Tf, Pi and Gc Variants: a Study by Isoelectric Focusing with Immobilized pH Gradients

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INTRODUCTION

The extremely high resolution of isoelectric focusing (IEF) on immobilized pH gradients (IPG) with ultra-narrow pH ranges has successfully been applied for the analysis of serum proteins polymorphism.

In the former studies (Tagliabracci and Gianni 1986; Tagliabracci et al. 1986; Tagliabracci and Paoli 1987) carried out by polyacrylamide gel isoelectric focusing (PAGIF) with carrier ampholytes we have investigated the transferrin (Tf), alpha-1-antitrypsin (Pi) and group-specific component (Gc) polymorphisms in the population living in Ancona (Middle Italy). Gels were prepared using the specifications advised by Bargagna et al. (1983), with minor modifications. The genic frequencies are shown in Tables 1, 2 and 3.

In order to obtain a better resolution and a sure identification we have utilized the IPG method in the investigation of some variants found in these studies.

MATERIALS AND METHODS

Immobiline pK 3.6, pK 4.6, pK 6.2 and pK 9.3 and N,N,N',N'-tetramethylethylendiamine (TEMED) were from LKB; acrylamide, N,N'-methylene-bis-acrylamide (Bis), ammonium persulfate and Coomassie Brilliant Blue R-250 were from BIO-RAD; glycerol was from MERCK.

The 0.5 mm thin gels were cast with a microgradient mixer (LKB) and in the pH ranges as described by Görg et al. (1983) for Tf, Weidinger and Cleve (1984) for Pi and Cleve et al. (1982) for Gc.

IEF was carried out in a Multiphor II Chamber (LKB 2117) together with a LKB 2197 Power Supply. The running conditions were 10 W, 25 mA and 2500 V overnight at 10°C. The pH gradient in the gel was misured with a Beckman surface electrode immediately afterwards the end of the focusing.

The phenotypes were revealed using the conventional method for serum

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proteins: fixation in a mixture of methanol and sulfosalicylic acid, staining with Coomassie Brilliant Blue R-250, destaining in a solution of glacial acetic acid, ethanol and distilled water.

RESULTS AND DISCUSSION

The Figures 1, 2 and 3 show the results obtained and the Tf, Pi and Gc variants classification as we tentatively have made.

It has been possible to carry out the family study of the Gc variants only. The investigation revealed their hereditary transmission.

In conclusion, our work has confirmed the usefulness of IEF with IPG in the resolution of the common subtypes and of the rare variants closely located on them. Moreover it has confirmed the difficulty or impossibility - due to the narrow pH gradient - in the visualization of other types or variants more cathodic or anodic.

Table 1. Distribution of Tf phenotypes in Ancona

Phenotype	Observed		Expected		Gene frequency
	n	%	n	%	
C1	420	58.17	419.78	58.14	
C1-C2	216	29.92	213.49	29.57	Tf^{C1} = .7625
C1-C3	37	5.12	40.41	5.60	
C2	25	3.46	27.15	3.76	Tf^{C2} = .1939
C2-C3	12	1.66	10.28	1.42	
C3	2	0.28	0.97	0.13	Tf^{C3} = .0367
C1-rare	8	1.11	7.60	1.05	
C2-rare	2	0.28	1.93	0.27	Tf^{rare} = .0069
C3-rare	0	0.00	0.36	0.05	
rare	0	0.00	0.03	0.01	
Total	722	100.00	722.00	100.00	

$\sum\chi^2 = .9520$[1] $.80 < P < .90$ 3 d.f.

1 In the χ^2 test the variants were pooled with common subtypes.

Table 2. Distribution of Pi phenotypes in Ancona

Phenotype	Observed		Expected		Gene frequency
	n	%	n	%	
M1	405	49.82	401.79	49.42	
M1-M2	182	22.39	188.38	23.17	Pi^{M1} = .7030
M1-M3	97	11.93	98.42	12.11	
M2	23	2.83	22.08	2.72	Pi^{M2} = .1648
M2-M3	25	3.07	23.07	2.84	
M3	6	0.74	6.03	0.74	Pi^{M3} = .0861
M1-S	38	4.67	37.95	4.67	
M2-S	12	1.48	8.90	1.09	Pi^{S} = .0332
M3-S	4	0.49	4.65	0.57	
S	0	0.00	0.90	0.11	Pi^{Z} = .0031
M1-Z	3	0.37	3.54	0.44	
M2-Z	1	0.12	0.83	0.10	Pi^{rare} = .0098
M3-Z	1	0.12	0.43	0.05	
M1-rare	13	1.60	11.20	1.38	
M2-rare	2	0.25	2.63	0.32	
M3-rare	1	0.12	1.37	0.17	
Others	0	0.00	0.83	0.10	
Total	813	100.00	813.00	100.00	

$\Sigma\chi^2 = 2.8198^2$ $.80 < P < .90$ 6 d.f.

