Genetic studies on a new deficiency gene (PI*Z_{tun}) at the PI locus

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SUMMARY During a study of the α_1 antitrypsin (AAT) protein and its locus (PI) by high resolution isoelectric focusing and direct molecular analysis of 106 PIZ probands and their families, a new allele (Z_{tun}) was identified that resembles Z in many of its properties. Two sibs, both compound heterozygotes for Z_{tun} and Z, showed similar evidence of mild liver involvement that was indistinguishable from that associated with classical ZZ homozygotes. The Z_{tun} protein appeared to be deficient in the plasma to about the same degree as the Z protein. Allele specific oligonucleotide analysis of amplified genomic DNA indicated that the new allele is the result of a mutation in exon V that is identical to the classical G→A transition at codon 342 that results in the Glu→Lys substitution characteristic of the Z allele. An analysis of DNA haplotypes constructed from polymorphic restriction enzyme recognition sites in and around the PI locus confirmed that Z_{tun} probably represents a new mutation at codon 342 that has occurred on an M2-like genetic background.

Alpha₁ antitrypsin (AAT) is a major proteinase inhibitor present in high concentrations in the plasma. It is a glycoprotein of mw 52 000 which is synthesised mainly in the liver. It inhibits a wide range of serine proteinases, although its principal target is thought to be neutrophil elastase.¹ The structural gene locus encoding AAT is called PI (for proteinase inhibitor) and is located on chromosome 14q31-32.3.^{2,3} The PI locus displays high levels of genetic polymorphism at the protein level. Using acid starch gel electrophoresis, three common alleles, M, S, and Z, were originally found in the European population.⁴ However, with the introduction of isoelectric focusing (IEF) in narrow pH gradients, three common subtype alleles that control variant M proteins, M1, M2, and M3, have been characterised and more than 50 genetic variants of the AAT protein have now been identified.⁵

AAT is associated with early onset pulmonary emphysema and a form of neonatal liver disease⁶⁻⁸ both of which are associated with abnormally low levels of plasma AAT. The majority of subjects

with genetic deficiency of AAT are homozygous for the PI*Z allele, which occurs at a frequency of about 0.02 in the United Kingdom and on average causes an 85% reduction of the Z protein in the plasma relative to the level of the normal M protein. The plasma deficiency results from a blockage in secretion of the Z protein from the rough endoplasmic reticulum of the hepatocytes rather than a reduced rate of synthesis of the Z polypeptide.^{8,9} The Z gene is the consequence of a point mutation in codon 342 giving rise to a protein in which glutamic acid is replaced by lysine.^{10,11} On the basis of gene frequency estimates in populations of European origin and its association with a unique DNA haplotype, the Z mutation is thought to have occurred just once, about 6000 years ago, in a North European subject.¹² The Z gene is extremely rare among other ethnic groupings.

Several other PI alleles are associated with reductions of plasma AAT, including S, P, and I which result in moderate deficiency and rare variants such as M_{malton}, M_{heerlen}, M_{procida}, M_{duarte}, M_{like}, M_{rouen}, PI_{null}, and Z_{augzburg}, all of which result in severe deficiencies.^{6,9,13} The pathological conse-

quences of moderate deficiency of AAT are not clear. However, on the basis of current evidence, homozygosity or compound heterozygosity for any severe deficiency alleles carry a high risk of chest disease, while the risk of childhood liver disease is, as yet, only associated with homozygosity for the Z gene.

We have investigated a large series of subjects with the ZZ genotype, both with clinical disorders and apparently healthy, by high resolution isoelectric focusing and DNA analysis, in an attempt to identify genetic heterogeneity in the Z gene that might be related to disease susceptibility. Two types of variation of the Z protein have been found; one is non-genetic in origin and is described elsewhere.¹⁴ Here we describe the genetical analysis of a new PI variant that resembles Z in several of its properties. The variant allele was encountered in a family in which both children were compound heterozygotes for the normal Z and the new variant. Our studies suggest that the variant resulted from a recurrence of the Z mutation but on a different genetic background from the classical Z reported by Cox *et al.*¹²

