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Alpha$_1$-Antitrypsin: Evidence for a Fourth Pi^M Allele. Distribution of the PiM Subtypes in Southern Germany* **

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Summary. Subtypes of the protease inhibitor (Pi) α_1-antitrypsin were determined in sera from 752 unrelated individuals from Southern Germany. By isoelectric focusing nine common PiM subtypes were distinguished and several rare Pi variants were observed. Family studies confirm the existence of a fourth Pi^M suballele. The frequency of Pi^{M4} was found to be 0.018. A survey of the distribution of Pi alleles is given; the application of Pi subtyping in cases of disputed paternity is discussed.

Key words: Blood groups, α_1-antitrypsin – PiM subtypes – Paternity examinations

Zusammenfassung. Die Untergruppen des Protease-Inhibitors (Pi) α_1-Antitrypsin wurden im Serum von 752 nichtverwandten Personen aus Süddeutschland bestimmt. Mit Hilfe der isoelektrischen Fokussierung ließen sich dabei neun häufige PiM-Untergruppen neben mehreren seltenen Pi-Varianten unterscheiden. Die Existenz eines vierten Pi^M-Subtypenallels wurde durch Familienuntersuchungen bewiesen. In unserer Stichprobe errechnete sich für Pi^{M4} eine Allelfrequenz von 0.018. Die übrigen Allelfrequenzen wurden mit den Untersuchungsergebnissen anderer Autoren verglichen. Die Anwendung der Pi-Untergruppenbestimmung in der Vaterschaftsuntersuchung wird besprochen.

Schlüsselwörter: Blutgruppen, α_1-Antitrypsin – PiM-Untergruppen – Vaterschaftsuntersuchung

Hereditary variants of the protease inhibitor α_1-antitrypsin have been disclosed in recent years at an increasing rate. For classification their differences in electro-

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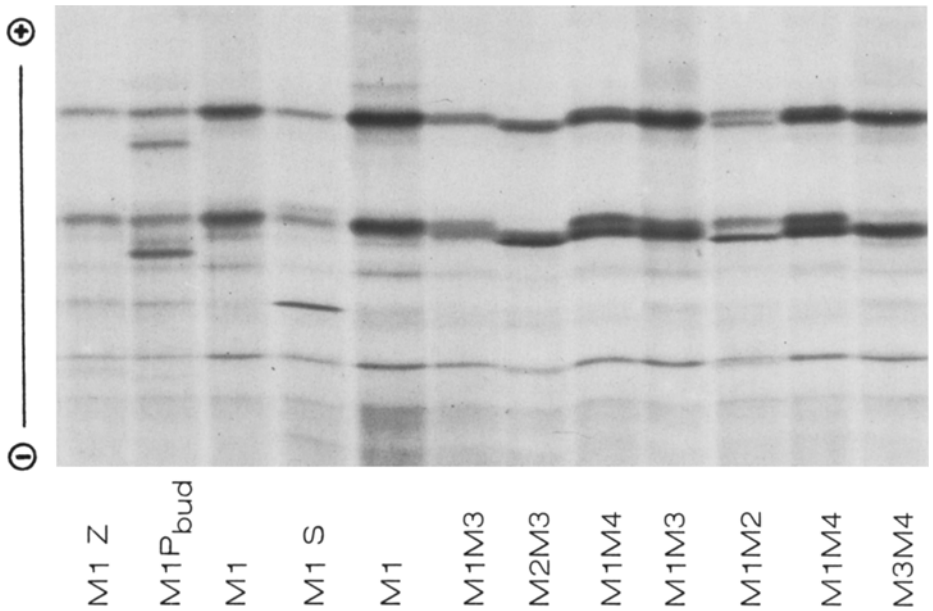

Fig. 1. Pi phenotypes as revealed by isoelectric focusing (pH range 4–5). Demonstrated are the PiM4 subtypes PiM1M4 and PiM3M4 and their small, but reproducible differences in comparison to PiM1M3

phoretic mobilities were used (Fagerhol and Braend 1965; Cox et al. 1980). PiB is the fastest anodal variant, PiZ the slowest migrating cathodal variant. The most common PiM is placed in the middle of this spectrum. Isoelectric focusing has permitted the delineation of six PiM subtypes (Klasen et al. 1977; Genz et al. 1977; Frants and Eriksson 1978; Kühnl and Spielmann 1978; Weidinger et al. 1980; Charlionet et al. 1981). The codominant mode of inheritance of the suballeles Pi^{M1}, Pi^{M2}, and Pi^{M3} was confirmed by family studies (Cleve et al. 1979). Recently, evidence for a fourth Pi^{M} suballele has been obtained (Constans et al. 1980; Weidinger 1980). Also a silent gene is known in the Pi system (Talamo et al. 1973).

