What is Pi (proteinase inhibitor) null or PiQO?: a problem highlighted by the α_1 antitrypsin Mheerlen mutation

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Abstract

α_1 antitrypsin deficiency is associated with predisposition to the development of pulmonary emphysema and childhood cirrhosis. There are two common deficiency alleles in the European population, proteinase inhibitor (Pi) Z and S. In addition, there are rare Pinull or QO variants which can be difficult to diagnose. A family assigned as having the PiQO allele by AAT protein quantification and isoelectric focusing was shown by DNA sequencing to have the PiMheerlen mutation (Pro[369]-Leu). This highlights the difficulties of diagnosis of PiQO.

α_1 antitrypsin (AAT) is the major serine proteinase inhibitor in human plasma.[1] One of the main physiological functions of AAT is protection of the lower respiratory tract from damage by the proteinase human neutrophil elastase (HNE).

During inflammation 'primed' neutrophils leave the circulation and release their contents.[2] These include HNE which is capable of degrading the connective tissue of the lung and whose action is controlled by AAT. Consequently, subjects with AAT deficiency have an increased risk of developing progressive lung damage particularly if they smoke. Pulmonary emphysema usually develops in the fourth to fifth decade of life.[1]

AAT deficiency affects about 1 in 3000 of northern Europeans and a major deficiency allele is referred to as proteinase inhibitor (Pi) Z. PiZ also predisposes to liver disease in childhood as AAT protein accumulates in hepatocytes. Most Pi variants are detected by isoelectric focusing (IEF) which relies on the properties of charge to distinguish the variants. The normal allele is referred to as M and there are rare variants including Pinull or PiQO. The null variants arise from a number of different mutations in the AAT gene which are characterised typically by undetectable AAT protein in the plasma. In contrast, although PiZ subjects present with deficiency they have approximately 10% of the mean AAT concentration of the normal PiM allele.

Subjects with PiQO appear to present with lung disease one or two decades earlier than those with PiZ,[3] suggesting that the low serum AAT concentrations associated with PiZ may be sufficient to confer some protection to the lower respiratory tract.

Currently the detection of PiQO requires several criteria including undetectable serum AAT associated with the allele, a pattern on isoelectric focusing which does not show a detectable abnormality associated with deficiency, and family studies to confirm the mendelian inheritance of the allele. The first two criteria are influenced by a number of variables. For example, a variety of mutations have been described in the AAT gene which give rise to PiQO.[4][5] Many of these mutations result either from premature termination of translation or a mutation which results in a profound conformational change in the tertiary structure of the protein resulting in an unstable protein which is presumably degraded intracellularly. What is not clear about these mutations is whether protein is secreted at all from hepatocytes and, if it is secreted, whether the protein is stable in the blood in which it is measured. Fresh plasma samples analysed immediately after collection may show detectable AAT which may be undetectable if the samples are stored for a few hours.

We present a family with AAT deficiency where the allele was a presumed PiQO by IEF and AAT quantification and the coding sequences of the AAT gene were determined in all affected family members.

Methods

IEF
This was performed as previously described.[6]

AMPLIFICATION AND DNA SEQUENCING
Exons II to V of the AAT gene were amplified with the oligonucleotides used previously[7] and sequencing was also performed as previously described.[7]

FAMILY STUDY
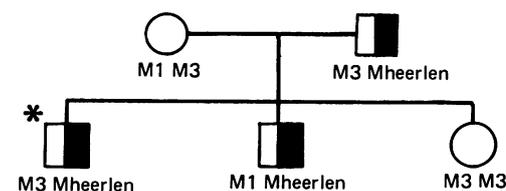The family pedigree is shown in fig 1. The 24 year old proband presented three years ago

