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GROUP SPECIFIC COMPONENT (Gc) AND TRANSFERRIN (Tf) SUBTYPES IN CZECH POPULATION – ASCERTAINED BY ISOELECTRIC FOCUSING

*ABSTRACT: Gc and Tf subtypes were determined by isoelectric focusing in a random sample from 243 unrelated donors from Prague. Following gene frequencies were found: Gc * 1F = 0.0905, * 1S = 0.5618, * 2 = 0.3477, and Tf * C1 = 0.7655, * C2 = 0.1358, * C3 = 0.0925, * B = 0.0041, * D = 0.0021. In the Gc-system a clear lower frequency was estimated for * 1F and a higher one for * 2. The frequency for Tf alleles was quite similar to those observed in other populations of Middle Europe. The ultrathin-layer isoelectric focusing on polyacrylamide gels has been proved as a convenient cost-saving method.*

KEY WORDS: Gc subtypes — Tf subtypes — Isoelectric focusing.

INTRODUCTION

The group specific component (Gc) is the transport protein for vitamin D in the human plasma, first detected as a diallelic system by immuno-electrophoresis (Hirschfeld 1959, Hirschfeld et al. 1960). Using the isoelectric focusing Constans and Viau (1977) demonstrated 3 suballeles (Gc * 1F, * 1S, * 2). The six phenotypes (Gc 1F, 1S, 1F 1S, 2–1S, 2–1F, 2) showed a good distribution. The exclusion rate of paternity increased from about 15 % to 30 %. In addition to the three suballeles a lot of rare variants were found (Constans and Cleve 1979). Until recently, about 160 populations have been investigated all over the world showing extensive racial differences (Kamboh and Ferrell 1986).

The biological function of transferrin (Tf) is the transport of iron in the human plasma. Genetical determined variants (Tf * B, * C, * D) were first observed by Smithies (1957) using the starch gel electrophoresis. The most common allele Tf * C has a frequency of about 98 % in European populations resulting in a low exclusion rate in paternity testing. After application of isoelectric focusing Kühnl and Spielmann (1978, 1979) detected 3 suballeles (Tf * C1, * C2, * C3). The six phenotypes (C1, C1–2, C1–3, C2, C2–3, C3) occured in a good distribution, and the exclusion rate increased to about 20 %. Further rare variants have been detected (overview see Weidinger et al. 1984).

At present Gc and Tf belong to the established useful genetic markers in paternity testing.

In the present paper we report of allele frequencies and phenotypes of Gc and Tf in Czech population using isoelectric focusing.

MATERIAL AND METHODS

Fresh serum samples of unrelated apparent healthy persons from Prague were stored at a temperature of –20 °C. For Tf determination prior focusing each serum was diluted in a fresh 0.25 % ferrous ammonium-II-sulfate × 10 H_2O solution (1 drop serum, 4 drops solution) and kept at 4 °C overnight (appr. 17 h). IEF was performed with a self-made flat bed apparatus and a power supply (Statron/GDR), specially equipped with power stabilizing and volthour integration. Cooling temperature was 8 °C. Polyacrylamide gels (T 5, C 3, concentration of ampholytes 2.5%) with dimensions 200 × 120 × 0.2 mm were cast accordingly to the method of Radola (1980). Maximal electrical parameters: current 6mA, voltage 2500 V, power 7 W. Electrode solutions: 0.025 M aspartic acid and 0.025 M glutamin acid (anode), and 0.2 M NaOH (cathode), respectively.

Gc determination: 0.2 ml Ampholine (LKB) pH range 4 – 6 and 0.1 ml Ampholine (LKB) pH range 3.5 – 5 were used. Filter paper pieces 3 × 5 mm (DESAGA or FN 1/GDR) were soaked with undiluted serum and placed on the gel surface 2 cm from the cathode. Prefocusing took place 15 minutes (50 Vh), focusing with samples 15 minutes (to 120 Vh), focusing without samples at least 2.5 hours (to 4000 Vh). For sample detection after the run the gel was immersed in fixing solution (30 g sulfosalycilic acid, 330 ml methanol, 660 ml aqua). The Gc pattern appeared after a few minutes as fine white bands on a dark background. For some samples a blotting method (Yuasa et al. 1985) was employed. Anti-Gc by Behring (Marburg/FRG), anti-rabbit-POD-antibody by Sifim (GRD).

Tf determination: 0.20 ml Servalyt (SERVA) pH range 3 – 5 and 0.10 ml servalyt (SERVA) pH range 5 – 6 were used. Filter paper pieces (see above) soaked in ferrum salt pretreated serum were placed on the gel surface 3 cm from the cathode. Prefocusing took place 15 minutes (60 Vh), focusing without samples 2.5 hours (to 4000 Vh). After completion of the run the gel was immersed in fixing solution for 5 minutes to remove the ampholytes and then stained for 30 minutes in 0.1 % Coomassie-blue-G solution. Destaining took place for 1 hour (if necessary for a longer time) in a solution of ethanol (25 %) and acetic acid (8 %). The dried gel plates are storable for an unlimited time.

