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Leading article

Alpha-1 antitrypsin deficiency

α-1 antitrypsin is synthesised in the liver and protects lung alveolar tissues from destruction by neutrophil elastase.[1] α-1 antitrypsin deficiency is a common autosomal recessive condition (1:1600 to 1:1800) in which liver disease results from retention of abnormal polymerised α-1 antitrypsin in the endoplasmic reticulum of hepatocytes, and emphysema results from alveolar wall damage. The clinical consequences of α-1 antitrypsin deficiency in childhood are haemorrhagic disease in infancy, cholestasis in infancy, or chronic liver disease. Lung disease attributable to α-1 antitrypsin deficiency does not occur in childhood, but is closely linked to smoking in adults. Membranoproliferative glomerulonephritis, panniculitis, and necrotising vasculitis are associations with α-1 antitrypsin deficiency in adult life.[2–4] Diagnostic methods are summarised in table 1.

Phenotypes

α-1 antitrypsin is a protease inhibitor, and common *Pi* variants have been named by their electrophoretic mobility. PiM, of which there are several minor variants, is the normal protein. PiZ, the mutant responsible for more than 95% of cases of pulmonary and hepatic disease associated with α-1 antititrypsin deficiency, is most frequent in Scandinavia and progressively less common as one travels south in Europe. PiS, by contrast, is most common in the Iberian peninsula.[5]

Emphysema is associated both with null mutations (no protein produced) and with mutations producing defective or non-exported protein. Liver disease is associated only with those mutations that produce a peptide which forms loop sheet polymers that are retained in the liver, namely homozygotes for PiZ, PiM (Malton), and the compound heterozygotes PiZ−, PiSZ, and PiZI.[6] The nomenclature of genetic variants is slightly confusing: presumed homozygous abnormalities such as PiZZ are conventionally referred to as PiZ unless the null gene has been excluded from the phenotype; thus PiZ might actually be PiZ− (Z plus null), or PiZZ. The infant with α-1 antitrypsin deficiency is considered to be PiZZ if both parents carry the Z allele; this may be confirmed by genotype.

Molecular pathology

There are several recent reviews of the molecular pathology of α-1 antitrypsin deficiency.[7–9] Newly formed α-1 antitrypsin peptide enters the endoplasmic reticulum, where a series of molecular chaperones superintends an orderly process of glycosylation, disulphide bridge formation, and folding. Having achieved its tertiary structure, α-1 antitrypsin leaves the endoplasmic reticulum and travels by Golgi and secretory vesicles to the plasma membrane, where it is secreted as a 55 kDa glycoprotein at a rate of approximately 34 mg/kg/day. Newly synthesised α-1 antitrypsin is subject to a quality control process—protein that fails to achieve the correct tertiary structure enters a degradative pathway in which misfolded α-1 antitrypsin remains bound to calnexin in the endoplasmic reticulum, induces conjugation with ubiquitin, and leaves the endoplasmic reticulum to be degraded in proteasomes.

The mutant peptide found in α-1 antitrypsin deficiency of the common PiZZ phenotype (α-1 antitrypsin Z, AAT-Z) differs from α-1 antitrypsin M (AAT-M) only in a single amino acid change, gly 342 → lys. The effect of this is a severe reduction in the rate at which the peptide folds.[10] Slow folding allows peptide monomers to come together by a loop sheet insertion mechanism to form an AAT-Z polymer which is retained within the endoplasmic reticulum.[12] In liver biopsies from patients with α-1 antitrypsin deficiency, polymerised AAT-Z may be demonstrated in the endoplasmic reticulum by electron microscopy and is apparent histochemically as characteristic PAS positive, diastase resistant globules.

