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Classification of Transferrin (Tf) Subtypes by Isoelectric Focusing*

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Summary. A sample of 450 sera from unrelated individuals from Southern Germany was examined by isoelectric focusing on polyacrylamide gels. Three common subtypes, Tf C1, C2-1, and C2, were differentiated. In addition, the rare variants Tf B1, B1-2, B2, D1, D1-2, D2, and D3 were observed. The frequencies of the Tf alleles in our sample were found to be: $Tf^{C1} = 0.8544$, $Tf^{C2} = 0.1367$, $Tf^{B1} = 0.0011$, $Tf^{B1\text{-}2} = 0.0022$, $Tf^{B2} = 0.0045$, and $Tf^{D1} = 0.0011$. Analysis of 73 parents with 73 children did not show deviations from the expected mode of inheritance. Modification of the method by addition of 0.01 M $FeCl_3$ to the sera prior to examination did, however, reveal further variation and permitted the distinction of six subtypes, C1, C2-1, C2, C3, C3-1, and C3-2.

Key words: Serum groups, transferrin subtypes – Transferrin subtypes

Zusammenfassung. Mit Hilfe der isoelektrischen Fokussierung in Polyacrylamidgelen (PAGIF) wurden 450 Proben von nicht verwandten Personen aus Süddeutschland untersucht. Es wurden drei häufige Untergruppen Tf C1, C2-1 und C2 differenziert, sowie die seltenen Varianten Tf B1, B1-2, B2, D1, D1-2, D2 und D3 beobachtet. Die Allelfrequenzen in dieser Stichprobe betrugen: $Tf^{C1} = 0{,}8544$, $Tf^{C2} = 0{,}1367$, $Tf^{B1} = 0{,}0011$, $Tf^{B1\text{-}2} = 0{,}0022$, $Tf^{B2} = 0{,}0045$, and $Tf^{D1} = 0{,}0011$. Die Untersuchung von 73 Elternpaaren mit ihren Kindern ergab keine Abweichung vom angenommenen autosomal kodominanten Erbgang. Modifizierung der Methode durch Zusatz von 0,01 M $FeCl_3$ zu den Seren vor der Auftrennung ließ weitere Variation erkennbar werden und erlaubte sechs häufige Untergruppen zu differenzieren, nämlich C1, C2-1, C2, C3, C3-1 und C3-2.

Schlüsselwörter: Serumgruppen, Transferrin-Untergruppen – Transferrin-Untergruppen

* This paper is dedicated to Fritz Hartmann, MD, Professor of Medicine, Hannover Medical School, FRG, on the occasion of his 60th birthday

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Transferrin is the transport protein for iron present in the plasma. Inherited variants of transferrin were observed first by Smithies (1957) with the aid of starch gel electrophoresis. At least 22 different variants of transferrin have been observed until today which occur mostly at low frequencies (Smithies 1957; Giblett 1969; Walter 1975). The common transferrin type has been named Tf C, the faster migrating variants are named Tf B variants, the slow migrating variants are called Tf D variants. The common allele Tf^C has a frequency of 0.98 to 0.99 in European populations. Recently, the application of the isoelectrofocusing method has permitted the demonstration of subtypes of the common transferrin C. Kühnl and Spielmann (1978) as well as Thymann (1978) reported the presence of three common subtypes Tf C1, C2-1, and C2 which are determined by two alleles, Tf^{C1} and Tf^{C2}. While our study was in progress, Kühnl and Spielmann (1979) presented evidence that the use of an improved separatory procedure permitted the differentiation of further common subtypes. They were able to distinguish six common Tf subtypes called Tf C1, C2-1, C2, C3, C3-1, and C3-2. The additional three subtypes are due to a third allele Tf^{C3}. The original allele Tf^{C1} was thus split into Tf^{C1} and Tf^{C3}.

In this paper classification of Tf subtypes in a sample from Southern Germany is described. The results of Kühnl and Spielmann (1979) are confirmed and the usefulness of the Tf-subtyping procedure is demonstrated for application in cases of disputed paternity.

Material and Methods

The first sample comprised 450 unrelated healthy individuals from Southern Germany. Sera were analyzed for the subtypes Tf C1, C2-1, and C2 by isoelectric focusing on polyacrylamide gels (260 × 125 × 1 mm) at pH 4–6.5. Ampholine (LKB) was used at a concentration of 2.2%. Sera (5 µl) were applied with filter paper (Whatman Nr. 1) at a distance of 5 cm from the anodal edge of the gel. The Multiphor chamber (LKB) was used. Isoelectric focusing was carried out at 10° C with maximally 1500 V, 35 mA, and 25 W for 180 min. After 90 min, the paper pieces were removed.

A second sample of 184 unrelated healthy individuals from Southern Germany was analyzed for the six common subtypes of Tf C. Conditions for isoelectrofocusing were not altered. 0.01 M $FeCl_3$ was added to the sera prior to examination.

Immunofixation was carried out according to Ritchie and Smith (1976) as modified by Hamann (1977) and Cleve et al. (1978). Cellulose acetate strips soaked with monospecific Tf-antiserum (DAKO-Immunglobuline Boehringer) were used.

Polyacrylamide gels were fixed with sulfosalicylic acid and stained with Coomassie Brillant Blue R 250.

Results and Discussion

The distribution of Tf subtypes in samples of unrelated healthy individuals from Southern Germany is shown in Table 1. The distribution found in the sample ($n = 450$) in which three common subtypes are distinguished (Table 1, A) differs only slightly from those observed by Kühnl and Spielmann (1978) and by Thymann

Table 1.

