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Pi Subtyping by Isoelectric Focusing: Further Genetic Studies and Application to Paternity Examinations

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Summary. Genetic variation of the protease inhibitor (Pi) α_1-antitrypsin was analyzed by isoelectric focusing on polyacrylamide gels in a sample of 347 unrelated individuals from Southern Germany. Six common subtypes of Pi M were observed as well as the relatively frequent variants Pi S and Pi Z and the rare variants Pi T, Pi < L, Pi L, Pi I and Pi F. Also, a variant called Pi Z1 was found. The frequency of alleles in this sample was $Pi^{M1} = 0.6917$, $Pi^{M2} = 0.1686$, $Pi^{M3} = 0.0865$, $Pi^{S} = 0.0230$, $Pi^{Z} = 0.0187$, and $Pi^{*} = 0.0115$. In 82 families the distribution of Pi types was in agreement with an autosomal codominant mode of inheritance. The application of Pi classification in cases of disputed paternity is discussed.

Key words: Serum groups, α_1-antitrypsin – Pi-subtypes, isoelectric focusing – Paternity examinations, Pi-subtypes

Zusammenfassung. Die genetischen Variationen des Protease-Inhibitors (Pi) α_1-Antitrypsin wurden mit Hilfe der Isoelektrofokussierung in einer Stichprobe von 347 nicht verwandten Personen aus Süddeutschland untersucht. Es wurden sechs häufige Pi M-Untergruppen und die relativ häufigen Varianten Pi S und Pi Z differenziert; zudem fanden sich die seltenen Varianten Pi T, Pi < L, Pi L, Pi I, Pi F sowie eine als Pi Z1 bezeichnete Variante. In dieser Stichprobe wurden folgende Allelfrequenzen berechnet: $Pi^{M1} = 0{,}6917$, $Pi^{M2} = 0{,}1686$, $Pi^{M3} = 0{,}0865$, $Pi^{S} = 0{,}0230$, $Pi^{Z} = 0{,}0187$ und $Pi^{*} = 0{,}0115$. In 82 Familien fand sich keine Abweichung vom angenommenen autosomal kodominanten Vererbungsmodus. Die Verwendbarkeit des Pi-Systems für die Paternitätsbegutachtung wird diskutiert.

Schlüsselwörter: Pi-Untergruppen, Isoelektrofokussierung – Vaterschaftsbegutachtung, Pi-Untergruppen – Blutgruppen, α_1-Antitrypsin

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Alpha$_1$-antitrypsin is the most prominent protease inhibitor (Pi) of human plasma. At least 25 different inherited Pi variants have been demonstrated by various electrophoretic procedures (Fagerhol and Laurell 1970; Cox and Celhoffer 1974; Johnson 1976). Application of the method of isoelectric focusing on polyacrylamide gels (PAGIF) revealed further genetic heterogeneity (Allen et al. 1974; Arnaud et al. 1975; Kueppers 1976; Genz et al. 1977; Klasen et al. 1977; Frants and Eriksson 1978; Kühnl and Spielmann 1978). By this method, six common PiM subtypes can be distinguished which are called PiM1, PiM1M2, PiM2, PiM1M3, PiM2M3, and PiM3. Family studies indicate that these six subtypes are determined by three alleles Pi^{M1}, Pi^{M2}, and Pi^{M3} (Frants and Eriksson 1978; Kueppers and Christopherson 1978; Kühnl and Spielmann 1978; Cleve et al. 1979). PiZ in the homozygous state is associated with severe chronic obstructive pulmonary disease (Eriksson 1965) or with childhood liver cirrhosis (Sharp 1973). Also, a rare allele Pi^0 (null) has been described (Talamo et al. 1973).

In this study an improved separatory procedure is used. We provide data on the distribution of Pi subtypes and Pi variants in a sample of unrelated individuals from Southern Germany. Distribution of Pi types in 82 families is demonstrated. An unusual phenotypic variant of PiZ is presented. We discuss also the application of the Pi classification in cases of disputed paternity.

