REVIEW SERIES

 α_1 -Antitrypsin deficiency · 4: Molecular pathophysiology

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The molecular basis of α_1 -antitrypsin deficiency is reviewed and is shown to be due to the accumulation of mutant protein as ordered polymers within the endoplasmic reticulum of hepatocytes. The current goals are to determine the cellular response to polymeric α_1 -antitrypsin and to develop therapeutic strategies to block polymerisation in vivo.

Alpha₁-antitrypsin (AAT) deficiency was reported in an Alaskan girl who died 800 years ago¹ and may have accounted for the premature death of Frederic Chopin in 1849.^{2–3} It was first described as a clinical entity in 1963 by Laurell and Eriksson who noted an absence of the α_1 band on serum protein electrophoresis.⁴ The major function of AAT is to protect the tissues against the enzyme neutrophil elastase.^{5–6} Its role in protecting the lungs against proteolytic attack is underscored by the association of plasma deficiency with early onset panacinar emphysema.⁷ This finding, together with the observation that the intrapulmonary instillation of elastolytic enzymes also results in emphysema,^{8–11} gave rise to the proteinase-antiproteinase hypothesis of lung disease. In health there is a balance between proteinases and antiproteinases, but when proteinases are in excess, tissue destruction will ensue. The proteinase-antiproteinase hypothesis was developed over 35 years ago and still remains central to our understanding of the pathogenesis of lung disease. We review here the molecular mechanisms that underlie AAT deficiency and show how an understanding of this mechanism has allowed us to explain the deficiency of other members of the serine proteinase inhibitor or serpin superfamily. These include the deficiency of antithrombin, C1 inhibitor, α_1 -antichymotrypsin, and neuroserpin in association with thrombosis, angio-oedema, airflow obstruction, and dementia, respectively. We have grouped these conditions together as the “serpinopathies”.^{12–14} Their common pathophysiology provides a platform for the development of strategies to treat the associated clinical syndromes.

STRUCTURE AND FUNCTION OF α_1 -ANTITRYPSIN (AAT)

AAT is a 394 amino acid, 52 kDa, acute phase glycoprotein encoded on chromosome 14q31–32.1.^{15–17} It is synthesised by hepatocytes^{18–19} and secreted into the plasma at a concentration of 1.9–3.5 mg/ml. It is also synthesised by and secreted from macrophages²⁰ and intestinal²¹ and bronchial epithelial cells.²² The protein was

originally named because of its ability to inhibit pancreatic trypsin.²³ Subsequently it has been found to be an effective inhibitor of a variety of other proteinases including neutrophil elastase,⁵ cathepsin G,⁵ and proteinase 3.²⁴ The broad spectrum of proteinase inhibition gave rise to its alternative name of α_1 -proteinase inhibitor,²⁵ although this too is inaccurate as other proteins in the α_1 band of serum (such as α_1 -antichymotrypsin) are also proteinase inhibitors.

Crystal structures have shown that AAT is composed of three β -sheets (A–C) and an exposed mobile reactive loop (fig 1) that presents a peptide sequence as a pseudosubstrate for the target proteinase.^{26–30} The critical amino acids within this loop are the P1–P1' residues, methionine serine, as these act as a “bait” for neutrophil elastase.³¹ After docking, the enzyme cleaves the P1–P1' peptide bond of AAT³² and the proteinase is inactivated by a mousetrap action (fig 1) that swings it from the upper to the lower pole of the protein in association with the insertion of the reactive loop as an extra strand in β -sheet A.^{33–37} This altered conformation of AAT bound to its target enzyme is then recognised by hepatic receptors and cleared from the circulation.³⁸

The remarkable mouse trap action of AAT is central to its role as an effective inhibitor of serine proteinases. Paradoxically, it is also its Achilles heel as point mutations in these mobile domains make the molecule vulnerable to aberrant conformational transitions such as the one that underlies AAT deficiency.

α_1 -ANTITRYPSIN (AAT) DEFICIENCY

AAT deficiency is the most widely recognised abnormality of a proteinase inhibitor that causes lung disease. Over 70 naturally occurring variants have been described and characterised by their migration on isoelectric focusing gels—the proteinase inhibitor or Pi system.³⁹ The commonest deficiency variants, S and Z, result from point mutations in the AAT gene^{40–42} and are so named as they make the protein migrate more slowly than normal M AAT. Mutations that cause more rapid migration of AAT are labelled A to L.

A recent review of 70 surveys has provided an assessment of the frequency and distribution of the S and Z AAT alleles throughout Europe.⁴³ The greatest frequency of the S allele occurs within the Iberian peninsula and gradually reduces in the direction of south to north and from west to east. S AAT (²⁶⁴glutamic acid→valine) is found in up to 28% of southern Europeans and, although it results in plasma AAT levels that are 60% of the M allele, it is not associated with any pulmonary sequelae. In contrast, the Z allele is most common in northwest Europe with

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Figure 1 α_1 -antitrypsin can be considered to act as a mousetrap.^{26, 37, 138} Following docking (left), the neutrophil elastase (grey) is inactivated by movement from the upper to the lower pole of the protein (right). This is associated with insertion of the reactive loop (red) as an extra strand into β -sheet A (green). Reproduced from Lomas and Carrell¹² with permission.

frequencies declining from west to east and from north to south. The Z variant (³⁴²glutamic acid→lysine) results in a more severe deficiency that is characterised in the homozygote by plasma AAT levels of 10% of the normal M allele and 60% in the MZ heterozygote (50% from the M allele and 10% from the Z allele). The Z mutation results in the accumulation of AAT as inclusions in the rough endoplasmic reticulum of the liver.⁴⁴ These inclusions predispose the homozygote to juvenile hepatitis, cirrhosis,^{45, 46} and hepatocellular carcinoma.⁴⁷ Furthermore, the lack of circulating protein predisposes the homozygote to early onset panlobular emphysema.^{7, 48, 49}

MOLECULAR PATHOLOGY OF THE LIVER DISEASE ASSOCIATED WITH PI Z AAT

There is now overwhelming evidence that the liver disease associated with the Z variant of AAT is due to the accumulation of aggregated protein rather than a plasma deficiency. Strong support is provided by the recognition that null alleles, which produce no AAT, are not associated with cirrhosis.³⁹ Moreover, the overexpression of Z AAT in animal models results in liver damage.^{50, 51} Our understanding of the molecular basis of AAT deficiency came from a recognition that the normal active protein undergoes a profound conformational transition to inhibit its target proteinase, neutrophil elastase (see above). The Z mutation of AAT is at residue P17 (17 residues proximal to the P1 reactive centre) at the head of strand 5 of β -sheet A and the base of the mobile reactive loop (fig 2). The mutation opens β -sheet A, thereby favouring the insertion of the reactive loop of a second AAT molecule to form a dimer.^{26, 52–54} This can then extend to form polymers that tangle in the endoplasmic reticulum of the hepatocyte to form inclusion bodies (fig 3). Support for this came from the demonstration that purified plasma Z AAT formed chains of polymers when incubated under physiological conditions.⁵² The rate of polymer formation was accelerated by raising the temperature to 41°C and could be blocked by peptides that competed for annealing to