There are many genetic disorders in which a mutated peptide fails to achieve correct conformation and is retained in the endoplasmic reticulum. As with AAT-Z, the mutated protein may be functionally active but disease results from failure of trafficking to its correct location, whether that be the plasma membrane (for example, cystic fibrosis or sucrase-isomaltase deficiency), other organelle membranes (Wilson's disease), or the extracellular fluid (fibrinogen, protein C, or thyroglobulin deficiency).[7 8]

Why is it that α-1 antitrypsin deficiency differs from all these disorders in that the retained mutant peptide damages the liver cell? It might simply be that α-1 antitrypsin is synthesised in large amounts, but probably more important is the fact that polymerised AAT-Z resists degradation. It may fancifully be imagined as doing to the endoplasmic reticulum what haemoglobin S does to the erythrocyte in sickle cell anaemia.

Why then do only 10% of PiZZ infants develop liver disease? Among the suggested acquired contributory factors are the following:

Table 1 *Diagnostic methods for identification of α-1 antitrypsin deficiency*

Method	Comment	Limitations of method
Serum level	Normal 0.8 to 1.8 g/l (5th and 95th centiles at 6 months). In PiZZ, serum level usually <0.6 g/l	AATD an acute phase reactant Liver inflammation may raise serum level in AATD. Therefore always request phenotype
Protease inhibitor (Pi) phenotype	Assessed by isoelectric focusing on polyacylamide gels. Normal = PiMM; common AATD = PiZZ	Experienced lab essential; CMV infection may cause a spurious Z band Sometimes PiZZ may spuriously appear like PiSZ in children with liver disease[49]
Genotype (149)	Primers available for M, Z, and S alleles	Only available in reference laboratories
Histochemistry	PAS positive diastase resistant granules in hepatocytes	Only obvious after 3 months of age

AATD, α-1 antitrypsin deficiency.

- *intrauterine infection*, because PiZ babies with liver disease tend to have been small for gestational age;
- *gut derived proteases*, suggesting that breast feeding might be protective;
- *autoimmunity*, but liver directed antibodies are likely to be secondary to liver damage rather than primary;
- *fever*, because a raised temperature increases both the production and the polymerisation rate of α-1 antitrypsin. Therefore, transient elevations of plasma transaminases do indeed occur during infections in α-1 antitrypsin deficient children[14];
- *cryptic hepatitis B or C infection*,[12] but this has not been substantiated.[13]

None of these acquired factors seems sufficient to explain fully the enormous variation in hepatic phenotype. A more attractive hypothesis addresses possible genetic differences in AAT-Z degradation. In transfected skin fibroblasts from PiZZ patients who had developed liver disease, AAT-Z was degraded less rapidly than in fibroblasts from PiZZ individuals who had not developed liver disease.[15] This suggests that PiZZ patients who develop liver disease have one of a number of possible inborn errors of AAT-Z clearance. It is tempting to extrapolate this concept to other trafficking disorders.

Epidemiology of α-1 antitrypsin deficiency liver disease in childhood

In a classic piece of prospective epidemiology,[14 16–20] Sveger studied 200 000 infants in 1972–74, identifying 127 PiZ infants among these and following them to the age of 18 years. Only 14 developed cholestatic jaundice in infancy. Eight more had hepatosplenomegaly and mildly disturbed liver function tests.

PROGNOSIS OF THE α-1 ANTITRYPSIN DEFICIENT CHILD WHO DOES NOT DEVELOP INFANTILE CHOLESTASIS

The frequency of abnormal liver function tests in PiZZ individuals with α-1 antitrypsin deficiency who do not develop jaundice or hepatomegaly in infancy falls from 60% at 6 months to a plateau of approximately 15% at 12–18 years, with no clinical evidence of liver disease (table 2). Abnormal liver function tests may be transient—most of those with abnormal liver function tests at 16 years were normal at 18 and vice versa. There are occasional reports of PiZZ children who developed new or worsening liver dysfunction in childhood, often in association with other severe illnesses: one case each of appendicitis and pneumonia in Sveger's series, and one of pancreatitis in another report. These cases seem to be rare, and justify excluding α-1 antitrypsin deficiency in the work up of undiagnosed liver disease in childhood.

