Alpha-1-Antitrypsine (Pi) Polymorphism. Improved Pi-M-Subtyping by PAGIF with Immobilized Gradients.

F. Ferstl and H. H. Hoppe
Zentralinstitut für Transfusionsmedizin, Hamburg, D-2000 Hamburg
Filbektal 111

The demonstration of the genetically determined alpha-1-antitrypsine (PI) phenotypes may be achieved generally by isoelectrofocusing (IEF) on polyacrylamide gels (PAGIF) with carrier ampholytes. This method which originally was applied at a pH-gradient pH 3.5 - pH 6 does not produce a sufficient resolution quality though recently carrier ampholytes with a pH gradient pH 4.2 - 4.9 are offering an improved resolution (3).

The reason for this problem is the poor resolution of the PiM-gene products which show a narrow difference of about 0.01 pH only.

However a substantial improvement can be achieved by IEF with immobilized pH gradients. This method is based on a new concept of the preparation of linear pH-gradients. Compared to pH-gradients achieved by carrier ampholytes, Immobilines are covalently fixed on the polyacrylamide gel matrix during polymerization. So Immobilines incorporated into the gel offer a stabile buffering capacity.

The advantages of isoelectrofucusing with immobilized pH gradients are above all the application of extremely narrow pH gradients. Furthermore some problems have been solved, e.g. the plateau-phenomenon (1, 2).

Simultaneously PAGIF with carrier ampholytes (APAGIF) and Immobilines^{R} (IPAGIF) were carried out. The evaluation of the investigation described below has the following objects in view:

- Pi-Gene Frequencies of the North German Population
- Estimation of PiM-Variants
- Evaluation of Pi-tests by PAGIF with immobilized pH-gradients in paternity testing.

Material and Methods

600 serum samples of blood donors and 300 serum samples of not related persons involved in cases of disputed paternity (mothers, presumptive fathers) were tested.

Simultaneously APAGIF with carrier ampholytes for basic Pi-typing according to Dr. Martins method (3) and IPAGIF with immobilized pH-gradients (6) was carried out.

IPAGIF (pH 4.5 - 4.7) is performed on polyacrylamide gels 0.5 mm. The preparation of the gels follows the modified method originally described by Görg et al. (4).

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Acid Solution pH 4.50 (High Density)		Basic Solution pH 4.70 (Low Density)	
Immobiline pK 4.6	1,50 ml	Immobiline pK 4.6	1,50 ml
Immobiline pK 6.2	0,62 ml	Immobiline pK 6.2	0,88 ml
Stock solution	2,50 ml	Stock solution	2,50 ml
(29,1 % Acrylamide, 0,9 % Bis)		(29,1 % Acrylamide 0,9 % Bis)	
Glycerole (87 %)	4,20 ml		
Aqua bidest. ad	15,00 ml	Aqua bidest. ad	15,00 ml
7,5 ml into mixing chamber of mixer		7,5 ml into reservoir chamber of mixer	
TEMED (10 %)	60 µl	TEMED (10 %)	60 µl
Ammoniumpersulfate (10%)	30 µl	Ammoniumpersulfate (10%)	30 µl

Table 1: Composition of acid and basic solution for the preparation

The gels are ready for use after a polymerization time of 1 hrs without prefocusing. The serum amount of 25 µl has to be applied on sample application pieces (LKB 1850-901). Equipment, running conditions and staining is summarized on table 2.

Equipment: LKB Ultrophor 2217-001
LKB Power Supply 2197
LKB Gradient Mixer 2117-902

Running Conditions: Voltage 2000 V
Current 25 mA
Power 10 W

Electrofocusing time (total) 15 hrs (over night)
Electrofocusing with Filter paper pieces: 45 min.

Staining: Fixation: (57,5 g Trichloroacetic acid; 17,25 g Sulfosalicylic acid; 150 ml Methanol; 350 ml Aqua dest.) ca. 30 min.
Staining: 1,15 g Servaviolett in 1000 ml Destaining solution (60°C): ca. 60 min.
(Destaining Solution: 50 ml Methanol; 20 ml Acetic acid; 130 ml Aqua dest)

Table 2: Equipment, Running Conditions and Staining

Results and Discussion

The gene frequencies for the North German population were calculated from the observed phenotypes:
Pi*M1 = 0.7033; Pi*M2 .....

The observed frequencies are in agreement with the Hardy Weinberg equilibrium (x^2 = 2.4791; d.f. 5). Compared to other European frequencies no significant differences could be stated (Table 3).