β -sheet A.^{52, 55} The role of polymerisation in vivo was confirmed by the finding of AAT polymers in inclusion bodies from the liver of a Z AAT homozygote with cirrhosis,^{52, 56} and in hepatic cell lines expressing the Z variant.⁵⁷ Moreover, point mutations that block polymerisation increased the secretion of mutants of AAT from a *Xenopus* oocyte expression system.⁵⁸

The pathway of AAT polymerisation has been assessed by biochemical, biophysical, and crystallographic analysis and is shown in fig 2.^{33, 59} Step 1 represents the conformational change of AAT to a polymerogenic monomeric form (M^*), step 2 represents the formation of polymers (P), and step 3 represents a side pathway which leads to the formation of the stable monomeric latent conformation (L). The Z mutation causes most of the unstable protein to form polymers. The presence of the unstable polymerising intermediate M^* was predicted from the biophysical analysis of polymer formation,⁵³ the demonstration of an unfolding intermediate,^{60–62} and solving the crystal structure of a polymerogenic mutant of α_1 -antichymotrypsin.⁵⁹ Our latest data suggest that the Z mutation forces AAT into a conformation that approximates the unstable M^* and hence favours polymer formation.⁶³

The quality control mechanisms within the hepatocyte that handle polymers are now being elucidated.^{64–66} Elegant studies have shown that it is the trimming of asparagine linked oligosaccharides that target Z AAT polymers into an efficient non-proteosomal disposal pathway within hepatocytes. However, the proteosome has an important role in metabolising Z AAT in some hepatic⁶⁷ and extrahepatic^{68, 69} mammalian cell lines. Moreover, there is increasing evidence that the retained Z AAT stimulates an autophagic response within the hepatocyte.^{70, 71} Despite our increased understanding of the disposal pathway, it still remains unclear how the accumulation of Z AAT causes cell death and liver cirrhosis.

The temperature and concentration dependence of polymerisation,^{52, 53} together with genetic factors,^{72, 73} may account for the heterogeneity in liver disease among individuals who are homozygous for the Z mutation. The synthesis of AAT rises during episodes of inflammation as part of the acute phase response. 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. This hypothesis has been challenged by cell studies which do not show an increase in intracellular Z AAT in response to raised temperatures.⁷⁴ However, our recent data in a *Drosophila* model of AAT deficiency show a clear temperature dependence of polymerisation in vivo.⁷⁵ There is also anecdotal clinical evidence to support the role of temperature in exacerbating the liver disease associated with Z AAT from the prospective study of Sveger and colleagues in Sweden.^{45, 46} They screened 200 000 newborn babies and identified 120 Z homozygotes whom they have followed into late adolescence. Two of these patients developed progressive jaundice during the course of the study; in one this followed an acute appendicitis and in the other severe pneumonia. Other asymptomatic infants developed marked derangement of liver function tests in association with corneal illnesses and eczema. Further prospective studies are required to assess whether pyrexial episodes occur more frequently and increase the burden of intrahepatic polymers in Z AAT homozygotes who develop liver disease compared with those individuals who remain asymptomatic.

Although many AAT deficiency variants have been described, only two mutants (other than the Z allele) have similarly been associated with plasma deficiency and hepatic inclusions: AAT Siyama (³³serine→phenylalanine) which is the commonest cause of AAT deficiency in Japan,^{76, 77} and Mmalton (also known as Mnichinan⁷⁸ and Mcagliari,⁷⁹

Figure 2 The structure of α_1 -antitrypsin (AAT) is centred on β -sheet A (green) and the mobile reactive centre loop (red). Polymer formation results from the Z variant of AAT (Glu342Lys at P17, arrowed) or the Siiyama, Mmalton, S or I mutations in the shutter domain (blue circle) that open β -sheet A to favour partial loop insertion (step 1) and the formation of an unstable intermediate (M^*).^{59–63} The patent β -sheet A can either accept the loop of another molecule (step 2) to form a dimer (D) which then extends into polymers (P)^{26–52–54} or its own loop (step 3) to form a latent conformation (L).^{139–140} The individual molecules of AAT within the polymer are coloured red, yellow and blue. Reproduced from Goopu *et al*⁵⁹ with permission.

deletion of phenylalanine at position 52) which is the most common cause of AAT deficiency in Sardinia. Both of these mutants destabilise and open β -sheet A (fig 2) to allow the formation of folding intermediates^{61–62} and loop-sheet polymers *in vivo*.^{80–81} Polymerisation also underlies the mild

plasma deficiency of the S (Glu264Val) and I (Arg39Cys) variants of AAT.^{82–83} The point mutations that are responsible for these variants cause less disruption to β -sheet A than does the Z variant. Thus, the rates of polymer formation are much slower than that of Z AAT⁵³ and this results in less retention

Figure 3 Z α_1 -antitrypsin (AAT) is retained within hepatocytes as intracellular inclusions. (A) These inclusions are PAS positive and diastase resistant (arrow) and are associated with neonatal hepatitis and hepatocellular carcinoma. (B) Electron micrograph of a hepatocyte from the liver of a patient with Z AAT deficiency shows the accumulation of AAT within the rough endoplasmic reticulum. These inclusions are composed of chains of AAT polymers shown here from the plasma of a Siiyama AAT homozygote (C) and from the liver of a Z AAT homozygote (F). Similar mutations in AAT and neuroserpin result in similar intracellular inclusions of AAT and neuroserpin shown here in (A) hepatocytes and (D) neurones with PAS staining, and as endoplasmic aggregates of the abnormal proteins on electron microscopy (B and E, respectively). Electron microscopy confirms that the abnormal neuroserpin forms bead-like polymers and entangled polymeric aggregates identical to those shown here with Z AAT (C and F, respectively). Magnification left to right: $\times 200$, $\times 20\,000$, $\times 220\,000$. Reproduced from Carrell and Lomas⁵⁴ with permission.

of protein within hepatocytes, milder plasma deficiency, and the lack of a clinical phenotype. However, if a mild slowly polymerising I or S variant of AAT is inherited with a rapidly polymerising Z variant, the two can interact to form heteropolymers within hepatocytes leading to inclusions and finally cirrhosis.^{83–85}

