The PI System: Genetic Variation, Forensic Application and Clinical Aspects

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INTRODUCTION

In the past two decades few polymorphic plasma proteins have attracted more interest among scientists than the PI (protease inhibitor) system of alpha-1-antitrypsin (α1AT). There are several reasons for this interest: (1) The PI system was, from the beginning, associated with disease; (2) With the development of high-resolution techniques, it was shown that the PI system comprises a large number of codominant alleles, and (3) The findings of linkage between the PI system and the Gm system of human IgG has contributed to chromosome mapping.

On the basis of protein sequence homology, α1AT has been recognized as one member of the large serine protease inhibitor (serpin) gene family (Hunt and Dayhoff 1980). Although called antitrypsin, its prime role is an inhibitor of neutrophil leucocyte elastase, and for this reason it is alternatively called alpha-1-protease inhibitor. The PI locus has been mapped to chromosome 14q32.1, near the immunoglobulin heavy chain gene cluster (Rabin et al. 1986; Purrello et al. 1987). The α1AT gene is composed of seven exons spanning 12.2 kb genomic DNA (Long et al. 1984). α1AT is a highly polymorphic glycoprotein consisting of a single polypeptide chain of 394 amino acids and a carbohydrate content of 12% (Carrell et al. 1982). The 52-kD protein is synthesized primarily in hepatocytes. There are three carbohydrate attachment sites at asparagine residues 46, 83 and 247, which result in heterogeneous molecular species. For classification of PI phenotypes isoelectric focusing (IEF) is a widely used technique (Weidinger 1992).

GENETIC VARIATION

Alpha-1-antitrypsin shows a considerable amount of genetic variability. To date, approximately 100 variants have been identified in the PI system by either IEF of serum and/ or sequence analysis (Faber et al. 1994). The variants can be conveniently categorized into four groups: normal, deficient, null, and dysfunctional. Most of the PI alleles (including the M family) are associated with normal concentrations of α1AT. PI M, which can be divided into 10 subtypes (M1, M1M2, M2, M1M3, M2M3, M3, M1M4, M2M4, M3M4, and M4) by high resolving IEF, is the most common phenotype in all populations (frequency >90%). The nomenclature is very complex and is based on the focusing position of the α1AT in IEF gels (Cox et al. 1980). The anodal variants are designated from B to L, and variants cathodal to M are designated from N to Z. In contrast to the normal alleles a number of deficiency alleles (including null alleles and deficient M alleles) in the PI system is associated with reduced (or no detectable) serum α1AT levels. All rare alleles are also named with the birthplace of the earliest known carrier. Figure 1 shows the band patterns of several normal and deficient PI phenotypes which were obtained by IEF and immunoprinting.

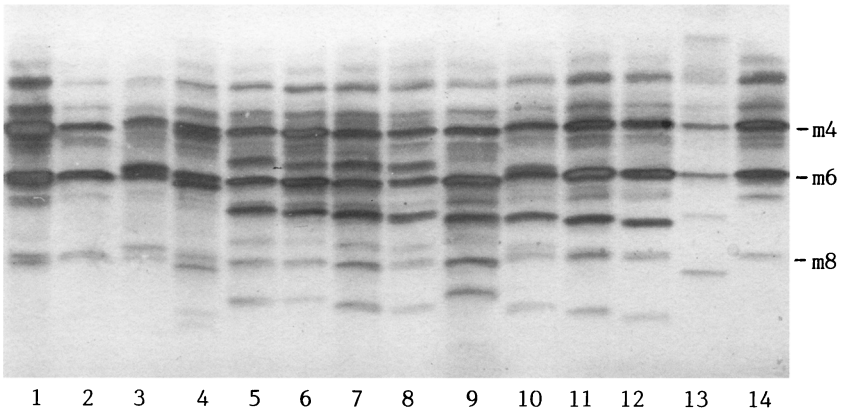

Figure 1. Banding patterns of several normal and deficient PI phenotypes obtained from sera by high-resolution IEF in a narrow pH gradient (pH range 4.2-4.9), followed by immunoprinting with a monospecific α1AT-antiserum. The isoprotein zones m4, m6, and m8 are indicated. Anode at the top. Lanes: (1) M1M2; (2) M3riedenburg-Q0riedenburg; (3) M3-Lfrankfurt; (4) M2-Loffenbach; (5) M1-P; (6) M1-Pduarte; (7) M3-Psaint albans; (8) M1-Pdonauwoerth; (9) M3-Smunich; (10) M1-V; (11) M1-S; (12) M1-T; (13) M1-Z; (14) M1-Mpalermo.

Distribution of the PI alleles has now been determined in many populations (Table 1). The frequency of PI*M1 is the highest in all populations studied. M2 and M3 are the next most frequent. The additional subtype allele PI*M4 has been described in several ethnic groups, at a frequency of 0.01 to 0.05. The PI*S allele is rare or absent in black and oriental populations, highest in Spain and Portugal, next most frequent in other parts of Europe. PI*Z, which is the most common deficiency allele, seems to be absent in oriental and black populations.