Material and Methods

Blood was collected from healthy individuals who were residents from Southern Germany. They were investigated in the course of paternity examinations. Sera were analyzed freshly and without prior treatment. Isoelectric focusing was carried out on thin-layer polyacrylamide gels (PAGIF). Gels (245 × 110 × 1 mm) were prepared from 5 ml acrylamide solution (28%), 5 ml N,N′-methylenbisacrylamide solution (2%), 1 ml Servalyt pH 4–4.5, 0.5 ml Servalyt pH 4–5, 4 g saccharose, and 20 ml aqua dest. The mixture was freed from air bubbles by evacuation for 5 min. One milliliter ammonium persulphate solution (1%) was added. The mixture was filled into a prepared gel mould (LKB) with syringe. The gels polymerized within 30 min. Five microliter serum was applied on filter paper and placed on the gel 2 cm off the cathodal edge of the plate. The Multiphor chamber of LKB was used with the cooling system at a temperature of +10° C for the circulating fluid. At 40 mA and 24 W with a maximum of 1,600 V isoelectric focusing was carried out for 180 min. At the cathode 1 M glycine, at the anode 1 M H_3PO_4 was employed. Some gels were analyzed by immunofixation with monospecific α_1-antitrypsin-antisera (Atlantic Antibodies from Merz + Dade) soaked onto cellulose acetate strips and applied to the gel for 2 min immediately following the isoelectric separation. Fixation of proteins was accomplished by precipitation with sulfosalicylic acid for 30 min. Gels were stained with Coomassie Brillant Blue R 250.

Results and Discussion

In Table 1 the distribution of Pi phenotypes in 347 unrelated individuals from Southern Germany is shown. The observed distribution and the distribution expected at population equilibrium are in close agreement. The phenotype- and allele frequencies in this sample are different to the distributions reported earlier from our group only in two minor respects: In our first study (Genz et al. 1977) a relative deficit of the subtype M1M3 was observed which was not found in this

Table 1. Distribution of Pi phenotypes and Pi alleles in a sample from Southern Germany

Phenotypes	Observed		Expected		Allele frequencies
	n	%	*n*	%	
PiM1	171	49.28	166.02	47.84	$Pi^{M1} = 0.6917$
M1M2	79	22.77	80.93	23.32	$Pi^{M2} = 0.1686$
M1M3	35	10.09	41.52	11.97	$Pi^{M3} = 0.0865$
M2	9	2.59	9.86	2.84	$Pi^{S} = 0.0230$
M2M3	13	3.75	10.12	2.92	$Pi^{Z} = 0.0187$
M3	3	0.86	2.60	0.75	$Pi^{*} = 0.0115$
M1S	9	2.59	11.04	3.18	
M2S	4	1.15	2.69	0.78	
M3S	3	0.86	1.38	0.40	
M1Z	10	2.88	8.98	2.59	
M2Z	1	0.29	2.19	0.63	
M3Z	2	0.58	1.12	0.32	
M1Z1	2				
M1T	1				
M2<L	1				
M1L	1	2.31	8.55	2.46	
M1I	1				
M2I	1				
M3F	1				
Total	347	100.00	347.00	100.00	

$\Sigma\chi^2 = 2.3037$, df = 5, $P > 0.20$; Pi* = Frequency of rare Pi alleles

Note: Phenotypes of Pi^{S}, Pi^{Z} and Pi* were combined for χ^2 calculation. PiZ1 has recently been found in a child and his presumptive father. Further genetic studies indicate the existence of further suballeles of Pi^{M} which are called Pi^{M4} and Pi^{M5}

sample presumably due to more complete ascertainment of this class as a consequence of the improved separatory procedure. In our second study (Cleve et al. 1979) the frequency for Pi^{Z} was unexpectedly low, probably caused by an artefact, viz., the destruction of PiZ in older serum specimen as a consequence of longer storage and repeated freezing and thawing. The distribution found in this study and given in Table 1 is in close agreement with the findings of Kühnl and Spielmann (1978) in a sample from Hessen/FRG. Apparently, there are no significant regional differences in the German population as far as the PiM subtypes are concerned. The subtype distribution is also similar in the Dutch population (Klasen et al. 1977; Frants and Eriksson 1978). Other European populations or populations of European origin may have slightly different distributions (Frants and Eriksson 1978; Kueppers and Christopherson 1978).

In Table 2 the results of a family study are summarized. In 82 families with a total of 82 children the distribution of PiM and Pi phenotypes corresponded to an autosomal codominant mode of inheritance. Including this study, 273 families with a total of 397 children have been analyzed for the inheritance of PiM subtypes and Pi types at the present time. The results confirm fully the genetic hypothesis.