MOLECULAR PATHOLOGY OF THE LUNG DISEASE ASSOCIATED WITH PI Z AAT

The single most important factor in the development of emphysema in patients with AAT deficiency is smoking.^{49–86} The combination of antiproteinase deficiency and cigarette smoke can have a devastating effect on lung function,^{48–87} probably by allowing the unopposed action of proteolytic enzymes. AAT levels are greatly reduced in the lungs of individuals with AAT deficiency.⁸⁸ Moreover, the AAT that is available to protect the lungs is approximately five times less effective at inhibiting neutrophil elastase than normal M AAT.^{55–89–91} The inhibitory activity of Z AAT can be further reduced as AAT is susceptible to inactivation by oxidation of the P1 methionine residue by free radicals from leucocytes or direct oxidation by cigarette smoke.^{5–6–92–93} Finally, the Z mutation favours the spontaneous formation of AAT loop-sheet polymers within the lung.⁹⁴ This conformational transition inactivates AAT as a proteinase inhibitor, thereby further reducing the already depleted levels of AAT that are available to protect the alveoli (fig 4). The mechanisms that drive the formation of Z AAT polymers within the lung are unknown. It is possible that polymerisation may be accelerated by the inflammatory milieu that exists within the lungs of individuals with Z AAT deficiency. Moreover, cigarette smoke is mildly acidic and previous studies have shown that polymerisation of AAT is accelerated at low pH.⁵³ Thus, cigarette smoke may act in several ways to promote the inactivation of Z AAT in vivo.

Patients with Z AAT deficiency have an excess number of neutrophils in bronchoalveolar lavage fluid⁹⁵ and in tissue sections of lung parenchyma¹³ compared with controls. This may reflect an excess of chemoattractant agents such as leukotriene B4 (LTB4) and interleukin (IL)-8.^{96–97} However, recent studies have shown that the polymers are themselves chemotactic for human neutrophils in vitro.⁹⁸ The magnitude of this effect was similar to the chemoattractant C5a and was present over a range of physiological concentrations (EC₅₀ 4.5 (2) µg/ml). Polymers also induced neutrophil shape change and stimulated myeloperoxidase release and neutrophil adhesion.⁹⁸ It is possible that polymers of Z AAT form in vivo and then act as a chronic chemoattractant to cause an influx of inflammatory cells.¹³ They may evade the defence mechanisms of the lung by adhering to the interstitium. Any proinflammatory effect of polymers is likely to be exacerbated

by inflammatory cytokines, cleaved or complexed AAT,⁹⁹ elastin degradation products,¹⁰⁰ and cigarette smoke which themselves cause neutrophil recruitment. Our understanding of the biological properties of AAT thus provides novel pathways for the pathogenesis of emphysema in individuals who are homozygous for the Z mutation (fig 4). Indeed, the presence of polymers may explain the progression of lung disease in Z AAT homozygotes after smoking cessation despite adequate intravenous replacement with plasma AAT. The relationship between intrapulmonary Z AAT polymers and smoking, infection, cytokine production, and rate of decline in lung function requires assessment in both cell and animal models of disease, and prospective studies in Z homozygotes

MOLECULAR PATHOLOGY OF OTHER CONDITIONS ASSOCIATED WITH PI Z AAT

Pi Z AAT deficiency is reported with panniculitis that is characterised by an acute inflammatory infiltrate of the skin and fat necrosis.^{101–102} There is also an association of the Z allele of AAT with asthma,^{103–104} vasculitis,^{105–106} bronchiectasis,¹⁰⁷ pancreatitis,¹⁰⁸ and vascular aneurysms,^{109–110} although the association with bronchiectasis and vascular disease have been disputed by other studies.^{108–111} The feature that links many of these conditions is neutrophil mediated inflammation, and it is possible that AAT polymers are one of the factors that drive this inflammation and disease progression.⁹⁸

DISEASE CAUSED BY THE POLYMERISATION OF OTHER SERPINS

AAT is the archetypal member of the serine proteinase inhibitor or serpin superfamily. This family includes members such as α_1 -antichymotrypsin, C1 inhibitor, antithrombin, and plasminogen activator inhibitor-1 which have an important role in the control of proteinases involved in the inflammatory, complement, coagulation, and fibrinolytic cascades, respectively.^{25–112} The family is characterised by more than 30% sequence homology with AAT and conservation of tertiary structure.^{15–113} Consequently, physiological and pathological processes that affect one member may be extrapolated to another. The phenomenon of loop-sheet polymerisation is not restricted to AAT and has now been reported in mutants of other members of the serpin superfamily to cause disease (the serpinopathies). Mutants of C1 inhibitor, antithrombin and α_1 -antichymotrypsin can also destabilise the protein architecture to form inactive polymers that are associated with plasma deficiency and angio-oedema, thrombosis, and chronic obstructive pulmonary disease, respectively.^{59–114–120}

The process is most strikingly displayed by the inclusion body dementia, familial encephalopathy with neuroserpin inclusion bodies (FENIB).¹²¹ We have shown that this dementia is caused by mutations in neuroserpin that are homologous to those causing liver cirrhosis in AAT deficiency.¹²¹ Moreover, both the liver cirrhosis and the neurodegenerative disease have an identical pattern of intracellular polymerisation and inclusion body formation (fig 3). Further kindreds with polymerogenic neuroserpin mutations have been described and it is becoming clear that there is a direct relationship between the magnitude of intracellular accumulation of neuroserpin and the severity of the clinical syndrome.¹²² Moreover, our recent work has shown that one of the neuroserpin mutants that causes FENIB (Ser49Pro) polymerises up to 13-fold faster than wild type protein.¹²³ This provides strong support for the role of aberrant neuroserpin polymerisation in the pathogenesis of FENIB.

Figure 4 Proposed model for the pathogenesis of emphysema in patients with Z α_1 -antitrypsin (AAT) deficiency. The plasma deficiency and reduced inhibitory activity of Z AAT may be exacerbated by the polymerisation of AAT within the lungs. These processes inactivate the inhibitor, thereby further reducing the antiproteinase screen. AAT polymers may also act as a proinflammatory stimulus to attract and activate neutrophils, thereby increasing tissue damage. Reproduced from Lomas and Mahadeva¹³ with permission.