The prognosis of PiSZ infants is even better. None in Sveger's series developed jaundice in infancy, and the frequency of abnormal liver function tests was lower throughout childhood (table 2).

The risk of cirrhosis in adults is much more difficult to quantitate because the available data are derived either from patients known to have α-1 antitrypsin deficient lung disease or from studies of α-1 antitrypsin deficiency in cases of cirrhosis. Thus 12% of affected Swedish adults identified through hospital admission had cirrhosis.[21] Whether alcohol has anything to do with liver disease in α-1 antitrypsin deficiency is unknown; we are not therefore justified in insisting on abstinence but do advise moderation.

Whereas the heterozygous state, PiMZ, carries no risk in childhood, it may be associated with liver disease in adults. The PiMZ phenotype was found in 8% of adult transplant candidates with chronic liver failure, including 27% of those with cryptogenic cirrhosis, compared with 2–4% in the general population.[22] Inverting that statistic, however, shows that the risk of liver disease in a particular PiMZ individual must be small.

PROGNOSIS OF THE α-1 ANTITRYPSIN DEFICIENT CHILD WHO DOES DEVELOP INFANTILE CHOLESTASIS

Of the 22 clinically affected Swedish babies with α-1 antitrypsin deficiency, two died of cirrhosis at around 7 years, while one died of aplastic anaemia and had cirrhosis at necropsy.[14 16–20] Of 74 children referred to King's College Hospital and followed to 17 years, 20 died, 20 had cirrhosis, 19 had persistently abnormal liver function tests, and only 15 made a complete recovery.[23] However, these were patients whose infantile cholestasis was sufficiently severe for tertiary referral.

A more recent review[24] has shown, perhaps unsurprisingly, that those children who progressed to end stage liver disease had more severe abnormalities in infancy. They were more likely to have remained jaundiced for more than 6 weeks, to have had higher aspartate aminotransferase at presentation, and to have had more severe changes on the initial biopsy (comprising severe bile duct reduplication, severe fibrosis with bridging septa, and established cirrhosis). Nevertheless, one should be cautious about the outlook in an individual jaundiced infant with α-1 antitrypsin deficiency. Volpert *et al* point out that among affected children with established liver disease in whom transplant was not immediately indicated is a group that remains clinically stable for a prolonged period after the presence of cirrhosis or portal hypertension is established.[13] These are not initially distinguishable from those children whose liver function declines more rapidly.

Three diagnostic points in the cholestatic infant merit emphasis. First, the early liver biopsy appearances, with prominent portal tract changes and with PAS positive, diastase negative granules not yet apparent, may mimic those of biliary atresia. Giant cell transformation of hepatocytes is uncommon in α-1 antitrypsin deficiency. Second, the plasma concentration of α-1 antitrypsin in a deficient patient may be raised as an acute phase reactant, so the α-1 antitrypsin phenotype must be obtained. Third, determining the α-1 antitrypsin phenotype may be the most time consuming laboratory investigation, so must (like the coagulation screen) be requested promptly.

The indications for liver transplantation are the same as for other hepatic disorders. After biliary atresia, α-1 antitrypsin deficiency is the most frequent reason for liver transplantation in childhood. The lung does not appear to pose any particular problem, and the perioperative and postoperative care does not differ from the usual routines. Outcomes are good.[25 26] Orthotopic liver transplantation from a donor parent has been successfully performed.[27] After transplant the recipient manifests the phenotype of the donor and is expected not to be at risk of emphysema.

What is the risk of severe liver disease in the subsequent PiZ sibling of a severely affected proband? Psacharopoulos *et al* reported that the risk was 78%,[23] while others have reported less pessimistic figures. Parents who have had a

Table 2 Frequency (%) of abnormality of alanine transaminase and/or γ glutamate transferase in prospectively identified Swedish α-1 antitrypsin deficient children who did not develop infantile cholestasis or hepatomegaly

Age	PiZZ	PiSZ
3 months	52	24
6 months	60	5
4 years	41	2
8 years	36	2
12 years	15	2
16 years	17	8
18 years	12	15

No clinical features of liver disease found at any age group.[4 16–20]

severely affected child are more likely to opt for termination of a subsequent PiZ fetus, so it may be difficult to acquire further data to refine this risk.

