Alpha₁ antitrypsin deficiency due to Pi null: clinical presentation and evidence for molecular heterogeneity

F J BAMFORTH AND N A KALSHEKER

**From the Department of Medical Biochemistry, University of Wales College of Medicine, Royal Infirmary, Cardiff CF2 1SZ.*

SUMMARY The proteinase inhibitor null (Pi-) allele is a rare cause of α_1 antitrypsin (AAT) deficiency. In three families, all the subjects with AAT deficiency due to PiZ- presented in early childhood with recurrent chest infections and wheezing presumably related to passive smoking. In Pi- the AAT gene is present and there is no evidence for a gene deletion. In one family a restriction fragment length polymorphism (RFLP) detected with the enzyme *Xba*I segregates with the Pi- allele. In a family where a consanguineous marriage occurred, the *Xba*I polymorphism segregates with the normal M1 allele rather than Pi-, suggesting that Pi- may have originated from M1. In contrast, a third family and 20 normal unrelated subjects do not show the RFLP.

It is estimated that about 50 000 people in the United Kingdom have AAT deficiency^{1,2} resulting mainly from two common protein variants, the S and Z. Each of these variants results from a single base mutation in the coding sequence of the gene for the normal M allele.^{3,4} The Pi null (Pi-) allele is a rare cause of deficiency which is associated with undetectable amounts of AAT.^{5,6} Subjects who have AAT deficiency develop pulmonary emphysema in their fourth or fifth decade.² Only a few patients with homozygous Pi- have been documented and these subjects present with pulmonary emphysema in their early 20s.^{5,6} The frequency of the Pi- allele in an American population has been estimated to be about 1.7 per 10 000⁷ and the frequency in the United Kingdom is not known. We have investigated the nature of the Pi- allele in three families who presented with AAT deficiency and related disease.

Case reports

CASE 1, FAMILY 1.4

This boy, aged three years, had recurrent episodes of bronchitis with wheezing from the age of three months, five episodes severe enough to warrant hospital admission. No persistent abnormality was apparent on chest x ray. At the age of nine months he was noted to have a palpable liver but no clinical

evidence of liver disease. His serum aspartate transaminase (AST) was persistently raised with a mean level of 70 IU/l. Serum transaminase levels at two years of age were near normal, with a mean AST of 45 IU/l and alanine transaminase (ALT) of 14 IU/l. Reference ranges are up to 28 IU/l for both enzymes. There was no family history of chest or liver disease. Both parents smoke more than 20 cigarettes per day and have done so for the past five years.

CASE 2, FAMILY 1.5

Case 2, aged one year six months, is the brother of case 1. His AAT deficiency was detected shortly after birth. He had a persistent productive cough from the age of one month. He developed bronchiolitis when three months old which required hospital admission. Chest x rays revealed shadowing in the upper lobe of the right lung which subsequently cleared. There was no clinical evidence of liver disease but he had mildly raised serum AST and ALT with mean levels of 62 and 57 IU/l respectively. This patient also suffers from eczema.

CASE 3, FAMILY 3.13

Case 3 is aged 45 years. He had a history of recurrent chest infections from the age of seven months and developed pneumonia at the age of three years. He was regularly absent from school because of recurrent chest infections and he was treated for asthma at this time. He was relatively

Received for publication 11 September 1986.

Revised version accepted for publication 8 December 1986.

asymptomatic from the age of 14 years onwards. At the age of 41 he developed pneumonia. He subsequently developed recurrent chest infections (about four episodes annually) which were worse in the autumn. These episodes were severe enough to keep him in bed for three to four days at a time. He has established emphysema. Serum transaminases were not measured in childhood and he has no evidence of liver dysfunction in later life. He smoked about 20 cigarettes a day for about 20 years until five years ago but now smokes a pipe. His father has always been a cigarette smoker who rolls his own tobacco.