PREVENTION OF POLYMER FORMATION

There is substantial evidence that polymers of AAT and, indeed, of all other serpins form by an aberrant linkage between the reactive centre loop of one molecule and β -sheet A of another.^{26 52 54 124–127} This has allowed the development of new strategies to attenuate polymerisation and so treat the associated disease. We have shown previously that the polymerisation of Z AAT can be blocked by annealing of reactive loop peptides to β -sheet A.^{52 128} Such peptides were 11–13 residues in length and could bind to other members of the serpin superfamily.^{128 129} This was most clearly demonstrated by the finding that the reactive loop peptide of antithrombin inserted more readily to β -sheet A of AAT and vice versa.¹³⁰ These peptides, although useful in establishing the mechanism of polymerisation, are too long and too promiscuous to be suitable for rational drug design. More recently we have designed a 6-mer peptide that specifically anneals to Z AAT alone and blocks polymerisation.⁶³ Indeed, trimer peptides have been developed that will also anneal to a patent β -sheet A of antithrombin in vitro.¹³¹ The aim now is to convert these peptides into small drugs that can be used in vivo.

A second strategy comes from the identification of a hydrophobic pocket in AAT that is bounded by strand 2A and helices D and E.^{29 132} The cavity is patent in the native protein but is filled as β -sheet A accepts an exogenous reactive loop peptide during polymerisation.²⁹ We have shown that introducing mutations into this pocket retards the polymerisation of M AAT and increases the secretion of Z AAT from a *Xenopus* oocyte expression system.¹³³ This cavity is therefore an ideal target for the development of drugs that will stabilise β -sheet A and so ameliorate polymer formation.

An alternative strategy is to use chemical chaperones to stabilise intermediates on the folding pathway. Osmolytes such as betaine, trimethylamine oxide, and sarcosine all stabilise AAT against polymer formation.¹³⁴ The chaperone trimethylamine oxide had no effect on the secretion of Z AAT in cell culture⁷⁴ as it favoured the conversion of unfolded Z AAT to polymers.¹³⁵ In contrast, glycerol increased the secretion of Z AAT from cell lines⁷⁴ most likely as it binds to and stabilises β -sheet A.¹³¹ 4-phenylbutyrate (4-PBA) also increased the secretion of Z AAT from cell lines and transgenic mice.⁷⁴ This agent has been used for several years to treat children with urea cycle disorders and, more recently, 4-PBA has been shown to increase the expression of mutant (Δ F508) cystic fibrosis transmembrane regulator protein in vitro¹³⁶ and in vivo.¹³⁷ These encouraging findings have led to a pilot study that is currently ongoing to evaluate the potential of 4-PBA to promote the secretion of AAT in patients with AAT deficiency.

CONCLUSION

The molecular basis of Z AAT deficiency has now been elucidated with biochemical, cellular, and structural studies. The current goals are to determine the cellular response to polymeric AAT and to develop therapeutic strategies to block polymerisation in vivo.

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REFERENCES

- Kiernan V. Warm hearts in a cold land. *New Scientist* 1995;4 March:10.
- Kuzemko JA. Chopin's illnesses. *J R Soc Med* 1994;87:769–72.
- Kubba AK, Young M. The long suffering of Frederic Chopin. *Chest* 1997;113:210–6.
- Laurell C-B, Eriksson S. The electrophoretic α_1 -globulin pattern of serum in α_1 -antitrypsin deficiency. *Scand J Clin Lab Invest* 1963;15:132–40.
- Beatty K, Bieth J, Travis J. Kinetics of association of serine proteinases with native and oxidized α_1 -proteinase inhibitor and α_1 -antitrypsin. *J Biol Chem* 1980;255:3931–4.
- Carrell RW, Jeppsson J-O, Laurell C-B, et al. Structure and variation of human α_1 -antitrypsin. *Nature* 1982;298:329–34.
- Eriksson S. Studies in α_1 -antitrypsin deficiency. *Acta Med Scand* 1965;432(Suppl):1–85.
- Gross P, Pfitzer EA, Tolker E, et al. Experimental emphysema. Its production with papain in normal and silicotic rats. *Arch Environ Health* 1965;11:50–8.
- Senior RM, Tegner H, Kuhn C, et al. The induction of pulmonary emphysema with human leukocyte elastase. *Am Rev Respir Dis* 1977;116:469–75.