Late haemorrhagic disease in infancy

Whereas early neonatal haemorrhagic disease in breast fed infants is prevented by oral vitamin K at birth, cases of late vitamin K dependent bleeding continue to occur, usually in babies with undiagnosed cholestasis, of which α-1 antitrypsin deficiency and biliary atresia are the most common. Of 182 000 babies in the United Kingdom North East region given 1 mg of oral vitamin K at birth and, for the breast fed babies, recommended to receive three further 1 mg doses at fortnightly intervals, four developed late haemorrhagic disease.[28] Two of these had not received vitamin K and two had α-1 antitrypsin deficiency. Given that the breast feeding rate was approximately 30% (Wariyar U, personal communication) and the incidence of α-1 antitrypsin deficiency is approximately 1:1800, it seems that haemorrhagic disease occurred in 2/30 breast fed infants with α-1 antitrypsin deficiency.

Of 332 686 Swedish babies, about 80% of whom received 1 mg or 2 mg of oral vitamin K at birth, and among whom breast feeding rates were reportedly high, 17 developed late haemorrhagic disease.[29] Fifteen of these had cholestatic liver disease, comprising three with α-1 antitrypsin deficiency, five with biliary atresia, and seven with other conditions. Thus 1/35 α-1 antitrypsin deficient infants bled. α-1 Antitrypsin deficiency was also responsible for cases of late haemorrhagic disease in other reports.[30 31] This tragic consequence of α-1 antitrypsin deficiency and other infantile cholestases is prevented by the more physiological Dutch protocol of giving breast fed babies 1 mg of vitamin K at birth and 25 μg daily from 2–13 weeks of life, though late haemorrhagic disease may still occur because of failure of compliance.[32]

Despite the liver disease, the grossly prolonged prothrombin time dramatically improves within hours of giving parenteral vitamin K.

Subsequent affected siblings of an α-1 antitrypsin deficient proband should always receive intramuscular vitamin K at birth.

Lung disease in α-1 antitrypsin deficiency

The damage wrought by uninhibited neutrophil elastase in the lung takes many years to manifest itself clinically.[33] The characteristic pathology seen in α-1 antitrypsin deficiency is emphysema, caused by loss of elastic recoil. Children and adolescents with α-1 antitrypsin deficiency have not been shown to have significant lung function abnormalities.[34 35] Although a study of affected children with liver disease suggested a tendency to hyperinflation,[36] this was not found in Sveger's subsequent study of 150 adolescents.[34] After the age of 30–35 years there is an accelerated decline in forced expiratory volume in one second (FEV_1), which is considerably worsened by cigarette smoking. In a non-smoker, symptoms are generally seen at around 50 years of age, while smokers will be symptomatic by 30–40 years. Although life expectancy is more difficult to estimate with accuracy, a combination of three studies gives a mean age of death of 50 years in smokers, compared with 66 years in non-smokers.[37–39] Interestingly, only 3% of adolescents with α-1 antitrypsin deficiency smoked in Sveger's study,[34] suggesting that health education may be effective in this group of children.

Paediatricians tend to include an α-1 antitrypsin phenotype in the panel of tests for unexplained pulmonary symptoms. There is little evidence in support of this, although it is theoretically possible that a coexisting inflammatory disease might be worsened by α-1 antitrypsin deficiency, even in childhood. In a study of adults with bronchiectasis there was no increase in the prevalence of α-1 antitrypsin deficiency alleles, but more emphysema if both diseases coexisted.[40] If α-1 antitrypsin deficiency is found in a child with lung symptoms it should not therefore be accepted as the underlying cause of the problem, but it might be an exacerbating factor in disease progression.