Table 1. PI allele frequencies in selected populations

Population origin	No. tested	PI alleles M1	M2	M3	M4	S	Z	Other
Denmark	909	0.728	0.136	0.082	-	0.022	0.023	0.009
Netherlands	357	0.679	0.147	0.081	0.047	0.029	0.013	0.004
Germany	752	0.689	0.165	0.090	0.018	0.017	0.013	0.008
France	1030	0.667	0.143	0.101	-	0.063	0.018	0.008
Italy	965	0.661	0.162	0.107	-	0.024	0.041	0.005
Spain	340	0.596	0.156	0.112	0.013	0.112	0.007	0.004
U.S.(white)	904	0.724	0.137	0.095	-	0.023	0.014	0.007
U.S.(black)	549	0.981	-	-	-	0.015	0.004	-
Japan	746	0.785	0.153	0.062	-	-	-	-
China	1010	0.709	0.209	0.070	-	-	-	0.012

In addition to the extensive variation found in the protein by electrophoretic methods, further variation is found in the DNA sequence, as recognized by restriction enzymes, producing RFLP's (restriction fragment length polymorphism). Several genomic sequences have been obtained by using the probes 4.6 and 6.5. The allele frequencies for DNA polymorphisms are shown in Table 2.

Table 2. Allele frequencies for DNA polymorphisms

Probe	Restriction enzyme	Alleles (kb)	Allele frequency*			No. of haplotypes	PIC
			+	-	0		
4.6	Sst I	1.8, 1.9	0.69	0.31	-	2	0.33
	Msp I	0.95, 0.98	0.47	0.53	-	2	0.38
	Ava II	0.9, 1.1	0.65	0.35	-	2	0.35
6.5	Mae III	2.3, 2.5	0.71	0.29	-		
	Mae III(3')	0.5, 0.7	0.65	0.35	-	4	0.33
	Ava II(5/7)	0.48, 0.68	0.22	0.78	-	4	0.44
	Ava II(1/4)(3')	0.72, 2.7	0.29	0.71	-		
	Taq I	1.4, 2.0	0.97	0.03	-		
	Taq I(3')	4.8, 6.7, 0	0.53	0.26	0.21	4	0.58
	Eco RI(3')	5.7, 8.6	0.23	0.77	-	2	0.29

*) + = Presence of restriction site; - = absence of site; 0 = no fragment.
PIC = Polymorphism information content.

The probe 4.6 includes the first exon of α1AT, and the probe 6.5 includes the coding region and some of the 3' flanking region (Kidd et al. 1983). Three polymorphisms have been described with probe 4.6, using the restriction enzymes Sst I, Msp I, and Ava II. Seven polymorphisms were detected with probe 6.5. Ava II detects polymorphisms both in the α1AT gene and the homologous sequence and was the first enzyme shown to produce a unique DNA haplotype for PI ZZ individuals (Cox et al. 1985).

FORENSIC APPLICATION

The considerable number of alleles, together with the highly reproducible techniques available for phenotyping and genotyping, make the PI system

Table 3. Comparison of highly polymorphic serum markers

System	Chromosomal assignment	Method	No. of alleles*	Silent alleles	Exclusion chance (%)
HP	16q	PAGIF	4 (> 20)	+	30.6
GC	4q	PAGIF/IP	3 (>120)	+	29.8
PI	14q	PAGIF	6 (> 90)	+	27.1
TF	3q	PAGIF	3 (> 30)	+	19.5
ORM1	9q	PAGIF/IP	3 (> 30)	+	21.0
AHSG	3q	PAGIF/IP	2 (> 30)	?	18.5
PLG	6q	AGIF/IF	2 (> 20)	+	19.8
F13B	1q	AGIF/IF	3 (> 20)	?	22.7

Abbreviations: HP = haptoglobin; GC = group-specific component; PI = protease inhibitor; TF = transferrin; ORM = orosomucoid; AHSG = alpha2-HS-glycoprotein; PLG = plasminogen; F13B = B subunit of coagulation factor XIII.
PAGIF = polyacrylamide gel isoelectric focusing; AGIF = agarose gel isoelectric focusing; IF = immunofixation; IP = immunoprinting.
*) No. of rare alleles in parenthesis.

valuable for forensic haemogenetics, especially paternity testing. In fact, a so-called classical exclusion constellation at the PI locus is considered to disprove paternity. The single exclusion chance in paternity cases is 27.1%, indicating that the protease inhibitor is a very powerful marker in forensic individualization and paternity testing. Table 3 shows a comparison of highly polymorphic serum markers in forensic haemogenetics. The theoretical exclusion probability of PI is similar to that of GC or vitamin D-binding protein (DBP), which is the highest polymorphic serum marker. Silent alleles were revealed for most of these proteins, and may lead to erroneous assumption of inverse homozygosity. Chromosomal assignment either from somatic cell hybrids or recombinant DNA has shown, with exceptance of TF and AHSG, that the structural genes of these markers are located on distinct chromosomes.