In this study, the distribution of PiM subtypes in a larger sample from Southern Germany is reported. The presence of the fourth Pi^{M} suballele is confirmed by family studies. The application of the Pi system in paternity examinations is presented.

Material and Methods

The sera were obtained from apparently healthy individuals in the course of paternity examinations. Sera were analyzed by isoelectric focusing on thin-layer polyacrylamide gels of 0.5 mm thickness. Gels were made of 2.5 ml acrylamide solution 28% (w/v), 2.5 ml bis-acrylamide solution 2% (w/v), 0.6 ml Servalyt pH 4–4.5, 0.2 ml Servalyt pH 4–5, 5 µl TEMED, 2 g saccharose, and 10 ml aqua dest. After addition of 0.5 ml ammonium persulfate (1%) gels polymerized within 30 min.

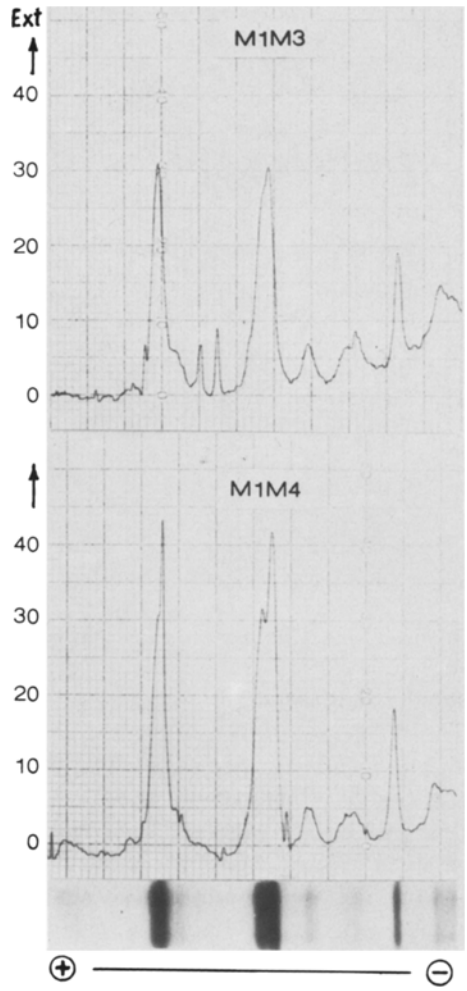

Fig. 2. Densitograms of the PiM1M3 and PiM1M4 banding pattern

For the application of samples filter paper pieces were used on which 5 µl of serum had been pipetted. For electrofocusing the Multiphor chamber of LKB was used. The cooling temperature was +10° C. At maximum values of 1,800 V, 25 mA, and 18 W separation was carried out for 150 min. At the cathode 0.5 *M* Glycine was used, at the anode a mixture of 0.025 *M* aspartic acid and 0.025 *M* glutamic acid was employed.

After isoelectrofocusing gels were placed for 20 min in a mixture of methanol and sulfosalicylic acid for protein fixation. Gels were stained with Coomassie Brilliant Blue R 250. Staining and destaining were carried out within 30 min.

For identifications of Pi types some gels were analyzed by immunofixation with a monospecific anti-human-α_1-antitrypsin antiserum (Atlantic Antibodies purchased from Merz & Dade).

In some cases banding pattern of Pi types and Pi subtypes were recorded by densitometry with a Vitatron TDL 100 Universal-Densitometer at a wave length of 578 nm and a slit width of 0.1 mm.

