile conformational changes of antithrombin Rouen-V1 (187 AsnAsp). J Clin Invest 1994;94:2265–74.

Lindo VS, Kakkar VV, Learmonth M, Melissari E, et al. Antithrombin-TRI (Ala352 to Thr) causing severe thromboembolic tendency undergoes the S-to-T transition and is associated with a plasma-inactive high-molecular-weight complex of aggregated antithrombin. Br J Haematol 1995;99:589–601.

Poller W, Faber J-P, Weidinger S, Tief K, et al. A leucine-to-proline substitution causes a defective α1-antichymotrypsin allele associated with familial obstructive lung disease. Genomics 1993;17:740–3.

Faber J-P, Poller W, Olek K, Baumann U, et al. The molecular basis of α1-antichymotrypsin deficiency in a heterozygote with liver and lung disease. J Hepatol 1993;18:831–21.

Gooptu B, Hazes B, Chang W-SW, Dafforn TR, et al. New inactive conformation of the serpin α1-antichymotrypsin indicates two stage insertion of the reactive loop; implications for inhibitory function and conformational disease. Proc Natl Acad Sci USA 2000;97:67–72.

Davis RL, Shrimpton AE, Holohan PD, Braidshaw C, et al. Familial dementia caused by polymerisation of mutant neuroserpin. Nature 1999;401:376–9.

Wewers MD, Casolaro MA, Sellers SE, Swayze SC, et al. Replacement therapy for α1-antitrypsin deficiency associated with emphysema. N Engl J Med 1987;316:1055–62.

The alpha-1-antitrypsin deficiency registry study group. Survival and FEV1 decline in individuals with severe deficiency of α1-antitrypsin. Am J Respir Crit Care Med 1998;158:49–59.

Dirksen A, Dijkman JH, Madsen E, Stoel B, et al. A randomised clinical trial of α1-antitrypsin augmentation therapy. Am J Resp Crit Care Med 1999;160:1468–72.

Massaro GD, Massaro D. Retinoic acid treatment abrogates elastase-induced pulmonary emphysema in rats. Nat Med 1997;3:675–7.

Carrell RW, Lomas DA. Conformational diseases. Lancet 1997;350:134–8.

Perutz MF. Glutamine repeats and inherited neurodegenerative diseases: molecular aspects. Curr Opin Struct Biol 1996;6:848–58.

Davis-Salinas J, Van Nostranl WE. Amyloid β-protein aggregation nullifies its pathogenic properties in cultured cerebrovascular smooth muscle cells. J Biol Chem 1995;270:20887–90.

Prusiner SB. Prion diseases of humans and animals. J Coll Physicians Lond 1994;28:153–70.

Soto C, Sigurdsson EM, Morelli L, Kumar RA, et al. β-sheet breaker peptides inhibit fibrillogenesis in a rat brain model of amyloidosis: implications for Alzheimer’s therapy. Nat Med 1998;4:822–6.

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