Table 3. Distribution of Gc phenotypes in Ancona

Phenotype	Observed		Expected		Gene frequency
	n	%	n	%	
1S	270	35.76	268.19	35.52	
1S-1F	130	17.22	126.89	16.81	Gc^{1S} = .5954
1F	14	1.85	15.01	1.99	
2-1S	228	30.20	235.79	31.23	Gc^{1F} = .1411
2-1F	55	7.29	55.78	7.39	
2	56	7.42	51.83	6.86	Gc^{2} = .2622
1S-rare	1	0.13	0.90	0.12	
1F-rare	0	0.00	0.00	0.00	Gc^{rare} = .0013
2-rare	1	0.13	0.40	0.05	
rare	0	0.00	0.00	0.00	
Total	755	100.00	755.00	100.00	

$\Sigma\chi^2 = .8503^2$ $.80 < P < .90$ 3 d.f.

2 In the χ^2 test the variants were pooled with common subtypes.

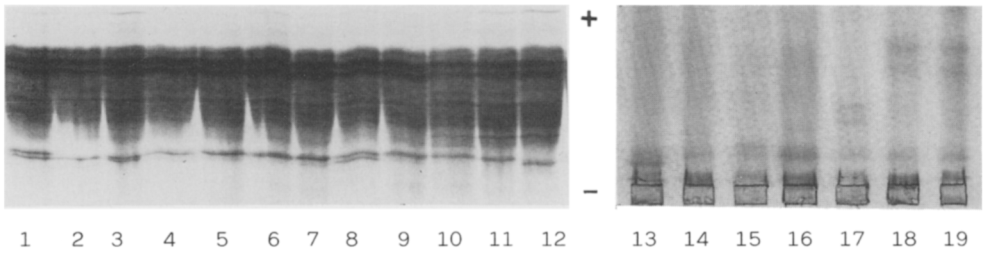

Fig. 1. Tf phenotypes after PAGIF with carrier ampholytes, pH range from 4 to 7 (left) and after IEF with IPG, pH range from 5.20 to 5.75 (right). From left to right: (1) C1-C2, (2) C2, (3) C2-C3, (4) C3, (5) C1, (6) C1-C3, (7) C1, (8) C1-C2, (9) C1-C4, (10) C1-B2, (11) C1-B1-2, (12) C2-B1-2, (13) C3, (14) C1-C15, (15) C1-C4, (16) C1-B4, (17) C1-B3, (18) C1-B2, (19) C1-B1-2[3].

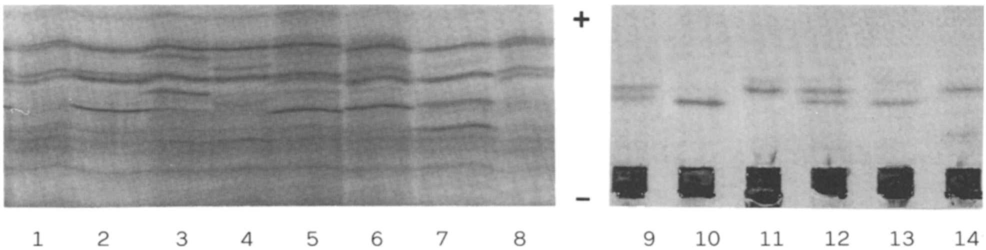

Fig. 2. Pi phenotypes after PAGIF with carrier ampholytes, pH range from 4 to 5 (left) and after IEF with IPG, pH range from 4.45 to 4.75 (right). From left to right: (1) M1M2, (2) M2S, (3) M1N, (4) M1R, (5) M1W, (6) M2S, (7) M3Z, (8) M1M2, (9) M1M3, (10) M2, (11) M1, (12) M1M3, (13) M3, (14) MIN.

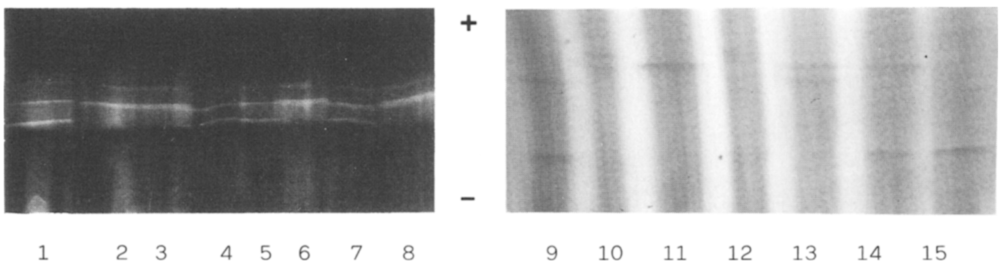

Fig. 3. Gc phenotypes after PAGIF with carrier ampholytes, pH range from 2.5 to 6.5 (left) and after IEF with IPG, pH range from 4.75 to 5.30 (right). From left to right: (1) 2-1F, (2) 1S, (3) 1S-1F, (4) 2-1C2, (5) 2-1S, (6) 1S-1F, (7) 2-1C2, (8) 1S-1C2, (9) 2-1C2, (10) 1S-1F, (11) 1S, (12) 2-1F, (13) 1S-1C2, (14) 2-1S, (15) 2.

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3 Some variants were kindly provided by doctor Giari (Pisa).

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