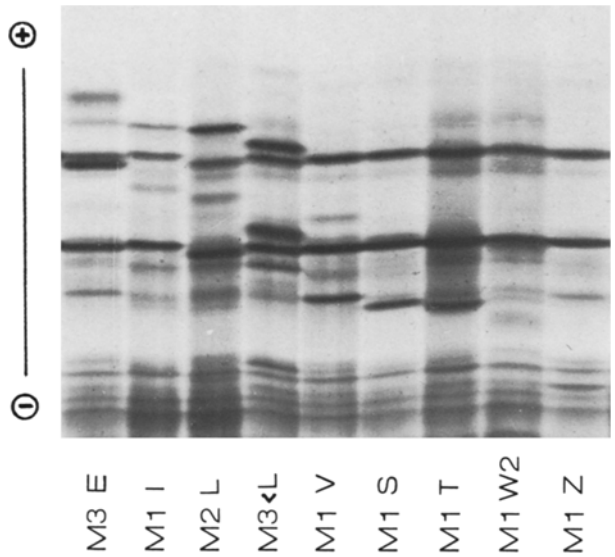

Fig. 3. Observed Pi variants after isoelectrofocusing (PAGIF, pH 4–5)

Results and Discussion

In Fig. 1 are demonstrated nine different Pi phenotypes as obtained by isoelectric focusing on polyacrylamide gels (PAGIF). Small, but reproducible differences are observed between subtype Pi M1M3 and Pi M1M4. Pi M4 is placed intermediate between M2 and M3.

In Fig. 2 densitograms of Pi M1M3 and Pi M1M4 are shown. In Pi M1M4 a double peak may be noticed, whereas in Pi M1M3 a peak with an asymmetric shoulder may be observed. Densitometry was found useful for classification of Pi M4 in the various combinations.

In Fig. 3 are illustrated eight different rare Pi variants in addition to Pi M1Z. The anodal Pi variants E, I, L, and < L may be observed as well as the cathodal Pi variants V, S, T, W2, and Z. Classification of the sample types as Pi M1Z was confirmed by immunofixation.

Figure 4 presents the Pi phenotypes as revealed by immunofixation. This complementary method is useful, in particular, for classification and identification of rare Pi variants. A Pi variant recently observed in our laboratory has a protein band in a position cathodal from Pi Z and Pi Z Pratt. The variant is called Pi Z1.

In Fig. 5 a schematic presentation is given of the phenotypes of the more than 30 alleles observed in the Pi system. Ten anodal variants from PiB to Pi < L, and 17 cathodal variants from PiM chapelhill to PiZ1 are shown. For the PiM subtypes the sequence from the anode to cathode is PiM1, PiM3, PiM4, and PiM2.

In Table 1 the distribution of Pi phenotypes and Pi alleles is given for a sample of 752 unrelated, healthy individuals from Southern Germany. We observed nine different PiM subtypes and the Pi variants S and Z as well as the rare variants F, I,