TABLE 1. *Distribution of phenotypes and alleles in the Gc system.*

Phenotypes	observed		expected		Allele-frequencies
	n	%	n	%	
Gc 1S	76	31.28	76.70	31.56	*1F = 0.0905
1F	1	0.41	1.99	0.82	*1S = 0.5618
1F1S	25	10.29	24.71	10.17	*2 = 0.3477
2–1S	96	39.50	94.93	39.06	
2–1F	17	7.00	15.29	6.30	
2	28	11.52	29.38	12.09	
total	243	100.00	243.00	100.00	

$\chi^2 = 0.10$ df = 4

TABLE 2. *Distribution of phenotypes and alleles in the Tf system.*

Phenotypes	observed		expected		Allele-frequencies
	n	%	n	%	
Tf C1	141	58.03	142.40	58.77	*C1 = 0.7655
C1–2	53	21.82	50.52	20.85	*C2 = 0.1358
C1–3	34	13.99	34.41	14.20	*C3 = 0.0925
C2	3	1.23	4.48	1.85	*B = 0.0041
C2–3	7	2.88	6.10	2.52	*D = 0.0021
C3	2	0.82	2.08	0.86	
BC1	2	0.82	1.52	0.63	
C1D	1	0.41	0.78	0.32	
total	243	100.00	242.29	100.00	

$\chi^2 = 0.13$ df = 3

TABLE 3. *Distribution of Gc alleles in some populations of Central Europe.*

Population	Number tested	1F	1S	2	Var.	Ref.
Southern Germany (FRG)	1523	0.1402	0.5975	0.2610	0.0130	15
Berlin (GDR)	636	0.1261	0.6022	0.2697	–	10
Magdeburg (GDR)[1]	1273	0.1375	0.5793	0.2832	–	6
Berlin (West)	821	0.1297	0.5779	0.2924	–	9
Vienna	513	0.1686	0.5526	0.2788	–	11
this paper	243	0.0905	0.5618	0.3477	–	

TABLE 4. *Distribution of Tf alleles in some populations of Central Europe.*

Population	Number tested	C1	C2	C3	B	D	Ref.
Berlin (GDR)	931	0.7701	0.1557	0.0677	0.0064	–	10
Southern Germany (FRG)	184	0.7772	0.1468	0.0706	0.0540	–	14
Magdeburg (GDR)[1]	439	0.7654	0.1561	0.0717	0.0068	–	6
this paper	243	0.7655	0.1358	0.0925	0.0041	0.0021	

[1]brought up to date (1988)

RESULTS AND DISCUSSION

Tables 1 and 2 show the subtypes and the allele frequencies of Gc and Tf, respectively. The difference between the observed and expected numbers in the phenotypes is not significant. Concerning the Gc system we found a clear lower frequency for 1F and a higher one for 2 in comparison with other European populations (see *Table 3*). The critical point in the Gc subtyping is the minimal position difference between the 1S and 1F bands connected with a risk of misinterpretation. In such cases the phenotypes 1F and 1F 1S frequently appear as 1S, and the gene frequency for * 1S clearly increases. But, in our investigation the frequency for * 1S is not higher than in other European populations. As far as we know, any other studies in the Gc system are not made in Czech population. Therefore, the comparison with other investigations is not possible.

The distribution of Tf phenotypes and Tf alleles corresponds to those observed in other populations of Central Europe (see *Table 4*). From our data we have calculated a theoretical exclusion rate of paternity of 27.4 % (Gc) and 20.1 % (Tf).

Gc and Tf subtypes were determined by isoelectric fosusing on ultrathin layer polyacrylamide gels consuming only a half of line chemicals compared with conventional thin layer gels (0.5 mm). Moreover, the ultrathin layer technique enables to shorten running times and speed up fixing, staining and destaining procedures. The size of filter paper pieces for sample application allows to analyse at least 40 samples per run.

Figures 1 and 2 represent some Gc and Tf subtypes showing the typical patterns. The difference between 1F and 1S bands in Gc and C1 and C3 bands in Tf is distinct. Fixing the focused proteins in sulfosalicylic acid (or trichloracetic acid) Gc patterns

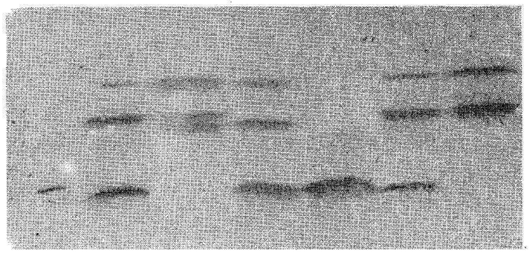

FIGURE 1. *Gc subtypes after isoelectric focusing and immunoblotting.*
The phenotypes are from left to right:
2—1F, 1F1S, 2—1S, 2, 2—1F, 1F.
Anode on top.

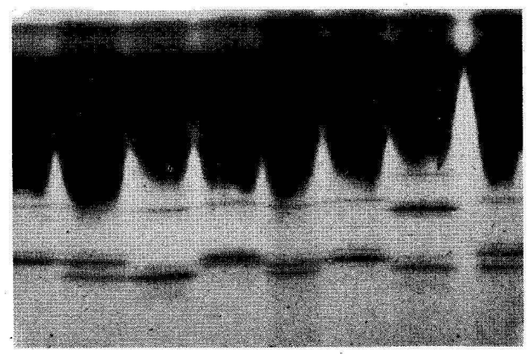

FIGURE 2. *Tf subtypes after isoelectric focusing and protein stain.*
The phenotypes are from left to right:
C1, C1—2, C2, C1—3, C2—3, C3, BC2, C1—2.
Anode on top.

appear as slight white bands on a dark background. However, the reliable classification of these bands requires a lot of experience connected with some difficulties in photographic reproduction. The immunoblotting method actually delivers better images but is much more expensive. So, the acid fixation of proteins has became a suitable procedure in routine testing with regard to economic demands.

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