Prospects for pharmacological treatment of α-1 antitrypsin deficiency

A logical therapeutic ambition is to devise a way of moving AAT-Z from the endoplasmic reticulum of the liver, where it causes damage, to the plasma, where its antiprotease activity—though less than the wild type AAT-M—would benefit the lung. This might be achieved either by inhibiting the polymerisation of AAT-Z[41] or by chaperoning the misfolded AAT-Z from endoplasmic reticulum to the secretory pathway.[2] The latter concept is common to all the endoplasmic reticulum retention diseases. Unlike cystic fibrosis ΔF508, incubation of cells at lower temperature does not improve the secretory defect in α-1 antitrypsin deficiency, but there are promising results from chemical chaperones. In cultured mouse hepatocytes or transfected skin fibroblasts, glycerol and 4-phenylbutyric acid achieved increases of secretion of AAT-Z, from 3% in controls to 25% and 17%, respectively. 4-Phenylbutyric acid also achieved increased plasma levels, reaching 20–50% of the levels present in PiM mice with transgenic α-1 antitrypsin deficiency.[42] Sodium phenylbutyrate is already in clinical use in urea cycle defects and is a potential form of treatment. Its mode of action, however, remains to be defined. Both the protein translation inhibitor cycloheximide and the specific inhibitor of proteasome function, lactacystin, prevented intracellular degradation of AAT-Z and partially restored its vesicular transport in transfected CHO cells and human alveolar macrophages.[43] In other cell culture work, glucosidase and mannosidase inhibitors also achieved increased secretion.[44]

Augmentation therapy with α-1 antitrypsin given intravenously has been shown to restore serum and sputum antiprotease levels. Obviously this will be of no benefit to infants with liver disease, but offers the prospect of prevention or treatment of lung disease. In non-randomised observational studies, intravenous augmentation therapy has been associated with slower declines in FEV_1[4] and improved survival, but as yet there is no evidence of long term benefit from a randomised controlled trial. If benefits are seen, they appear to be confined to a subgroup of patients with FEV_1 below 65% of predicted. The one small randomised trial which compared 28 treated patients and 28 controls showed marginal benefits in the appearances on computed tomography, but no significant differences in lung function after three years of treatment.[46] The treatment is not currently licensed in the United Kingdom. Recombinant α-1 antitrypsin is now becoming available, and trials of its administration by nebuliser are planned. However, the child with α-1 antitrypsin deficiency may never develop significant lung disease if other damaging factors are avoided, and replacement therapy (where available) is currently only advised for significant or rapidly progressive emphysema rather than as pre-emptive treatment.

There are promising early results with gene therapy. A normal α-1 antitrypsin gene in a plasmid–cationic liposome complex was delivered to one nostril of each of five affected patients. α-1 Antitrypsin protein increased, and interleukin 8 (as a marker of inflammation) decreased, in nasal lavage fluid, with a peak effect on day 5.[47] While it is technically possible to insert the AAT-M gene in an

AAT-Z mouse liver,[48] that clearly will not correct the cellular defect.

Conclusions

Late haemorrhagic disease remains a hazard for breast fed α-1 antitrypsin deficient babies with the current vitamin K administration protocol. Continued vigilance is necessary if cholestasis in infancy is to be promptly detected. We have good evidence about the hepatic prognosis of α-1 antitrypsin deficiency, and recent knowledge of the molecular pathology provides hope for newer therapeutic approaches to this and other endoplasmic reticulum retention disorders. The indications for, and outcomes of, liver transplantation have been delineated.

What advice should we give to young people with α-1 antitrypsin deficiency? The core message has not changed since it was baldly stated by Janus *et al* in 1985[39]: "If they smoke, they will develop crippling emphysema by middle age; if they do not smoke, they have a reasonable likelihood of a full life span." Until a simple and safe treatment is available, this remains the only way to ensure respiratory health in α-1 antitrypsin deficiency.

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