Materials and methods

SAMPLES

Blood samples were collected into plastic tubes containing K EDTA from subjects in families participating in a study of AAT deficiency and associated pathology. Plasma was collected after centrifugation of whole blood at 900 g for 10 minutes at 4°C and stored at -20°C. DNA was prepared from white cell pellets by a standard phenol/chloroform extraction procedure.¹⁵

ISOELECTRIC FOCUSING AND PROTEIN DETECTION PROCEDURES

The precise details of isoelectric focusing (IEF), treatment of plasma samples, non-specific detection of AAT, immunoblotting procedures, and functional detection of AAT are given by Whitehouse *et al.*¹⁴

SIALIDASE TREATMENT

Samples of plasma (25 µl) were incubated at 37°C overnight with 0.1 units of type V sialidase (Sigma) dissolved in 0.01 mol/l phosphate/citric acid buffer, pH 5.0. An aliquot of each reaction was used for isoelectric focusing without further treatment.

POLYMERASE CHAIN REACTION (PCR)

PCR was used to study two sites in the AAT gene. 25mer oligonucleotide primers (5'GGGCCGTCCAGGGGAGTGAGCGCT and 5'AGGGATTTACAGATCACATGCAGGC) were used to

amplify exon V DNA, the region including the site of the G→A transition at codon 342 diagnostic for the classical Z allele. Exon II DNA including the site of the G→A transition in codon 101 characteristic of the M2 allele¹⁶ was amplified using 18mer oligonucleotide primers (5'CCCATCTCTGTCTTGCAG and 5'CTGGTTGAGCAACCTTAC). Genomic DNA (1 µg) from each subject was used and PCR was carried out using *Taq* polymerase under standard conditions.¹⁷

OLIGONUCLEOTIDE ANALYSIS

An aliquot of each exon V PCR reaction was adjusted to 0.4 mol/l NaOH/0.025 mol/l EDTA and 5 µl of this denatured DNA was spotted onto each of two Hybond-N filters (Amersham). 19mer oligonucleotides were obtained from Celltech Ltd, that corresponded to the M and Z sequences, M: ACCATCGACGAGAAAGGGA, Z: ACCATCGACAAGAAAGGGA; 10 pmol of each oligonucleotide were end labelled with γ ³²P-ATP (Amersham) using polynucleotide kinase (Anglian Biotech Ltd) at 37°C for one hour and purified by passage through a Sephadex G-25 column in 3×SSC. Incorporation of the label was about 90%. The filters were prehybridised in 6×SSC at 45°C for two hours and then hybridised in 6×SSC/0.1% SDS containing either M or Z labelled oligonucleotides for two hours at 42°C. The blots were washed twice in 2×SSC at room temperature and then washed for 10 minutes at up to 56°C. The final wash was 2×SSC at 37°C for 30 minutes. The filters were dried and autoradiographed for four to six hours.

RESTRICTION ENZYME ANALYSIS

The PCR products of exon II were digested with *RsaI* (Amersham) and separated on a 1.5% agarose gel in a Tris borate EDTA buffer, pH 8.3, containing ethidium bromide. Each lane contained one tenth of the PCR reaction. The separated DNA fragments were visualised with UV light.

DNA PROBES AND SOUTHERN ANALYSIS

A total of 4 µg genomic DNA was digested with *MaeIII*, *AvaII*, or *TaqI*. The DNA fragments were separated on 0.8% agarose gels and transferred to Genescreen Plus (NEN) membranes. AAT and antitrypsin related sequence (ATR) specific probes were derived from a 6.5 kb *BamHI* genomic fragment for AAT¹⁸ and a 1.1 kb *HindIII*-*PstI* genomic fragment for ATR.¹⁹ Hybridisation to ³²P labelled probes was carried out in the presence of dextran sulphate and washed to a final stringency of 0.2×SSC/0.1% SDS at 50°C. Insert DNA was ³²P labelled to high specific activity using the random priming method.²⁰

Results

PROTEIN ANALYSIS

The AAT patterns of plasma samples from 75 PIZ patients with liver disease, 28 with chest disease, and 25 healthy subjects who were also PIZ were examined by high resolution IEF (gradient pH 4.2 to 4.9 plus ACES). A large proportion of the subjects had previously been classified as Z by acid starch gel electrophoresis or IEF in a pH 3.5 to 6.0 gradient.²¹ The objective of using higher resolution IEF was to assess any minor variation in the Z protein, especially in the patients with liver disease.