- Janoff A, Sloan B, Weinbaum G, et al. Experimental emphysema induced with purified human neutrophil elastase: Tissue localization of the instilled protease. *Am Rev Respir Dis* 1977;115:461–78.
- Snider GL, Lucey EC, Christensen TG, et al. Emphysema and bronchial secretory cell metaplasia induced in hamsters by human neutrophil products. *Am Rev Respir Dis* 1984;129:155–60.
- Lomas DA, Carrell RW. Serpinopathies and the conformational dementias. *Nature Reviews Genetics* 2002;3:759–68.
- Lomas DA, Mahadeva R. Alpha-1-antitrypsin polymerisation and the serpinopathies: pathobiology and prospects for therapy. *J Clin Invest* 2002;110:1585–90.
- Carrell RW, Lomas DA. Alpha-1-antitrypsin deficiency: a model for conformational diseases. *N Engl J Med* 2002;346:45–53.
- Huber R, Carrell RW. Implications of the three-dimensional structure of α_1 -antitrypsin for structure and function of serpins. *Biochemistry* 1989;28:8951–66.
- Aronsen KF, Ekelund G, Kindmark CO, et al. Sequential changes of plasma proteins after surgical trauma. *Scand J Clin Lab Invest* 1972;29(Suppl 124):127–36.
- Billingsley GD, Walter MA, Hammond GL, et al. Physical mapping of four serpin genes: α_1 -antitrypsin, α_1 -antichymotrypsin, corticosteroid-binding globulin, and protein C inhibitor, within a 280 kb region on chromosome 14q31.1. *Am J Hum Genet* 1993;52:343–53.
- Koj A, Regöczy E, Toews CJ, et al. Synthesis of antithrombin III and alpha-1-antitrypsin by the perfused rat liver. *Biochim Biophys Acta* 1978;539:496–504.
- Eriksson S, Alm R, Åstedt B. Organ cultures of human fetal hepatocytes in the study of extra- and intracellular α_1 -antitrypsin. *Biochim Biophys Acta* 1978;542:496–505.
- Mornex JF, Chytil-Weir A, Martinet Y, et al. Expression of the alpha-1-antitrypsin gene in mononuclear phagocytes of normal and alpha-1-antitrypsin-deficient individuals. *J Clin Invest* 1986;77:1952–61.
- Perlmutter DH, Daniels JD, Auerbach HS, et al. The α_1 -antitrypsin gene is expressed in a human intestinal epithelial cell line. *J Biol Chem* 1989;264:9485–90.
- Cichy J, Potempa J, Travis J. Biosynthesis of α_1 -proteinase inhibitor by human lung-derived epithelial cells. *J Biol Chem* 1997;272:8250–5.
- Schultze HE, Heide K, Haupt H. Alpha-1-antitrypsin aus humanserum. *Klin Wchschr* 1962;40:427–9.
- Rao NV, Wehner NG, Marshall BC, et al. Characterization of proteinase-3 (PR-3), a neutrophil serine proteinase. Structure and functional properties. *J Biol Chem* 1991;266:9540–8.
- Potempa J, Korzus E, Travis J. The serpin superfamily of proteinase inhibitors: structure, function, and regulation. *J Biol Chem* 1994;269:15957–60.
- Elliott PR, Lomas DA, Carrell RW, et al. Inhibitory conformation of the reactive loop of α_1 -antitrypsin. *Nat Struct Biol* 1996;3:676–81.
- Ryu S-E, Choi H-J, Kwon K-S, et al. The native strains in the hydrophobic core and flexible reactive loop of a serine protease inhibitor: crystal structure of an uncleaved α_1 -antitrypsin at 2.7 Å. *Structure* 1996;4:1181–92.
- Elliott PR, Abrahams J-P, Lomas DA. Wildtype α_1 -antitrypsin is in the canonical inhibitory conformation. *J Mol Biol* 1998;275:419–25.
- Elliott PR, Pei XY, Dafforn TR, et al. Topography of a 2.0 Å structure of α_1 -antitrypsin reveals targets for rational drug design to prevent conformational disease. *Protein Sci* 2000;9:1274–81.
- Kim S-J, Woo J-R, Seo EJ, et al. A 2.1 Å resolution structure of an uncleaved α_1 -antitrypsin shows variability of the reactive centre and other loops. *J Mol Biol* 2001;306:109–19.
- Johnson D, Travis J. Structural evidence for methionine at the reactive site of human α_1 -proteinase inhibitor. *J Biol Chem* 1978;253:7142–4.
- Wilczynska M, Fa M, Ohlsson P-I, et al. The inhibition mechanism of serpins. Evidence that the mobile reactive centre loop is cleaved in the native protease-inhibitor complex. *J Biol Chem* 1995;270:29652–5.
- Wilczynska M, Fa M, Karlin J, et al. Structural insights into serpin-protease complexes reveal the inhibitory mechanism of serpins. *Nat Struct Biol* 1997;4:354–7.
- Stratikos E, Gettins PGW. Major proteinase movement upon stable serpin-proteinase complex formation. *Proc Natl Acad Sci USA* 1997;4:453–8.
- Stratikos E, Gettins PGW. Mapping the serpin-proteinase complex using single cysteine variants of α_1 -antitrypsin inhibitor Pittsburgh. *J Biol Chem* 1998;273:15582–9.