CASE 4, FAMILY 2.10

This child, aged five years eight months, had neonatal hepatitis, described previously.⁸ Although she now has no clinical signs of liver disease, until recently she had persistently raised serum AST and ALT with mean levels of 91 and 76 IU/l respectively. Her liver function tests have returned to normal. At three years of age she developed asthma and had three to four episodes of bronchitis with wheezing each winter. She requires oral steroid therapy for her asthma. She recently had an episode of right middle lobe pneumonia. She also has mild ichthyosis. Her maternal grandfather died from chronic bronchitis. Both parents smoke five to 10 cigarettes per day.

Methods

AAT PI TYPES

The Pi typing was done by isoelectric focusing in agarose and immunoblotting⁹ and confirmed independently by the Protein Reference Unit in Sheffield by isoelectric focusing in polyacrylamide gels. The Pi typing was complemented by immunochemical quantification of serum AAT and the measurement of the elastase inhibitory activity of plasma, which is a measure of the functional activity of AAT.¹⁰

DNA RESTRICTION FRAGMENT STUDIES

DNA was prepared from anticoagulated blood.¹¹ DNA (10 µg) was digested to completion with restriction endonucleases *Bam*HI, *Eco*RI, *Hha*I, *Hinc*II, *Msp*I, *Pst*I, *Stu*I, *Taq*I, *Xba*I, and *Xho*I according to the manufacturer's instructions. The DNA fragments were separated on a 1% agarose gel and transferred to nitrocellulose filters by Southern blotting.¹² The conditions for prehybridisation and hybridisation were as previously described.¹³ Three DNA probes were used: (1) pAT 6-5, a generous gift from Dr S Woo of Baylor Medical College, Houston, Texas; (2) a1 4-8 RI a generous gift from Dr S Povey, University College, London; and (3)

NJ.¹⁴ Autoradiography was performed by exposing the filters to Fuji XR preflashed x ray film and intensifying screens at -70°C for two to seven days.

Results

The family pedigrees, their Pi types, and the *Xba*I polymorphism are shown in figs 1 to 3. The corresponding AAT concentrations and elastase inhibitory activity were compatible with the AAT Pi types (table).

Densitometric scanning (Quick Scan Junior, Helena Laboratories, Texas) of the signals obtained by autoradiography for the AAT gene did not reveal any differences in the intensities of the signals for the Pi-, M, or Z alleles (fig 4). This suggests that in the Pi-, the AAT gene is present and there is no detectable gene deletion. The AAT gene is shown diagrammatically in fig 5. For all of the enzyme

Family 1

FIG 1 Pedigree of family 1. ★ Subjects with *Xba*I polymorphism. ■ Subjects heterozygous for Pi-.

Family 2

FIG 2 Pedigree of family 2. ● Subjects heterozygous for Pi-.

used except *Xba*I, identical patterns were observed in the Pi-, PiM, and PiZ alleles by autoradiography of Southern blots, indicating that a potential gene deletion is also extremely unlikely. A polymorphism

Family 3

FIG 3 Pedigree of family 3 and genomic blots of DNA digested with *Xba*I. (Subject 13 did not show the *Xba*I polymorphism even after prolonged exposure.) m=molecular weight markers. ■ ● Subjects heterozygous for Pi-. **Xba*I polymorphism.

TABLE Serum AAT concentration, Pi types, and elastase inhibitory activity.

		Serum AAT (g/l) (0.9-2.2)	Pi type	Elastase inhibitory activity (UEI/ml) (9-19)
Family 1	1	1.1	M1Z	6.4
	2	1.14	M1-	8.0
	3	1.15	M1Z	7.0
	4	0.5	Z-	2.9
	5	0.4	Z-	2.4
Family 2	6	0.62	M2-	6.1
	7	1.13	M1Z	7.5
	8	1.57	M1M2	12.6
	9	1.75	M1M2	12.2
	10	0.09	Z-	3.5
Family 3	11	1.1	M1-	8.5
	12	0.8	M1Z	8.5
	13	0.2	Z-	<2.0
	14	1.6	M1M1	13.0
	15	1.8	M1M1	14.0
	16	1.0	M1Z	8.0
	17	1.1	M1Z	9.2