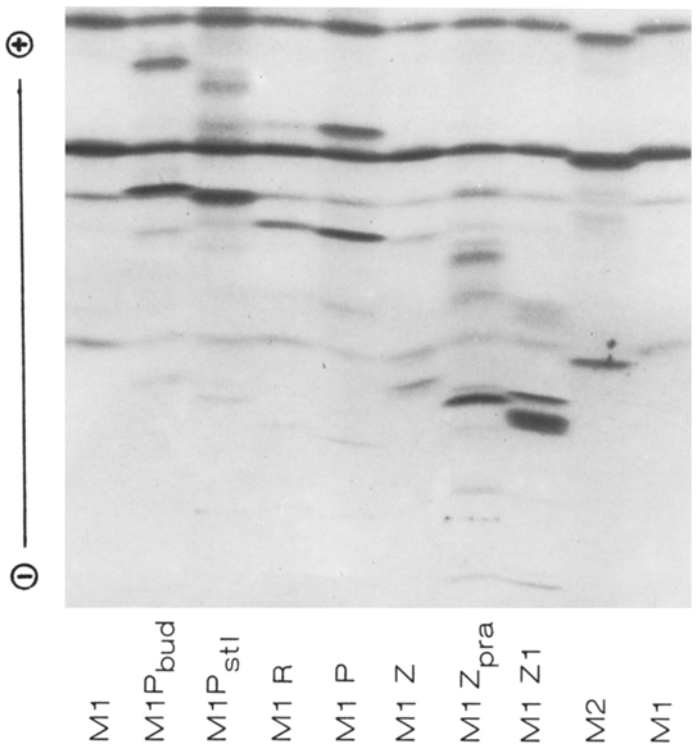

Fig. 4. Demonstration of Pi phenotypes after PAGIF immunofixation

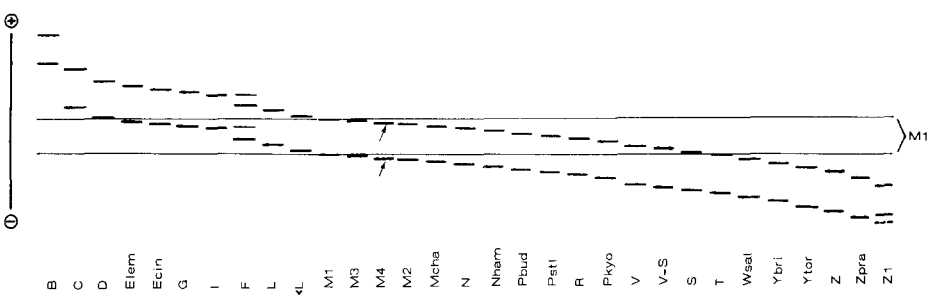

Fig. 5. Schematic presentation of the observed Pi phenotypes after PAGIF. *Arrows* indicate position of bands of the new subtype Pi M4. *Abbreviations:* Elem = Elemberg, Ecin = Ecincinnati, Mcha = Mchapelhill, Nham = Nhampton, Pbud = Pbudapest, Pstl = Pstlouis, Pkyo = Pkyoto, Wsal (W2) = Wsalerno, Ybri = Ybrighton, Ytor = Ytoronto, Zpra = Zpratt

L, < L, V, and Z1. There was good agreement between observed distribution and the distribution expected at population equilibrium. The frequency for the newly delineated allele Pi^{M4} is 0.0179.

In Table 2 a summary of the published data on the distribution of PiM suballeles is given. Only two populations have been analyzed for PiM4 so far. PiM1

Table 1. Frequencies of Pi phenotypes and Pi alleles in the South German population with the distinction of ten Pi M subtypes

Phenotypes	Observed		Expected		Allele frequencies
	n	%	n	%	
Pi M1	360	47.87	357.40	47.53	$Pi^{M1} = 0.6894$
M1 M2	169	22.47	170.98	22.74	$Pi^{M2} = 0.1649$
M2	21	2.79	20.45	2.72	
M1 M3	89	11.84	93.73	12.46	$Pi^{M3} = 0.0904$
M2 M3	24	3.19	22.42	2.98	$Pi^{M4} = 0.0179$
M3	8	1.06	6.15	0.82	$Pi^{S} = 0.0173$
M1 M4	18	2.39	18.56	2.47	
M2 M4	4	0.53	4.44	0.59	$Pi^{Z} = 0.0127$
M3 M4	3	0.40	2.43	0.32	$Pi^{a} = 0.0074$
M1 S	21	2.79	17.94	2.39	
M2 S	3	0.40	4.29	0.58	
M3 S	2	0.27	2.35	0.31	
M1 Z	14	1.86	13.18	1.75	
M2 Z	3	0.40	3.15	0.42	
M3 Z	1	0.13	1.73	0.23	
M4 Z	1	0.13	0.34	0.05	
M1 F	1				
M3 F	1				
M1 I	1				
M2 I	1				
M4 I	1				
M1 L	2	1.48	12.46	1.64	
M2 L	1				
M2 < L	1				
M1 V	1				
M1 Z1	1				
Total	752	100.00	752.00	100.00	

[a] Frequencies of rare Pi alleles

$\Sigma \chi^2 = 2.1929$; $df = 5$; $P > 0.20$

frequencies vary in European populations from 62% to 79%, PiM2 frequencies from 8% to 17%, and PiM3 frequencies from 6% to 12%.

The autosomal co-dominant mode of inheritance of Pi^{M4} was confirmed by family studies. In Table 3 the distribution of Pi types among the children is given which came from 12 parent-parent combinations involving one parent with PiM4.