Only in cases of low producing alleles errors may arise in precise subtyping by IEF if there are heterozygous carriers with a highly deficient (null allele, deficient M allele) and one normal allele. Figure 2A presents PI phenotypes of a case of paternity in which an inverse homozygosity was found between the child (PI M1) and the alleged father (PI M3). The man could not

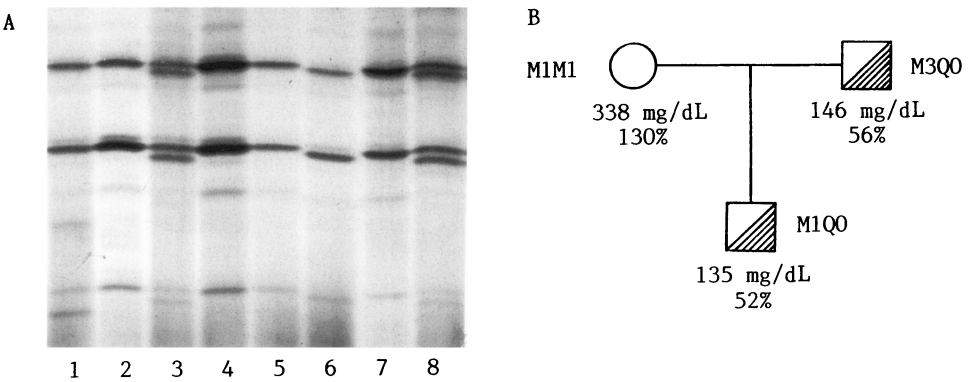

Figure 2. (A) Demonstration of PI M subtypes of mother, child, putative father and controls following high-resolution IEF of serum samples. Anode at the top. Lanes: 1= M1-Z; 2= M1M1; 3= M1M2; 4= M1M1 (mother); 5= M1Q0 (child); 6= M3Q0 (putative father); 7= M1M1; 8= M1M2. (B) Transmission of the PI*Q0 allele and α1AT serum levels.

be excluded as the father of this child in 26 other genetic systems. Biostatistical evaluation of the combined data yielded a paternity probability of W $\triangleq$ 99.99%. Because the band patterns of the two individuals were clearly reduced, it was assumed that they are PI M1Q0 and PI M3Q0 heterozygotes. This was consistent with their serum levels of 52% and 56% of normal, respectively (Figure 2B).

For characterization of the PI*Q0 allele direct sequencing of all exons after PCR amplification of genomic DNA was used (Faber et al. 1990). This method failed to reveal any molecular defect causing the PI Q0 phenotypes in these two individuals. DNA restriction analysis with two genomic probes of the α1AT gene revealed that the PI Q0 phenotype is caused by a major deletion of the gene (Poller et al. 1991). The newly deficiency allele has been preliminary named Q0riedenburg. The frequency of the PI*Q0riedenburg as well as other null alleles in the general population may be expected to be very low (0.001-0.0001).

CLINICAL ASPECTS

Alpha-1-antitrypsin deficiency is one of the most common inherited metabolic disorders. About 1 in 3000 of northern Europeans are homozygous for the PI Z mutation (Glu342 GAG →Lys AAG) (Cox 1989; Brantly et al. 1988). In comparison with some other disorders α1AT deficiency is only slightly less common than cystic fibrosis and it is more common than congenital adrenal hyperplasia (21-hydroxylase deficiency) and phenylketonuria (Table 4).

Table 4. Prevalence of inherited disorders

Disorder	Frequency
Cystic fibrosis	1/ 2 000
Alpha-1-antitrypsin deficiency	1/ 3 000
Congenital adrenal hyperplasia	1/ 7 000
Phenylketonuria	1/10 000
Sickle cell anemia	1/10 000
Cysteinuria	1/15 000
Galactosemia	1/40 000

Genetic deficiency of α1AT predisposes for the development of liver cirrhosis in early childhood (Sharp et al. 1969), and chronic degenerative lung disease in early adult life (Eriksson 1965). The most common sign of liver abnormality associated with α1AT deficiency is the "neonatal hepatitis syndrome". A deficiency should always be considered in a child with prolonged jaundice of unexplained origin, and PI typing should be an early diagnostic procedure. From 14 to 29% of infants with neonatal hepatitis have been found to possess PI ZZ (Moroz et al. 1976). Homozygote PI Z individuals have only about 15% of the normal α1AT level and a 20-30 fold increased risk of developing chronic obstructive pulmonary disease, specifically emphysema. In patients were shown that the basal lung regions are most severely affected. Although the majority of patients have emphysema, there are some symptoms of chronic bronchitis or bronchial asthma.

Longitudinal studies have demonstrated that PI ZZ individuals have reduced survival. The chance of being alive at the age of 50 is about 52%, compared with about 93% for the general population (Crystal et al. 1989). For those with a history of cigarette smoking, life expectancy is reduced by a further 10 years because of accelerated chronic degenerative lung disease. Heterozygotes of the type PI MZ are thought to have a moderately increased risk but only if they smoke. In addition to PI Z, there are a number of rare deficient PI M and PI null variants which are associated with diseases in the homozygous or compound heterozygous state.

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