- 36 **Stratikos E**, Gettins PGW. Formation of the covalent serpin-proteinase complex involves translocation of the proteinase by more than 70Å and full insertion of the reactive centre loop into β -sheet A. *Proc Natl Acad Sci USA* 1999;**96**:4808–13.
- 37 **Huntington JA**, Read RJ, Carrell RW. Structure of a serpin-protease complex shows inhibition by deformation. *Nature* 2000;**407**:923–6.
- 38 **Mast AE**, Enghild JJ, Pizzo SV, et al. Analysis of the plasma elimination kinetics and conformational stabilities of native, proteinase-complexed, and reactive site cleaved serpins: comparison of α_1 -proteinase inhibitor, α_1 -antichymotrypsin, antithrombin III, α_2 -antiplasmin, angiotensinogen, and ovalbumin. *Biochemistry* 1991;**30**:1723–30.
- 39 **Brantly M**, Nukiwa T, Crystal RG. Molecular basis of alpha-1-antitrypsin deficiency. *Am J Med* 1988;**84**(Suppl 6A):13–31.
- 40 **Owen MC**, Carrell RW, Brennan SO. The abnormality of the S variant of human α_1 -antitrypsin. *Biochim Biophys Acta* 1976;**453**:257–61.
- 41 **Jeppsson J-O**. Amino acid substitution Glu $\rightarrow$ Lys in α_1 -antitrypsin PiZ. *FEBS Lett* 1976;**65**:195–7.
- 42 **Yoshida A**, Lieberman J, Gaidulis L, et al. Molecular abnormality of human alpha₁-antitrypsin variant (Pi-ZZ) associated with plasma activity deficiency. *Proc Natl Acad Sci USA* 1976;**73**:1324–8.
- 43 **Blanco I**, Fernández E, Bustillo EF. Alpha-1-antitrypsin PI phenotypes S and Z in Europe: an analysis of the published surveys. *Clin Genet* 2001;**60**:31–41.
- 44 **Sharp HL**, Bridges RA, Krivit W, et al. Cirrhosis associated with alpha-1-antitrypsin deficiency: a previously unrecognised inherited disorder. *J Lab Clin Med* 1969;**73**:934–9.
- 45 **Sveger T**. Liver disease in alpha₁-antitrypsin deficiency detected by screening of 200,000 infants. *N Engl J Med* 1976;**294**:1316–21.
- 46 **Sveger T**. The natural history of liver disease in α_1 -antitrypsin deficient children. *Acta Paediatr Scand* 1988;**77**:847–51.
- 47 **Eriksson S**, Carlson J, Velez R. Risk of cirrhosis and primary liver cancer in alpha₁-antitrypsin deficiency. *N Engl J Med* 1986;**314**:736–9.
- 48 **Larsson C**. Natural history and life expectancy in severe alpha₁-antitrypsin deficiency, PiZ. *Acta Med Scand* 1978;**204**:345–51.
- 49 **Piitulainen E**, Eriksson S. Decline in FEV1 related to smoking status in individuals with severe alpha₁-antitrypsin deficiency. *Eur Respir J* 1999;**13**:247–51.
- 50 **Dycaico MJ**, Grant SGN, Felts K, et al. Neonatal hepatitis induced by α_1 -antitrypsin: a transgenic mouse model. *Science* 1988;**242**:1409–12.
- 51 **Carlson JA**, Barton Rogers B, Sifers RN, et al. Accumulation of PiZ α_1 -antitrypsin causes liver damage in transgenic mice. *J Clin Invest* 1989;**83**:1183–90.
- 52 **Lomas DA**, Evans DL, Finch JT, et al. The mechanism of Z α_1 -antitrypsin accumulation in the liver. *Nature* 1992;**357**:605–7.
- 53 **Dafforn TR**, Mahadeva R, Elliott PR, et al. A kinetic description of the polymerisation of α_1 -antitrypsin. *J Biol Chem* 1999;**274**:9548–55.
- 54 **Sivasothy P**, Dafforn TR, Gettins PGW, et al. Pathogenic α_1 -antitrypsin polymers are formed by reactive loop- β -sheet A linkage. *J Biol Chem* 2000;**275**:33663–8.
- 55 **Lomas DA**, Evans DL, Stone SR, et al. Effect of the Z mutation on the physical and inhibitory properties of α_1 -antitrypsin. *Biochemistry* 1993;**32**:500–8.
- 56 **Janciauskiene S**, Dominaitiene R, Sternby NH, et al. Detection of circulating and endothelial cell polymers of Z and wildtype alpha-1-antitrypsin by a monoclonal antibody. *J Biol Chem* 2002;**277**:26540–6.
- 57 **Le A**, Ferrell GA, Dishon DS, et al. Soluble aggregates of the human PiZ α_1 -antitrypsin variant are degraded within the endoplasmic reticulum by a mechanism sensitive to inhibitors of protein synthesis. *J Biol Chem* 1992;**267**:1072–80.
- 58 **Sidhar SK**, Lomas DA, Carrell RW, et al. Mutations which impede loop/sheet polymerisation enhance the secretion of human α_1 -antitrypsin deficiency variants. *J Biol Chem* 1995;**270**:8393–6.
- 59 **Goopu B**, Hazes B, Chang W-SW, et al. Inactive conformation of the serpin α_1 -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.
- 60 **Yu M-H**, Lee KN, Kim J. The Z type variation of human α_1 -antitrypsin causes a protein folding defect. *Nat Struct Biol* 1995;**2**:363–7.
- 61 **Kim J**, Lee KN, Yi G-S, et al. A thermostable mutation located at the hydrophobic core of α_1 -antitrypsin suppresses the folding defect of the Z-type variant. *J Biol Chem* 1995;**270**:8597–601.
- 62 **Kang HA**, Lee KN, Yu M-H. Folding and stability of the Z and Siiyama genetic variants of human α_1 -antitrypsin. *J Biol Chem* 1997;**272**:510–6.
- 63 **Mahadeva R**, Dafforn TR, Carrell RW, et al. Six-mer peptide selectively anneals to a pathogenic serpin conformation and blocks polymerisation: implications for the prevention of Z α_1 -antitrypsin related cirrhosis. *J Biol Chem* 2002;**277**:6771–4.
- 64 **Cabral CM**, Choudhury P, Liu Y, et al. Processing by endoplasmic reticulum mannosidases partitions a secretion-impaired glycoprotein into distinct disposal pathways. *J Biol Chem* 2000;**275**:25015–22.
- 65 **Cabral CM**, Liu Y, Sifers RN. Dissecting the glycoprotein quality control in the secretory pathway. *TIBS* 2001;**26**:619–23.
- 66 **Cabral CM**, Liu Y, Moremen KW, et al. Organizational diversity among distinct glycoprotein ER-associated degradation programs. *Mol Biol Cell* 2002;**13**:2639–50.
- 67 **Teckman JH**, Burrows J, Hidvegi T, et al. The proteasome participates in degradation of mutant α_1 -antitrypsin Z in the endoplasmic reticulum of hepatoma-derived hepatocytes. *J Biol Chem* 2001;**276**:44865–72.
- 68 **Qu D**, Teckman JH, Omura S, et al. Degradation of a mutant secretory protein, α_1 -antitrypsin Z, in the endoplasmic reticulum requires proteasome activity. *J Biol Chem* 1996;**271**:22791–5.
