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 α₁ 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 XbaI segregates with the Pi– allele. In a family where a consanguineous marriage occurred, the XbaI 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 deficiency¹ 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 bronchitis 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
asymptomatic from the age of 14 years onwards. At
the age of 41 he developed pneumonia. He subse-
quently 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. 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 ichthyo-
sis. 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 immunoblotting and confirmed inde-
dently 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.

**DNA Restriction Fragment Studies**

DNA was prepared from anticoagulated blood.
DNA (10 μg) was digested to completion with
restriction endonucleases BamHI, EcoRI, HhaI,
HincII, MspI, PstI, StuI, TaqI, XbaI, and XhoI
according to the manufacturer’s instructions. The
DNA fragments were separated on a 1% agarose gel
and transferred to nitrocellulose filters by Southern
blotting. The conditions for prehybridisation and
hybridisation were as previously described. Three
DNA probes were used: (1) pAT 6-5, a generous
gift from Dr S Woo of Baylor Medical College,
Houston, Texas; (2) a 4-8 RI a generous gift from
Dr S Povey, University College, London; and (3)

**NJ.** 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 XbaI
polymorphism are shown in figs 1 to 3. The cor-
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 enzymes

**Fig 1 Pedigree of family 1. * Subjects with XbaI polymorphism. □ Subjects heterozygous for Pi—.**

**Fig 2 Pedigree of family 2. ● Subjects heterozygous for Pi—.**
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used except XbaI, 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 in the AAT gene was demonstrated with XbaI 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 XbaI polymorphism segregated with the normal M1 allele and not Pi- (fig 3). With XbaI, 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 XbaI 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 XbaI 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

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

<table>
<thead>
<tr>
<th></th>
<th>Serum AAT (gil) (9-19)</th>
<th>Pi type</th>
<th>Elastase inhibitory activity (UEI/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family 1</td>
<td>6-4</td>
<td>M1Z</td>
<td></td>
</tr>
<tr>
<td>Family 2</td>
<td>6-1</td>
<td>M1Z</td>
<td></td>
</tr>
<tr>
<td>Family 3</td>
<td>8-5</td>
<td>M1Z</td>
<td></td>
</tr>
</tbody>
</table>

Reference ranges are given in parentheses.

UEI=units of elastase inhib/1ml serum.

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

TABLE Serum AAT concentration, Pi types, and elastase inhibitory activity.
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 Xbal. The arrow indicates the site of the polymorphism. Blocked areas denote coding sequences, open areas denote non-coding sequences.
Pi types ZZ and SZ, early respiratory disease was not a specific feature. 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. 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. 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. 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 XbaI 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 XbaI 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.

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References

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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.