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Figure 1   Family pedigree showing the PiMheerlen phenotypes. The proband is indicated by an asterisk.
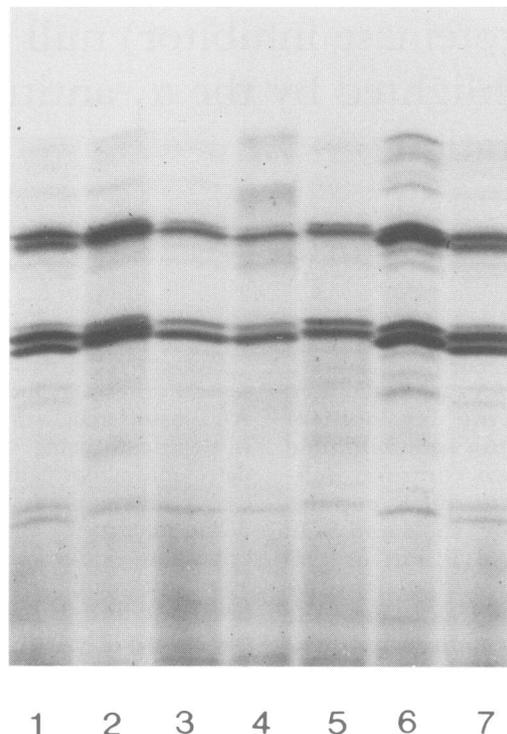
Figure 2   The IEF patterns for members of the PiMheerlen family (pH range of 4·2 to 4·9). From left (1) M1M2, (2) M1M3 = mother, (3) M3Mheerlen = father, (4) M3Mheerlen = proband, (5) M1Mheerlen = son, (6) M3M3 = daughter, and (7) M1M2. Anode is at the top.
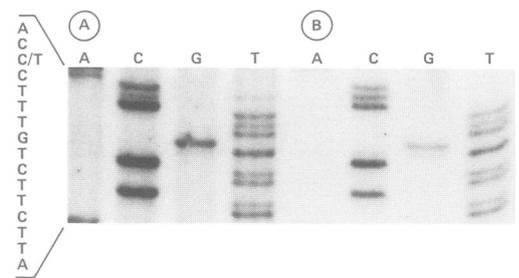

Figure 3   Exon V sequence in the proband. (A) The C to T mutation corresponding to the Pro[369]-Leu of the Mheerlen mutation and (B) a normal sequence are shown.

with respiratory disease and for the first time had difficulty in breathing. He was an active sportsman and a non-smoker. He was diagnosed as having bronchial asthma. His father also had a long standing cough and chronic respiratory disease. The AAT concentrations were measured by radial immunodiffusion. Of note is the occurrence of the Mheerlen mutation in the brother of the proband who is apparently well.

Results

The IEF and quantifications of AAT pattern are shown in fig 2 and the table, respectively. The sequencing of exon V is shown in fig 3 in a heterozygote who had the presumed null allele. There was a C to T mutation altering codon 369 from CCC to CTC. This would have the effect of altering the codon from proline to leucine. This corresponds to the previously described variant PiMheerlen.[8] The normal M subtypes M1 and M3 were confirmed by DNA sequencing. The codons for arginine at position 101 and glutamic acid at position 376 corresponded to the genotype expected for the M1 allele, and arginine at

position 101 and aspartic acid at position 376 corresponded to the M3 allele. The Mheerlen mutation occurs on the background of the normal M1 Val[213] allele.

Discussion

This report highlights the potential problems associated with the diagnosis of the null allele. The PiQO alleles of AAT characterised hitherto have been heterogeneous. We describe a family with PiMheerlen, a previously characterised deficiency variant of AAT.[8] The interesting aspect of this study is the original assignation of PiQO on the basis of IEF and protein quantification. In the original description of PiMheerlen, by IEF there were no detectable banding patterns corresponding to AAT on direct staining and the banding pattern could only be detected by immunoblotting.[9] The latter technique is not usually performed and indeed it is not clear in all the cases of putative homozygous nulls described whether immunoblotting was performed. For a strict definition of PiQO in the homozygous state, there should be no detectable AAT by immunoblotting.

It is apparent now that severe deficiency states arising from alleles other than PiZ may be the result of a variety of mutations. The PiQO is more likely to present in the heterozygous state and could easily be missed by IEF. The identification of specific mutations may make it feasible to consider the use of PCR and allele specific oligonucleotides for the direct detection of equivocal null alleles.

The tertiary structure of cleaved AAT is known and the structure of native AAT has been inferred. Mutations that cause folding abnormalities of the protein should perhaps not be designated PiQO unless it is certain that no protein is secreted from cells that synthesise the abnormally folded protein. This should require unequivocal evidence of the absence of detectable AAT in fresh plasma, the absence of a banding pattern corresponding to AAT by IEF and immunoblotting, and the failure to detect intracellular AAT.

Serum AAT concentrations and Pi types.

	AAT concentration (g/l)	Protein type
Mother	2·6	M1M3
Father	1·25	M3Mheerlen
Proband	1·25	M3Mheerlen
Brother	1·35	M1Mheerlen
Sister	4·70	M3M3

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2 Crystal RG. α₁-antitrypsin deficiency, emphysema and liver disease. J Clin Invest 1990;85:1343–52.
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