As reported earlier (Weidinger et al. 1980), the exclusion rate in paternity examinations is raised considerably by the application of the Pi subtype

Table 2. Pi allele frequencies in different populations

Population	n	Allele frequencies						
		Pi^{M1}	Pi^{M2}	Pi^{M3}	Pi^{M4}	Pi^{S}	Pi^{Z}	Pi^{Var}
Munich (Genz et al. 1977)	538	0.7500	0.1477	0.0623	—	0.0223	0.0121	0.0056
Munich	264	0.7633	0.1364	0.0777	—	0.0208	0.0019	0
Marburg (Cleve et al. 1979)	146	0.6815	0.1541	0.1096	—	0.0342	0.0137	0.0068
Southern Germany (Weidinger et al. 1980)	347	0.6917	0.1686	0.0865	—	0.0230	0.0187	0.0115
Southern Germany (this study)	752	0.6894	0.1649	0.0904	0.0179	0.0173	0.0127	0.0074
Hessia (Kühnl and Spielmann 1978)	229	0.7052	0.1638	0.0917	—	0.0240	0.0109	0.0044
Netherlands (Klasen et al. 1978)	708	0.7210	0.1228	0.1124	—	0.0297	0.0049	0.0092
Netherlands	131	0.7481	0.1298	0.0534	—	0.0420	0.0267	0
Finland (Frants and Eriksson 1978)	136	0.7900	0.0800	0.1200	—	0	0.0100	0
Northern Italy (Klasen et al. 1978)	202	0.7203	0.1485	0.0819	—	0.0297	0.0099	0.0097
Southern Italy (Klasen 1981)	150	0.7033	0.1700	0.0800	—	0.0267	0.0133	0.0067
Toulouse (Constans et al. 1980)	163	0.6260	0.0920	0.1040	0.0370	0.1410	0	0
Normandie (Charlionet et al. 1981)	1,030	0.6675	0.1427	0.1005	—	0.0631	0.0179	0.0083
Southern England (Arnaud et al. 1979)	926	0.7462	0.1177	0.0605	—	0.0475	0.0221	0.0060
USA (white people)	240	0.6400	0.1900	0.1100	—	0.0420	0.0130	0.0050
USA (black people) (Kueppers and Christopherson 1978)	304	0.9030	0.0280	0.0540	—	0.0050	0.0030	0.0070
Mali (Negroes) (Frants and Eriksson 1978)	102	0.9300	0.0200	0.0400	—	0	0.0100	0

Table 3. Distribution of PiM4 genotypes in 12 families with a total of 14 children

Matings	n	Children							
		M1	M1M2	M1M3	M1M4	M2M3	M2M4	M3M4	M1Z
M1 × M1M4	5	3	–	–	2	–	–	–	–
M1 × M2M4	1	–	1	–	1	–	–	–	–
M1 × M3M4	1	–	–	1	0	–	–	–	–
M1M2 × M1M4	1	0	1	–	0	–	0	–	–
M1M2 × M3M4	1	–	–	0	0	0	1	–	–
M1M2 × M4 Z	1	–	–	–	0	–	0	–	1
M1M3 × M4 S	1	–	–	–	1	–	–	0	–
M1 Z × M1M4	1	1	–	–	1	–	–	–	0
Total	12	4	2	1	5	0	1	0	1

Table 4. Paternity cases with an isolated Pi exclusion

Paternity cases			Pi phenotypes		
			Child	Mother	Putative father
1. One-man case	Gr. A./Gr. M.	132/80	M1M1	M1M1	M2 S
2. One-man case	Ku. C./Br. G.	142/80	M1M1	M1M1	M3M3
3. One-man case	Hä. R./Hä. H.	190/80	M1M2	M1M1	M1M1
4. Four-men case	Le. S. /Gö. H.	40/81	M1M1	M1 S	M2M2

classification. In Table 4 four cases from paternity examinations are presented. An exclusion was possible by Pi subtyping in these four cases. In eight blood group systems (AB0, MNSs, Rh, Fy, Kidd, Kell, Lu, P), in seven serum group systems (Gm, Inv, Hp, Gc, C3, Bf, Tf), and in nine enzyme systems (acP, PGM_1, AK, ADA, 6 PGD, GPT, EsD, GLO, Gt) an exclusion was not possible. Subtyping was made in the systems PGM_1, Gc, and Tf. The exclusions with the Pi system were confirmed with freshly obtained repeat samples of blood. In the case Gr. A./Gr. M. an additional exclusion combination was found in the HLA system. Involvement of a silent allele is theoretically possible in two of the four cases, but appears to be very unlikely as an explanation of the findings alternative to the assumption of an exclusion. With improvement of the method for Pi classification, and for PiM subtyping in particular, a useful marker system for paternity examinations has become available. Prerequisite for the application in paternity examinations is experience with the isoelectric focusing procedure. Rare variants should be classified also with the aid of immunofixation.

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