Reference ranges are given in parentheses.
UEI=units of elastase inhibited/ml serum.

in the AAT gene was demonstrated with *Xba*I which segregated with the Pi- allele in family 1 when probed with genomic clone pAT 6.5. The polymorphism was not seen in family 2, in 20 unrelated subjects with normal protein Pi types, and in three subjects with ZZ deficiency. In family 3, where a consanguineous marriage occurred, the *Xba*I polymorphism segregated with the normal M1 allele and not Pi- (fig 3). With *Xba*I, the size of the DNA fragments in the normal allele were 13.3 kb, 4.7 kb, and 2.4 kb, while subjects with the polymorphism had an additional band of 7.1 kb when their DNA was probed with pAT 6.5 (fig 3). The 7.1 kb band was consistent with the deletion of an *Xba*I site in intron IV (fig 5). The probable site for the mutation was confirmed by detection of the 7.1 kb band with a cDNA probe NJ which normally detects a 4.7 kb fragment at the 3' end of the gene. Because of the rarity of the Pi- allele it will be difficult to establish the frequency with which the *Xba*I polymorphism occurs in association with the Pi- allele.

Discussion

We describe three families with the Pi- allele in whom all members with the Pi type Z- have unusually early manifestations of respiratory disease associated with AAT deficiency. The occurrence of repeated chest infections in early childhood has not been previously documented in association with AAT deficiency. In a four year prospective Swedish study of 183 AAT deficient children, predominantly

FIG 4 (a) Southern blot. *EcoRI* digest of human DNA probed with pAT 6.5. AAT gene present in 9.8 kb fragment; additional band of 8.5 kb due to a gene related sequence. (b) Densitometric scanning of two *EcoRI* bands. The ratio of the signal intensities for AAT and gene related sequence were similar for Pi types Z-, MZ, and M- indicating that in Pi- the gene is present.

FIG 5 Diagram of the AAT gene and cleavage sites for *XbaI*. The arrow indicates the site of the polymorphism. Black areas denote coding sequences, open areas denote non-coding sequences.

Pi types ZZ and SZ, early respiratory disease was not a specific feature.¹⁵ In a follow up of these children at eight years old, 8% had evidence of asthma compared to an overall frequency of 2.7% in eight year olds in Sweden.¹⁶ In the families we have described, the presentation of recurrent chest infections may have been due to passive smoking which is thought to be associated with an increased incidence of respiratory tract infection.¹⁷ In all three families either one or both the parents were active smokers and in combination with AAT deficiency may contribute to early lung damage. There was some evidence of liver dysfunction in three of the four patients, presumably related to the Z allele they have inherited. Pi- deficiency is not usually associated with liver disease but there are insufficient data for an association to be excluded.

In agreement with previous reports we confirm that subjects with the Pi- allele do not show a detectable partial or complete deletion of the AAT gene.¹⁸ By electrophoresis in agarose gels it is difficult to separate DNA fragments which differ in size by a few nucleotides, so it is not possible to exclude totally a small deletion in the gene. In the families we have studied this possibility seems unlikely, as the use of a number of restriction enzymes which recognise DNA sequences in the AAT gene show identical patterns in the Pi- and Pi M alleles. It is still possible that a small deletion is present that cannot be resolved by current techniques. It seems probable that the Pi- allele results from a single point mutation in a critical region of the AAT gene and the abnormality may not be the same for all those with the Pi- allele. The deletion of the *Xba*I site in the AAT gene can be explained by a mutation in an intron sequence of the gene. As the polymorphism segregates with the M1 allele in one of the families, it seems likely that the null allele may have arisen from M1 and also that the polymorphism per se is not associated with decreased production of AAT.

It is also clear that there may be heterogeneity of Pi- since the *Xba*I polymorphism does not occur in another family with the allele. Currently, only family studies can confirm the presence of Pi- and better tests based on direct analysis of the gene should facilitate diagnosis. Although the frequency of the Pi- allele in the United Kingdom is not known, if there is a similar frequency to that observed in the American population, the incidence of PiZ- in the United Kingdom is about 1 in 500 000.