- 69 **Novorodovskaya N**, Lee J, Yu Z-X, et al. Inhibition of intracellular degradation increases secretion of a mutant form of α_1 -antitrypsin associated with profound deficiency. *J Clin Invest* 1998;**101**:2693–701.
- 70 **Teckman JH**, Perlmuter DH. Retention of mutant α_1 -antitrypsin Z in endoplasmic reticulum is associated with an autophagic response. *Am J Physiol Gastrointest Liver Physiol* 2000;**279**:G961–74.
- 71 **Perlmutter DH**. Liver injury in α_1 -antitrypsin deficiency: an aggregated protein induces mitochondrial injury. *J Clin Invest* 2002;**110**:1579–83.
- 72 **Wu Y**, Whitman I, Molmenti E, et al. A lag in intracellular degradation of mutant α_1 -antitrypsin correlates with liver disease phenotype in homozygous PiZZ α_1 -antitrypsin deficiency. *Proc Natl Acad Sci USA* 1994;**91**:9014–8.
- 73 **Teckman JH**, Perlmuter DH. The endoplasmic reticulum degradation pathway for mutant secretory proteins α_1 -antitrypsin Z and S is distinct from that for an unassembled membrane protein. *J Biol Chem* 1996;**271**:13215–20.
- 74 **Burrows JAJ**, Willis LK, Perlmuter DH. Chemical chaperones mediate increased secretion of mutant α_1 -antitrypsin (α_1 -AT) Z: a potential pharmacological strategy for prevention of liver injury and emphysema. *Proc Natl Acad Sci USA* 2000;**97**:1796–801.
- 75 **Green C**, Brown G, Dafforn TR, et al. Mutations in the *Drosophila* serpin necrotic midline disease-associated mutations of human serpins. *Development* 2003;**130**:1473–8.
- 76 **Seyama K**, Nukiwa T, Takabe K, et al. Siiyama (serine 53 (TCC) to phenylalanine 53 (TTC)). A new α_1 -antitrypsin-deficient variant with mutation on a predicted conserved residue of the serpin backbone. *J Biol Chem* 1991;**266**:12627–32.
- 77 **Seyama K**, Nukiwa T, Souma S, et al. α_1 -antitrypsin-deficient variant Siiyama (Ser⁵³[TCC] to Phe⁵³[TTC]) is prevalent in Japan. Status of α_1 -antitrypsin deficiency in Japan. *Am Rev Respir Dis* 1995;**152**:2119–26.
- 78 **Matsunaga E**, Shiokawa S, Nakamura H, et al. Molecular analysis of the gene of the α_1 -antitrypsin deficiency variant, Mnichinan. *Am J Hum Genet* 1990;**46**:602–12.
- 79 **Sergi C**, Consalez GC, Fabbretti G, et al. Immunohistochemical and genetic characterization of the M Cagliari α_1 -antitrypsin molecule (M-like α_1 -antitrypsin deficiency). *Lab Invest* 1994;**70**:130–3.
- 80 **Lomas DA**, Finch JT, Seyama K, et al. α_1 -antitrypsin Siiyama (Ser⁵³ØPhe); further evidence for intracellular loop-sheet polymerisation. *J Biol Chem* 1993;**268**:15333–5.
- 81 **Lomas DA**, Elliott PR, Sidhar SK, et al. Alpha₁-antitrypsin Mmalton (S⁵²Phe deleted) forms loop-sheet polymers in vivo: evidence for the C sheet mechanism of polymerisation. *J Biol Chem* 1995;**270**:16864–70.
- 82 **Elliott PR**, Stein PE, Bilton D, et al. Structural explanation for the dysfunction of S α_1 -antitrypsin. *Nat Struct Biol* 1996;**3**:910–1.
- 83 **Mahadeva R**, Chang W-SW, Dafforn T, et al. Heteropolymerisation of S, I and Z α_1 -antitrypsin and liver cirrhosis. *J Clin Invest* 1999;**103**:999–1006.
- 84 **Cruz M**, Molina JA, Pedrola D, et al. Cirrhosis and heterozygous α_1 -antitrypsin deficiency in a 4 year old girl. *Helv Paediatr Acta* 1975;**30**:501–7.
- 85 **Campra JL**, Craig JR, Peters RL, et al. Cirrhosis associated with partial deficiency of alpha-1-antitrypsin in an adult. *Ann Intern Med* 1973;**78**:233–8.
- 86 **Seersholm N**, Kok-Jensen A, Dirksen A. Survival of patients with severe α_1 -antitrypsin deficiency with special reference to non-index cases. *Thorax* 1994;**49**:695–8.
- 87 **Janus ED**, Phillips NT, Carrell RW. Smoking, lung function, and α_1 -antitrypsin deficiency. *Lancet* 1985;i:152–4.
- 88 **Wewers MD**, Casolaro MA, Sellers SE, et al. Replacement therapy for alpha₁-antitrypsin deficiency associated with emphysema. *N Engl J Med* 1987;**316**:1055–62.
- 89 **Ogushi F**, Fells GA, Hubbard RC, et al. Z-type α_1 -antitrypsin is less competent than M1-type α_1 -antitrypsin as an inhibitor of neutrophil elastase. *J Clin Invest* 1987;**80**:1366–74.
- 90 **Guzdek A**, Potempa J, Dubin A, et al. Comparative properties of human α_1 -proteinase inhibitor glycosylation variants. *FEBS Lett* 1990;**272**:125–7.
- 91 **Llewellyn-Jones CG**, Lomas DA, Carrell RW, et al. The effect of the Z mutation on the ability of α_1 -antitrypsin to prevent neutrophil mediated tissue damage. *Biochim Biophys Acta* 1994;**1227**:155–60.
- 92 **Gadek JE**, Fells GA, Crystal RG. Cigarette smoking induces functional antiprotease deficiency in the lower respiratory tract of humans. *Science* 1979;**206**:1315–6.
- 93 **Janoff A**, Carp H, Lee DK, et al. Cigarette smoke inhalation decreases α_1 -antitrypsin activity in rat lung. *Science* 1979;**206**:1313–4.
- 94 **Elliott PR**, Bilton D, Lomas DA. Lung polymers in Z α_1 -antitrypsin related emphysema. *Am J Respir Cell Mol Biol* 1998;**18**:670–4.
- 95 **Morrison HM**, Kramps JA, Burnett D, et al. Lung lavage fluid from patients with α_1 -proteinase inhibitor deficiency or chronic obstructive bronchitis: anti-elastase function and cell profile. *Clin Sci* 1987;**72**:373–81.
- 96 **Hubbard RC**, Fells G, Gadek J, et al. Neutrophil accumulation in the lung in alpha 1-antitrypsin deficiency. Spontaneous release of leukotriene B₄ by alveolar macrophages. *J Clin Invest* 1991;**88**:891–7.
- 97 **Woolhouse IS**, Bayley DL, Stockley RA. Sputum chemotactic activity in chronic obstructive pulmonary disease: effect of α_1 -antitrypsin deficiency and the role of leukotriene B₄ and interleukin 8. *Thorax* 2002;**57**:709–14.
- 98 **Parmar JS**, Mahadeva R, Reed BJ, et al. Polymers of α_1 -antitrypsin are chemotactic for human neutrophils: a new paradigm for the pathogenesis of emphysema. *Am J Respir Cell Mol Biol* 2002;**26**:723–30.
- 99 **Banda MJ**, Rice AG, Griffin GL, et al. The inhibitory complex of human α_1 -proteinase inhibitor and human leukocyte elastase is a neutrophil chemoattractant. *J Exp Med* 1988;**167**:1608–15.
- 100 **Senior RM**, Griffin GL, Mecham RP. Chemotactic activity of elastin derived peptides. *J Clin Invest* 1980;**66**:859–62.