Population	n	M1	M2	M3	M4	S	Z	VAR	
West-Japan	1.000	0.7065	0.2390	0.0480	0.0015	-	-	0.0050	*
Toscana	965	0.6772	0.1814	0.1073	-	0.0245	0.0410	0.0047	
Österreich	939	0.6731	0.1741	0.1097	-	0.0218	0.0138	0.0075	
Süddeutschland	752	0.6894	0.1649	0.0904	0.0179	-	-	0.0374	*
Niederlande	357	0.6793	0.1471	0.0812	0.0476	-	-	0.0448	*
Zentralspanien	103	0.6650	0.1699	0.0679	0.0146	-	-	0.0825	*
Süddeutschland	347	0.6917	0.1686	0.0865	-	0.0230	0.0187	0.0115	
Hessen (BRD)	282	0.6879	0.1720	0.0957	-	0.0284	0.0106	0.0053	
Toulouse (Frankreich)	163	0.6260	0.0920	0.1040	0.0370	-	-	0.1410	*
Niederlande	131	0.75	0.05	0.13	-	0.04	0.03	-	
Finnland	136	0.79	0.12	0.08	-	0.042	0.013	-	
USA-Weiße	240	0.64	0.19	0.11	-	-	0.01	0.005	
USA-Schwarze	304	0.903	0.028	0.054	-	0.005	0.003	0.007	
Norddeutschland	900	0.7033	0.1467	0.1089	-	0.0172	0.0167	0.0072	

* This value contains variants including PI S and PI Z

Table 3: Frequencies of PI-Alleles of different Populations

The particular value of IPAGIF as complementory method for Pi-subtyping may be demonstrated on Fig. 1 and Fig. 2.

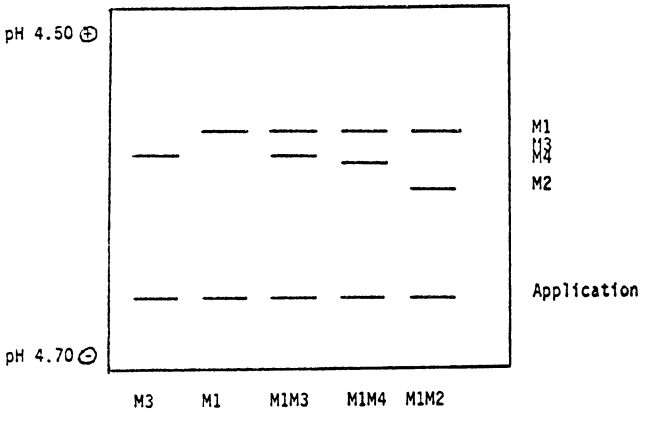

Fig. 1: Pi- Phenotypes (scheme)

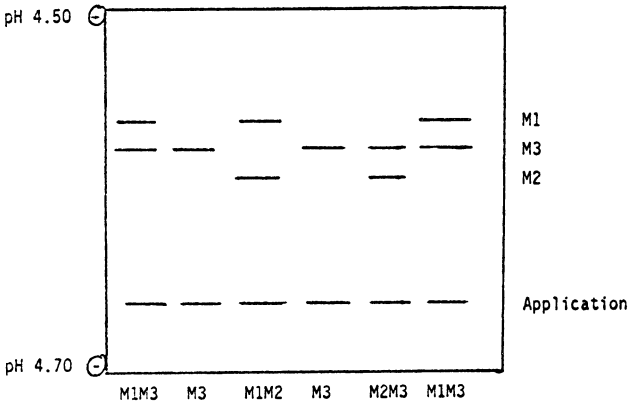

Fig. 2: Pi-Phenotypes (scheme)

The different bands of the PiM-subtypes are clearly separated by wide spaces. However due to the narrow pH gradient, cathodal resp. anodal Pi-types as PiS, PIZ and other variants are not visible. The PiM4-subtype identified by Constans et al. (5) could be found in our randomized sample of 900 sera only once (Fig. 1). Because of the narrow distance between the PiM3 and PiM4 bands PiM4 cannot be reliably identified in all cases, despite of the optimal resolution of IPAGIF. It seems to be easy to mis-interprete PiM4 as PiM3. Therefore gene frequencies of Pi*M4 published so far have to be cautiously evaluated.

Other PiM-variants were not observed.

For practical purposes at parentage problems of disputed paternity it seems to be advisable to employ APAGIF with carrier ampholytes within a range of pH 4.2 - 4.9 as basic method. IPAGIF with Immobilines (pH 4.5 - 4.7) may be used simultaneously in order to distinguish PiM-subtypes. After having acquired sufficient manual practice in handling and preparation of Immobiline-gels, this method is characterized by high reproducibility and technical stability.

For paternity tests the Pi-system should be highly recommended because of its efficiency: The general exclusion chance of the Pi-system based on the figures given above gave as a result 29.8 %.

Lit.:

(1) Fawcett, J.S. (1975) in Isoelectric Focusing and Isotachophoresis (Righetti, P.G. ed.), pp. 25 - 37 North Holland/American Elsevier, Amsterdam

(2) Rilbe, H. (1977) in Electrofocusing and Isotachophoresis (Radola, B. J. and Graesslin,D., eds.), pp. 35 - 50 de Gruyter, Berlin

(3) Martin, W.: Persönliche Mitteilung

(4) Görg, A., Postel, W., Westermeier,R., Gianazza, E., Righetti, P.G.: Biochem. Biophys. Methods 3 (1980) 273-284

(5) Constans, J., Viau, M., Gouaillard, C. (1980) Human. Genet. 55: 119 - 121

(6) Ferstl, F.: Inaugural Dissertation (in press)