When routine conditions of IEF and non-specific protein staining were used, all of the samples showed the expected Z phenotype. However, under optimal conditions of focusing it was clear that the Z bands in two sibs (from family MRC 4533) from the liver series were duplicated, such that the AAT pattern consisted of normal Z bands plus an additional set of slightly more basic bands of equal staining intensity and complexity (fig 1). Detailed analysis of their parents' AAT patterns indicated the mother was PIM₁Z, but the paternal pattern consisted of a combination of normal M₁ bands and a set of variant Z-like bands that cofocused precisely with the Z-like bands seen in the offspring's plasma (fig 1). Thus the variant bands were inherited from the father and appeared to be the products of an allelic gene. In accordance with convention we

have named the allele PI*Z_{tun} (for Tunbridge Wells).

Confirmation of the Z_{tun} bands as part of the AAT system was obtained by immunoblotting using monospecific antihuman AAT IgG antibodies (fig 2) and by functional detection (not shown). With both of these specific detection systems the resolution of the components is less clear than with Coomassie blue staining; consequently, the ZZ_{tun} phenotype appears as a single broad band as shown (fig 2). Nevertheless the difference between the Z and Z_{tun} bands is shown by immunoblotting and can be clearly seen by comparing the mother's and father's AAT patterns (fig 2).

Quantitative analysis indicated that the level of AAT in the M₁Z_{tun} phenotype plasma fell within the range for M₁Z and the ZZ_{tun} AAT levels were not different from the range of usual Z phenotypes.

After sialidase treatment, the patterns of AAT shown by IEF and immunoblotting indicated that the Z_{tun} protein remained distinct from the Z pattern in a manner that suggested a similar terminal glycosylation profile, since in both cases the sets of bands were shifted towards the cathode relative to the controls, but retained the same characteristic appearance (not shown).

OLIGONUCLEOTIDE ANALYSIS

In order to investigate the molecular basis of the Z_{tun} variant, DNA from Z, ZZ_{tun}, M₁Z, M₁Z_{tun}, and M₁M₃ subjects was amplified by polymerase chain reaction using primers that spanned the Z site

FIG 1 AAT patterns of the proband, immediate family, and control M1Z after IEF in gradient pH 4.2 to 4.9 plus ACES and Coomassie blue staining.

FIG 2 Specific immunodetection of AAT patterns of the proband, family members, and controls following IEF in gradient pH 4.2 to 4.9 plus ACES.

FIG3 Amplification of AAT exon V genomic DNA from various PI genotypes including ZZ_{tun} and $M1Z_{tun}$, followed by hybridisation with specific M and Z oligonucleotides.

in exon V. M and Z sequence specific 19mers that differed only by a single base were used to probe filter bound dots of amplified DNA (fig 3). The results clearly showed that the Z_{tun} DNA hybridised to the Z specific oligonucleotide probe but not the M probe. Therefore it can be concluded unequivocally that the Z_{tun} variant chromosome carries the G→A transition at codon 342 characteristic of the classical Z mutation, leading to the substitution of lysine in place of glutamic acid.

HAPLOTYPE ANALYSIS

The genetic background of the Z mutation in the Z_{tun} variant was investigated by analysing haplotypes derived from restriction fragment length polymorphisms. A useful feature of the AAT region is linkage disequilibrium, which occurs from the first intron of the gene and extends on the 3' side for at least 12 kb to the antitrypsin related sequence (ATR). This has enabled the identification of characteristic haplotypes.^{22 23} We used three enzymes for this analysis, *MaeIII*, *AvaII*, and *TaqI*. *MaeIII* and *TaqI* produce relatively simple RFLPs in which the classical Z allele is associated with a *MaeIII* 2.7 kb fragment in all cases and a *TaqI* 4.8 kb fragment in most cases. *AvaII* defines a complex pattern of polymorphic fragments as a result of multiple restriction sites in the AAT region. The *AvaII* fragments are numbered 1 to 7.¹² The classical Z gene is normally associated with the presence of bands 1 and 7 and rarely with their alleles, bands 4 and 5^{12 24} together with a usually invariant band 3. Fig 4 gives the haplotype information obtained from Southern blot analysis for family MRC 4533. Whereas the expected Z haplotype, *MaeIII* 2.7, *AvaII* 371, and *TaqI* 4.8, was found in association with the maternal Z, it is clear that the Z_{tun} variant is associated with *MaeIII* 2.4, which is associated with a neutral Ala→Val substitution at codon 213, *AvaII* 351, and *TaqI* 6.7. Since the *MaeIII* restriction site is in exon III and therefore to the 5' side of codon 342, and the *AvaII* 5/7 site is to the 3' side of exon V, it appears that the