- 101 **Warter J**, Storck D, Grosshans E, *et al.* Syndrome de Weber-Christian associe a un deficit en alpha-1-antitrypsine; enquete familiale. *Ann Med Interne (Paris)* 1972;**123**:877–82.
- 102 **O'Riordan K**, Blei A, Rao MS, *et al.* α_1 -antitrypsin deficiency-associated panniculitis. Resolution with intravenous α_1 -antitrypsin administration and liver transplantation. *Transplantation* 1997;**63**:480–2.
- 103 **Colp C**, Pappas J, Moran D, *et al.* Variants of α_1 -antitrypsin in Puerto Rican children with asthma. *Chest* 1993;**103**:812–5.
- 104 **Eden E**, Mitchell D, B M, *et al.* Atopy, asthma, and emphysema in patients with severe alpha-1-antitrypsin deficiency. *Am J Respir Crit Care Med* 1997;**156**:68–74.
- 105 **Griffith ME**, Lovegrove JU, Gaskin G, *et al.* 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.
- 106 **Baslund B**, Szpirt W, Eriksson S, *et al.* Complexes between proteinase 3, α_1 -antitrypsin and proteinase 3 anti-neutrophil cytoplasmic autoantibodies: a comparison between α_1 -antitrypsin PiZ allele carriers and non-carriers with Wegener's granulomatosis. *Eur J Clin Invest* 1996;**26**:786–92.
- 107 **King MA**, Stone JA, Diaz PT, *et al.* α_1 -antitrypsin deficiency: evaluation of bronchiectasis with CT. *Radiology* 1996;**199**:137–41.
- 108 **Seersholm N**, Kok-Jensen A. Extrapulmonary manifestations of alpha-1-antitrypsin deficiency. *Am J Respir Crit Care Med* 2001;**163**:A343.
- 109 **Schievink WI**, Prakash UBS, Piepgras DG, *et al.* α_1 -antitrypsin deficiency in intracranial aneurysms and cervical artery dissection. *Lancet* 1994;**343**:452–3.
- 110 **Cox DW**. α_1 -antitrypsin: a guardian of vascular tissue. *Mayo Clin Proc* 1994;**69**:1123–4.
- 111 **Cuvelier A**, Muir J-F, Hellot M-F, *et al.* Distribution of α_1 -antitrypsin alleles in patients with bronchiectasis. *Chest* 2000;**117**:415–9.
- 112 **Silverman GA**, Bird PI, Carrell RW, *et al.* The serpins are an expanding superfamily of structurally similar but functionally diverse proteins. Evolution, novel functions, mechanism of inhibition and a revised nomenclature. *J Biol Chem* 2001;**276**:33293–6.
- 113 **Whisstock JC**, Skinner R, Lesk AM. An atlas of serpin conformations. *Trends Biochem Sci* 1998;**23**:63–7.
- 114 **Aulak KS**, Eldering E, Hack CE, *et al.* A hinge region mutation in C1-inhibitor (Ala⁴³⁶→Thr) results in nonsubstrate-like behavior and in polymerization of the molecule. *J Biol Chem* 1993;**268**:18088–94.
- 115 **Eldering E**, Verpy E, Roem D, *et al.* COOH-terminal substitutions in the serpin C1 inhibitor that cause loop overinsertion and subsequent multimerization. *J Biol Chem* 1995;**270**:2579–87.
- 116 **Bruce D**, Perry DJ, Borg J-Y, *et al.* Thromboembolic disease due to thermolabile conformational changes of antithrombin Rouen VI (187 Asn→Asp). *J Clin Invest* 1994;**94**:2265–74.
- 117 **Lindo VS**, Kakkar VV, Learmonth M, *et al.* Antithrombin-TRI (Ala382 to Thr) causing severe thromboembolic tendency undergoes the S-to-R transition and is associated with a plasma-inactive high-molecular-weight complex of aggregated antithrombin. *Br J Haematol* 1995;**89**:589–601.
- 118 **Poller W**, Faber J-P, Weidinger S, *et al.* A leucine-to-proline substitution causes a defective α_1 -antichymotrypsin allele associated with familial obstructive lung disease. *Genomics* 1993;**17**:740–3.
- 119 **Faber J-P**, Poller W, Olek K, *et al.* The molecular basis of α_1 -antichymotrypsin deficiency in a heterozygote with liver and lung disease. *J Hepatol* 1993;**18**:313–21.
- 120 **Crowther DC**, Serpell LC, Dafforn TR, *et al.* Nucleation of α_1 -antichymotrypsin polymerisation? *Biochemistry* 2002;**42**:2355–63.
- 121 **Davis RL**, Shrimpton AE, Holohan PD, *et al.* Familial dementia caused by polymerisation of mutant neuroserpin. *Nature* 1999;**401**:376–9.
- 122 **Davis RL**, Shrimpton AE, Carrell RW, *et al.* Association between conformational mutations in neuroserpin and onset and severity of dementia. *Lancet* 2002;**359**:2242–7.
- 123 **Belorgey D**, Crowther DC, Mahadeva R, *et al.* Mutant neuroserpin (Ser49Pro) that causes the familial dementia FENIB is a poor proteinase inhibitor and readily forms polymers in vitro. *J Biol Chem* 2002;**277**:17367–73.
- 124 **Schulze AJ**, Baumann U, Knof S, *et al.* Structural transition of α_1 -antitrypsin by a peptide sequentially similar to β -strand s4A. *Eur J Biochem* 1990;**194**:51–6.
- 125 **Mast AE**, Enghild JJ, Salvesen G. Conformation of the reactive site loop of α_1 -proteinase inhibitor probed by limited proteolysis. *Biochemistry* 1992;**31**:2720–8.
- 126 **Huntington JA**, Pannu NS, Hazes B, *et al.* A 2.6Å structure of a serpin polymer and implications for conformational disease. *J Mol Biol* 1999;**293**:449–55.
- 127 **Dunstone MA**, Dai W, Whisstock JC, *et al.* Cleaved antitrypsin polymers at atomic resolution. *Protein Sci* 2000;**9**:417–20.
- 128 **Skinner R**, Chang W-SW, Jin L, *et al.* Implications for function and therapy of a 2.9Å structure of binary-complexed antithrombin. *J Mol Biol* 1998;**283**:9–14.
- 129 **Fitton HL**, Pike RN, Carrell RW, *et al.* Mechanisms of antithrombin polymerisation and heparin activation probed by insertion of synthetic reactive loop peptides. *Biol Chem* 1997;**378**:1059–63.
- 130 **Chang W-SW**, Wardell MR, Lomas DA, *et al.* Probing serpin reactive loop conformations by proteolytic cleavage. *Biochem J* 1996;**314**:647–53.
- 131 **Zhou A**, Stein PE, Huntington JA, *et al.* Serpin polymerisation is prevented by a hydrogen bond network that is centered on His-334 and stabilized by glycerol. *J Biol Chem* 2003;**278**:15116–22.
- 132 **Lee C**, Maeng J-S, Kocher J-P, *et al.* Cavities of α_1 -antitrypsin that play structural and functional roles. *Protein Sci* 2001;**10**:1446–53.
- 133 **Parfrey H**, Mahadeva R, Ravenhill N, *et al.* Targeting a surface cavity of α_1 -antitrypsin to prevent conformational disease. *J Biol Chem* 2003;**278**:33060–6.
- 134 **Chow MKM**, Devlin GL, Bottomley SP. Osmolytes as modulators of conformational changes in the serpins. *Biol Chem* 2001;**382**:1593–9.
- 135 **Devlin GL**, Parfrey H, Tew DJ, *et al.* Prevention of polymerization of M and Z α_1 -antitrypsin (α_1 -AT) with trimethylamine N-oxide. Implications for the treatment of α_1 -AT deficiency. *Am J Respir Cell Mol Biol* 2001;**24**:727–32.
- 136 **Rubenstein RC**, Egan ME, Zeitlin PL. In vitro pharmacologic restoration of CFTR-mediated chloride transport with sodium 4-phenylbutyrate in cystic fibrosis epithelial cells containing delta F508-CFTR. *J Clin Invest* 1997;**100**:2457–65.
- 137 **Rubenstein RC**, Zeitlin PL. A pilot clinical trial of oral sodium 4-phenylbutyrate (Buphenyl) in deltaF508-homozygous cystic fibrosis patients: partial restoration of nasal epithelial CFTR function. *Am J Respir Crit Care Med* 1998;**157**:484–90.
- 138 **Mahadeva R**, Lomas DA. Alpha₁-antitrypsin deficiency, cirrhosis and emphysema. *Thorax* 1998;**53**:501–5.
- 139 **Lomas DA**, Elliott PR, Chang W-SW, *et al.* Preparation and characterisation of latent α_1 -antitrypsin. *J Biol Chem* 1995;**270**:5282–8.
- 140 **Im H**, Woo M-S, Hwang KY, *et al.* Interactions causing the kinetic trap in serpin protein folding. *J Biol Chem* 2002;**277**:46347–54.