Table 2. Distribution of PiM subtypes in 82 parents with a total of 82 children

Parents	*n*	Children										
		M1	M1M2	M1M3	M2	M2M3	M3	M1S	M2S	M1Z	M3I	SZ
M1 × M1	17	17										
M1 × M2	2		2									
M1 × M3	1			1								
M1 × M1M2	11	5	6									
M1 × M1M3	8	4		4								
M1 × M2M3	5		2	3								
M1M2 × M2	3		1		2							
M1M2 × M1M2	5	2	2		1							
M1M2 × M1M3	4	1	1	0		2						
M1M2 × M2M3	4		2	0	1	1						
M1M3 × M1M3	2	1		1			0					
M1M3 × M2M3	1		1	0		0	0					
M1 × M1S	2	0						2				
M1 × M2S	1		0					1				
M2 × M1S	1		1						0			
M1M2 × M1S	1	0	1					0	0			
M1M2 × M2S	1		0		0			0	1			
M1M3 × M2S	1		1			0		0				
M1M3 × M3S	2			0			1	1				
M1 × M1Z	2	2								0		
M1 × M2Z	1		0							1		
M1M2 × M1Z	2	1	1							0		
M1M3 × M1Z	1	1		0						0		
M2M3 × M1Z	1		0	1								
M1S × M3Z	1			0						0		1
M1 × M1I	1	1										
M3S × M2I	1					0			0		1	
Total	82	35	21	10	4	3	1	4	1	1	1	1

In two different cases of disputed paternity an unusual Pi phenotype was observed. We have named this phenotypic variant PiZ1 because of its similarity to PiZ. As shown in Figs. 2 and 3 PiZ1 is characterized by a band slightly more cathodal than PiZ and, in addition, a broad cathodically located band. These bands were observed together with PiM1. The results were reproducible on repeated examinations and, in one case, on repeat samples from the same individual. The broad extra band reacts with a specific α_1-antitrypsin-antiserum on immunofixation (Fig. 3). A family study will be carried out. Kühnl (pers. commun. 1980) has observed a similar phenotypic variant for which he considers a non-genetic origin.

In Figs. 1–3 the Pi phenotypes are illustrated. In Fig. 1 the six common PiM subtypes are shown and the variants PiS and PiT. The arrows point to the M3 bands in the so-called M8 region, the slight differences indicate that further heterogeneity of M3 may exist. In Fig. 2 several phenotypes of the variants PiS, PiZ and PiI are shown together with PiM1Z1. In Fig. 3 the patterns obtained after

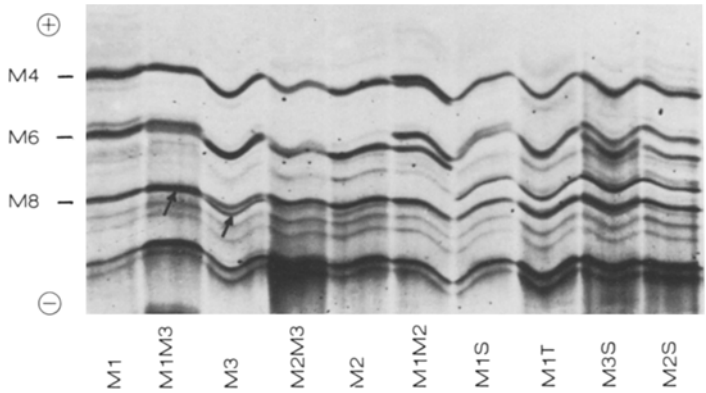

Fig. 1. Demonstration of Pi phenotypes by isoelectric focusing on polyacrylamide gels with a pH range of 4–5. Shown are the six common Pi M subtypes and the variants Pi S and Pi T. The arrows point to the Pi M3 bands (see text)