FIG4 Haplotype analysis of family MRC 4533 with the two $PIZZ_{tun}$ sibs (hatched symbols). Ma=*MaeIII*; A=*AvaII*; T=*TaqI*. The parental *AvaII* haplotypes were deduced from a combination of the sibs' *AvaII* haplotypes and the parental *MaeIII* and *TaqI* data. The arrow indicates the proband.

FIG5 *RsaI* digested fragments of AAT exon II PCR amplified DNA. U/C=uncut.

Z_{tun} mutation is flanked on either side by markers characteristic of non-Z chromosomes. Furthermore, the downstream ATR *TaqI* 6.7 kb allele found in the Z_{tun} variant gene is normally associated with the *AvaII* 351 genotype, a combination not previously observed in association with the classical Z mutation.

It has previously been shown that this extended haplotype is most commonly found in association with the PI^*M2 allele.²² All M2 genes that have been studied are characterised by the loss of an *RsaI* site in exon II,¹⁶ which corresponds to a G→A transition at codon 101 leading to the substitution of histidine for arginine. Fig 5 shows an *RsaI* digest of PCR amplified exon II DNA from the ZZ_{tun} sibs and both parents. The sibs are heterozygous for the 475 bp band diagnostic of M2 and the non-M2 395 bp band. Since the mother is homozygous for the 395 bp (phenotype M1Z) it can be concluded that the 475 bp band was of paternal origin and therefore that the Z_{tun} mutation has most probably occurred on an M2 genetic background.

Discussion

The Z_{tun} variant described here is unique among the 106 PIZ probands that we have studied by high resolution IEF, although it does bear a very close resemblance to Z_{augzburg} (S Weidinger, personal communication), another Z like variant protein that focuses on the basic side of the classical Z.¹³ In every other case the classical Z protein was found. We conclude that there are no common subtypes of the Z gene equivalent to the commonly occurring M subtypes.

The molecular basis of the Z_{tun} mutation appears to be identical to the classical Z mutation and the difference seen at the protein level reflects the genetic backgrounds of the two alleles. Haplotype analysis has shown that the Z_{tun} background differs from the classical Z both to the 5' and 3' sides of codon 342 at three polymorphic sites. In addition, there must be at least one further mutational difference to account for the charge difference shown by IEF, since this cannot be attributed to the Ala→Val 213 amino acid substitution resulting from the gain of the *MaeIII* site in exon III¹¹ and the *AvaII* sites do not occur in the exons. Furthermore, the *MaeIII* 2-4-*AvaII* 351-*TaqI* 6-7 haplotype associated with the Z_{tun} mutation is commonly associated with the M2 protein. Simply on the basis of the appearance of the Z_{tun} protein after IEF, it seemed quite likely that the Z_{tun} mutation occurred on an M2 or M3 genetic background. We have been able to confirm that the Z_{tun} genetic background is M2-like, since the loss of the *RsaI* site in exon II leads to the replacement of His 101 for Arg 101, which is one of the two mutational events that produces the minor charge change that differentiates M2 from M1 at the protein level. The second mutation, which produces the substitution of Asp 376 for Glu 376, has also been found in all M2 genes tested and may represent the molecular basis of the M3 allele whose gene product focuses between M1 and M2.¹⁶

Did the Z_{tun} allele arise as a result of a new point mutation or intragenic recombination? Although our data do not permit firm discrimination between these alternatives, the most economical explanation is a new point mutation, since this requires only one event. For the existing classical Z mutation to be placed on the Z_{tun} haplotype by recombination, at least two crossovers in close proximity to codon 342 are necessary.