We thank the physicians and patients for their

cooperation. Mr S Nethercott is thanked for doing the functional assays. This work was supported by the Welsh Scheme for Health and Social Research.

References

- 1 Cook PJJ. The genetics of α_1 -antitrypsin: a family study in England and Scotland. *Ann Hum Genet* 1975;**38**:275-87.
- 2 Carrell R, Jeppson JO, Laurell CB, *et al*. Structure and variation of human α_1 -antitrypsin. *Nature* 1982;**298**:329-34.
- 3 Long GL, Chandra T, Woo SLC, Davie EW, Kurachi K. Complete sequence of the cDNA for human α_1 -antitrypsin and the gene for the S variant. *Biochemistry* 1984;**23**:4828-37.
- 4 Kidd VJ, Wallace RB, Itakura K, Woo SLC. α_1 -antitrypsin deficiency detection by direct analysis of the mutation in the gene. *Nature* 1983;**304**:230-4.
- 5 Talamo RC, Langley CE, Reed CE, Makino S. α_1 -antitrypsin deficiency: a variant with no detectable α_1 -antitrypsin. *Science* 1973;**181**:70-1.
- 6 Bernheim JL, Arnaud P, Cellier C, Pokroy N, Traeger J. Apparent total α_1 -antitrypsin deficiency. *Isr J Med Sci* 1976;**12**:678-85.
- 7 Cox DW, Billingsley GD, Smyth S. Rare types of α_1 -antitrypsin associated with deficiency. In: *Electrophoresis*. Berlin: Walter de Gruyter, 1981:505-10.
- 8 Burn J, Dunger D, Lake B. Liver damage in a neonate with alpha-1-antitrypsin deficiency due to phenotype Pi Z null(Z-). *Arch Dis Child* 1982;**57**:311-3.
- 9 Kalsheker NA, Warner JT, Watkins GL. Phenotyping α_1 -antitrypsin (α_1 -AT) variants by isoelectric focusing in agarose and immunoblotting. *Clin Chim Acta* 1985;**148**:157-60.
- 10 Klumpp T, Bieth JG. Automated measurement of the elastase-inhibitory capacity of plasma with a centrifugal analyser. *Clin Chem* 1979;**25**:969-72.
- 11 Kunkel LM, Smith DK, Boyer SH, *et al*. Analysis of human Y-chromosome-specific reiterated DNA in chromosome variants. *Proc Natl Acad Sci USA* 1977;**74**:1245-9.
- 12 Southern EM. Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J Mol Biol* 1975;**98**:503-17.
- 13 Hodgson I, Kalsheker N. DNA polymorphisms of the human α_1 antitrypsin gene in normal subjects and in patients with pulmonary emphysema. *J Med Genet* 1987;**24**:47-51.
- 14 Rogers J, Kalsheker N, Wallis S, *et al*. The isolation of a clone for human α_1 -antitrypsin and the detection of α_1 -antitrypsin in mRNA from liver and leukocytes. *Biochem Biophys Res Commun* 1983;**116**:375-82.
- 15 Sveger T, Thelin T. Four-year-old children with α_1 -antitrypsin deficiency. *Acta Paediatr Scand* 1981;**70**:171-7.
- 16 Sveger T. Prospective study of children with α_1 -antitrypsin deficiency: eight-year-old follow up. *J Pediatr* 1984;**104**:91-4.
- 17 Ferris BG, Ware JH, Berkey CS, *et al*. Effects of passive smoking on health of children. *Environ Health Perspect* 1985;**62**:289-95.
- 18 Garver RI, Mornex JF, Nukiwa T, *et al*. Alpha₁-antitrypsin deficiency and emphysema caused by homozygous inheritance of non-expressing α_1 -antitrypsin genes. *N Engl J Med* 1986;**314**:762-6.

Correspondence and requests for reprints to Dr N A Kalsheker, Department of Medical Biochemistry, University of Wales College of Medicine, Cardiff Royal Infirmary, Newport Road, Cardiff CF2 1SZ.