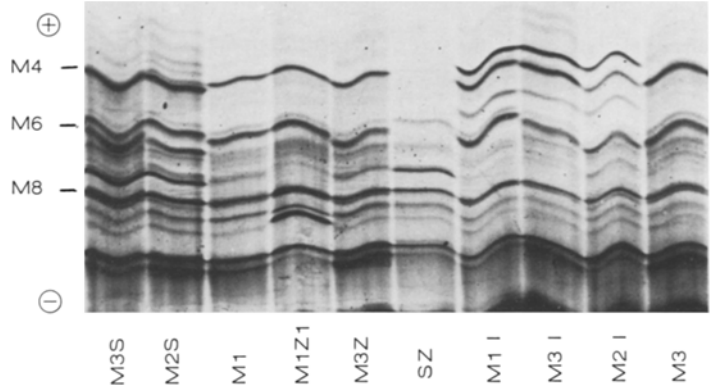

Fig. 2. Isoelectric focusing for Pi classification; illustrated are several Pi phenotypes of the variants S, Z, I, and Z1

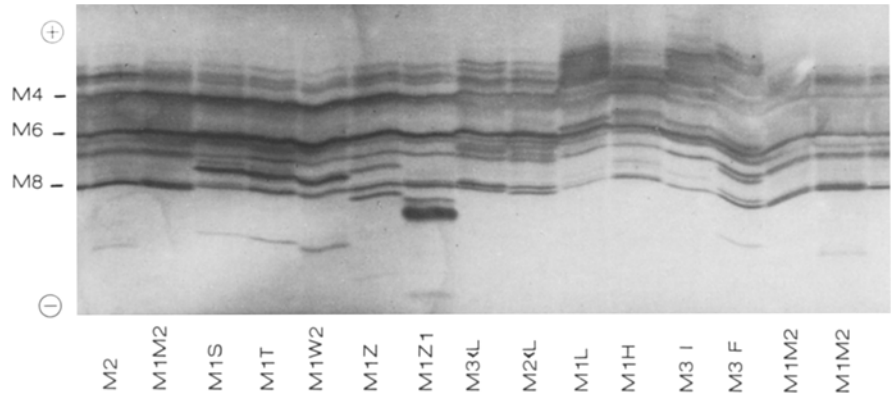

Fig. 3. Immunofixation of Pi phenotypes after isoelectric focusing at pH 4–5

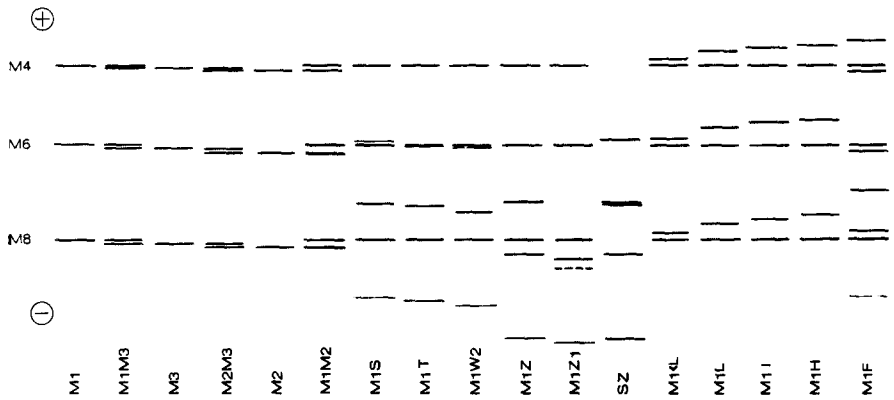

Fig. 4. Schematic presentation of Pi phenotypes as observed by polyacrylamide gel isoelectric focusing (PAGIF) at pH gradient 4–5

immunofixation are shown. The Pi banding pattern is very complex, reliable classification obviously requires a high degree of expertise.

In Fig. 4 we give a schematic presentation of the Pi phenotypes as observed by isoelectrofocusing at pH 4–5. M4, M6, and M8 refer to the different regions of the banding pattern in the pH gradient. The phenotypic notations are given at the bottom.

We applied Pi classification in 66 cases of disputed paternities. In six cases the constellation in the Pi system permitted an exclusion of paternity. This concerned two complainants, three defendants, and one witness. In each of this cases the exclusion was confirmed in other systems. From our results the practical exclusion rate appears to be 9.10%. The theoretical exclusion rate was calculated to be 23.37%. Pi classification which includes the determination of Pi M subtypes has, therefore, great potential for paternity examinations. Adequate separation by isoelectric focusing is mandatory for reliable classification. Furthermore, fresh sera or sera which have been frozen and thawed only once or twice are required, since in our experience the bands of Pi Z, Pi S, and Pi I are susceptible to repeated freezing and thawing during which treatment they gradually become fainter until they disappear.

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