The relationship between AAT deficiency in PiZ subjects and lung damage is understood in broad terms. The reduced concentration of plasma AAT is unable to prevent damage in the pulmonary tissues by proteinases, notably elastase, released by alveolar

neutrophils in response to tobacco smoke and other atmospheric pollutants. The pathogenesis of childhood liver disease associated with the PIZZ genotype is controversial and it seems that environmental and other genetic factors may be important.²⁵ It may not relate simply to reduced plasma levels of AAT since Pi_{null} homozygotes do not appear to be susceptible.²⁶⁻²⁷ Carrell⁸ and Brantly *et al*⁹ postulate that a more likely explanation is that liver damage is the consequence of accumulation of the Z protein in hepatocytes owing to secretory blockage associated with the specific structural change. However, there is no evidence that the severity of liver disease is related to the amount of storage observed. Indeed, at least 50% of neonates have no evidence of liver disease²⁸⁻²⁹ and the majority of adults who are PIZ have accumulation of AAT but also have no clinical evidence of liver disease.³⁰⁻³¹ In the case reported here, the proband showed prolonged neonatal jaundice, PIZ status being ascertained at the age of 10 weeks. Liver function tests were abnormal but subsequently improved and at the age of eight years showed only minor abnormality with no clinical evidence of liver disease. His older sib had a history of mild neonatal jaundice with minimal abnormality of liver function tests. When seen at the age of 16 years the proband showed no evidence of liver disease and his liver function tests were normal. However, since the proband was ascertained through the classical features of cholestatic jaundice associated with AAT deficiency, this study confirms that the Glu→Lys 342 amino acid substitution alone is necessary to produce the classical Z liver disease phenotype, irrespective of genetic background in the vicinity of the PI gene.

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References

- Heidtmann H, Travis J. Human α_1 -proteinase inhibitor. In: Barrett AJ, Salvesen G, eds. *Proteinase inhibitors: research monographs in cell and tissue physiology*. Vol 12. Amsterdam: Elsevier, 1986:441-56.
- Darlington GJ, Astrin KH, Muirhead SP, Desnick RJ, Smith M. Assignment of human α_1 -antitrypsin to chromosome 14 by somatic cell hybrid analysis. *Proc Natl Acad Sci USA* 1982;79: 870-3.
- Schroeder WT, Miller MT, Woo SLC, Saunders GF. Chromosomal localization of the human α_1 -antitrypsin gene (Pi) to 14q31-32. *Am J Hum Genet* 1985;37:868-72.
- Fagerhol M. The Pi-system. Genetic variants of serum α_1 -antitrypsin. *Ser Haematol* 1968;1:153-61.
- Cox DW, Levison H. Emphysema of early onset associated with a complete deficiency of alpha-1-antitrypsin (null homozygotes). *Am Rev Respir Dis* 1988;137:371-5.
- Fagerhol MK, Cox DW. The Pi polymorphism. Genetic, biochemical, and clinical aspects of human α_1 -antitrypsin. In:

- Harris H, Hirschhorn K, eds. *Advances in human genetics*. Vol 11. New York: Plenum Press, 1981:1–62.
- ⁷ Mowat AP. α_1 -Antitrypsin deficiency in liver disease. In: Williams R, Maddrey WC, eds. *Gastroenterology*. Vol 4. *Liver*. London: Butterworths, 1984:52–75.
 - ⁸ Carrell RW. α_1 -Antitrypsin: molecular pathology, leukocytes, and tissue damage. *J Clin Invest* 1986;**78**:1427–31.
 - ⁹ Brantly M, Nukiwa T, Crystal RG. Molecular basis of alpha-1-antitrypsin deficiency. *Am J Med* 1988;**84**(suppl 6A):13–31.
 - ¹⁰ Yoshida A, Lieberman J, Gaidulis L, Ewing C. Molecular abnormality of human alpha₁-antitrypsin variant (PiZZ) associated with plasma deficiency. *Proc Natl Acad Sci USA* 1976;**73**:1324–8.
 - ¹¹ Nukiwa T, Satoh K, Brantly ML, et al. Identification of a second mutation in the protein coding sequence of the Z-type alpha₁-antitrypsin gene. *J Biol Chem* 1986;**261**:15989–94.
 - ¹² Cox DW, Woo SLC, Mansfield T. DNA restriction fragments associated with α_1 -antitrypsin indicate a single origin for deficiency allele PI Z. *Nature* 1985;**316**:79–81.
 - ¹³ Weidinger S, Jahn W, Cujnik F, Schwarzfischer F. Alpha-1-antitrypsin: evidence for a fifth PIM subtype and a new deficiency allele PI*Z_{Augsburg}. *Hum Genet* 1985;**71**:27–9.
 - ¹⁴ Whitehouse DB, Lovegrove JU, Hopkinson DA. Variation in alpha-1-antitrypsin phenotypes associated with penicillamine therapy. *Clin Chim Acta* 1989;**179**:109–16.
 - ¹⁵ Maniatis T, Fritsch EF, Sambrook J. *Molecular cloning: a laboratory manual*. New York: Cold Spring Harbor Laboratory, 1982.
 - ¹⁶ Nukiwa T, Brantly ML, Ogushi F, Fells GA, Crystal RG. Characterization of the gene and protein of the common α_1 -antitrypsin normal M2 allele. *Am J Hum Genet* 1988;**43**:322–30.
 - ¹⁷ Saiki RK, Gelfand DH, Stoffel S, et al. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. *Science* 1988;**239**:487–91.
 - ¹⁸ Kelsey GD, Povey S, Bygrave AE, Lovell-Badge RH. Species- and tissue-specific expression of human α_1 -antitrypsin in transgenic mice. *Genes Dev* 1987;**1**:161–71.
 - ¹⁹ Kelsey GD, Parkar M, Povey S. The human alpha-1-antitrypsin-related sequence gene: isolation and investigation of its expression. *Ann Hum Genet* 1988;**52**:151–60.
 - ²⁰ Feinberg AP, Vogelstein B. A technique for radio-labelling DNA restriction endonuclease fragments to high specific activity. Addendum. *Anal Biochem* 1984;**137**:266–7.
 - ²¹ Frants RR, Eriksson AW. Reliable classification of six PiM subtypes by separator isoelectric focusing. *Hum Hered* 1978;**28**:201–9.
 - ²² Cox DW, Billingsley GD, Mansfield T. DNA restriction-site polymorphisms associated with the alpha₁-antitrypsin gene. *Am J Hum Genet* 1987;**41**:891–906.
 - ²³ Abbott CM, McMahon CJ, Kelsey GD, et al. α_1 -Antitrypsin related gene (ATR) for prenatal diagnosis. *Lancet* 1987;**i**:1425–6.
 - ²⁴ Meisen C, Poller W, Olek K. DNA haplotyping of PI Z and M alleles within the German population. *Am J Hum Genet* 1988;**43**:978–9.
 - ²⁵ Mieli-Vergani G, Doherty DG, Donaldson PT, et al. HLA status in children with alpha-1-antitrypsin deficiency (AATd) (PiZ) and chronic liver disease (CLD). *Pediatr Res* 1987;**22**:98.
 - ²⁶ Garver RI, Mornex JF, Nukiwa T, et al. Alpha₁-antitrypsin deficiency and emphysema caused by homozygous inheritance of non-expressing alpha₁-antitrypsin genes. *N Engl J Med* 1986;**314**:762–6.
 - ²⁷ Feldmann G, Martin JP, Sesboue R, et al. The ultrastructure of hepatocytes in alpha-1-antitrypsin deficiency with the genotype Pi-. *Gut* 1975;**16**:796–9.
 - ²⁸ Sveger T. Prospective study of children with α_1 -antitrypsin deficiency: eight-year-old follow-up. *J Pediatr* 1984;**104**:91–4.
 - ²⁹ Sveger T. The natural history of liver disease in α_1 -antitrypsin deficient children. *Acta Paediatr Scand* 1988;**77**:847–51.
 - ³⁰ 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.
 - ³¹ Eriksson S. α_1 -Antitrypsin deficiency and liver cirrhosis in adults: an analysis of 35 Swedish autopsied cases. *Acta Med Scand* 1987;**221**:461–7.

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