A Distribution of phenotypes and alleles in the Tf system in a sample from Southern Germany with the distinction of three common TfC subtypes (C1, C2-1, C2)

Phenotypes	Observed		Expected		Allele frequencies
	n	%	*n*	%	
TfC1	330	73.33	328.50	73.00	$Tf^{C1} = 0.8544$
C2-1	101	22.44	105.12	23.36	$Tf^{C2} = 0.1367$
C2	11	2.45	8.41	1.88	$Tf^{B1} = 0.0011$
C1B1	1	0.22	1.27	0.28	$Tf^{B2} = 0.0045$
C1B1-2	2	0.45	1.73	0.38	$Tf^{B1-2} = 0.0022$
C1B2	4	0.89	4.55	1.01	$Tf^{D1} = 0.0011$
C1D1	1	0.22	0.42	0.09	
Total	450.00	100.00	450.00	100.00	

$\Sigma\chi^2 = 1.9326$, df = 1; $P > 0.20$

B Distribution of phenotypes and alleles in a sample from Southern Germany with the distinction of six common TfC subtypes (C1, C2-1, C2, C3, C3-1, C3-2)

Phenotypes	Observed		Expected		Allele frequencies
	n	%	*n*	%	
TfC1	111	60.33	111.14	60.56	$Tf^{C1} = 0.7772$
C2-1	42	22.83	41.97	22.86	$Tf^{C2} = 0.1468$
C2	3	1.63	3.96	2.16	$Tf^{C3} = 0.0706$
C3-1	20	10.87	20.19	11.00	$Tf^{B2} = 0.0027$
C3-2	6	3.26	3.81	2.08	$Tf^{B1-2} = 0.0027$
C3	—	—	0.92	0.50	
C1B2	1	0.54	0.77	0.42	
C1B1-2	1	0.54	0.77	0.42	
Total	184.00	100.00	183.53	100.00	

$\Sigma\chi^2 = 2.5507$; df = 2; $P > 0.20$

The phenotype of the homozygous TfC3 has recently been observed by us

(1978). The distribution found in the sample ($n = 184$) analyzed for six common Tf subtypes is shown in Table 1, B. The results are similar to those obtained by Kühnl and Spielmann (1979). In particular, it is confirmed that the subdivision of three common Tf^C alleles, is a splitting of the "original" Tf^{C1} allele into Tf^{C1} ("new") and Tf^{C3}. The frequencies $Tf^{C1} = 0.7772$ and $Tf^{C3} = 0.0706$ are very similar (0.8478) to the Tf^{C1}-frequency of 0.8544 in the original sample.

The Tf phenotypes are demonstrated in Figs. 1–3. In Fig. 1, the Tf subtypes TfC1, C2-1, and C2 are shown together with several rare Tf variants. The Tf phenotypes are observed at the portion of the gel corresponding to a pH of

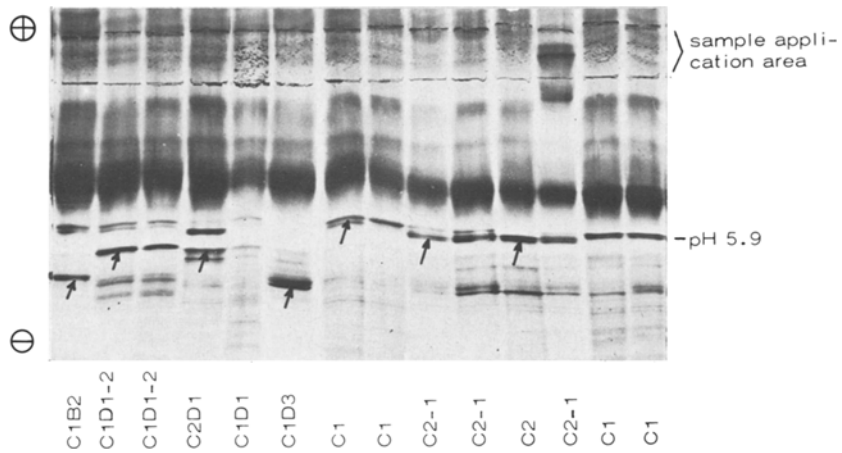

Fig. 1. Tf phenotypes after isoelectric focusing on polyacrylamide gels (PAGIF) at pH range 4–6.5

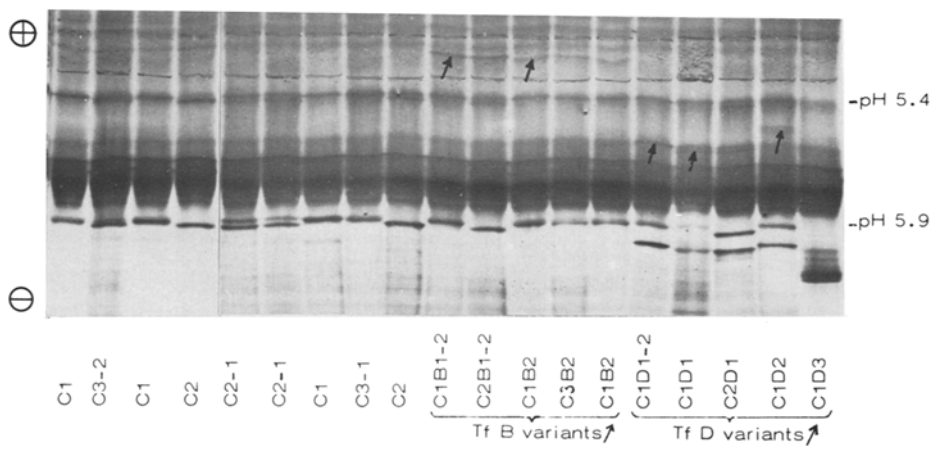

Fig. 2. Tf phenotypes after PAGIF at pH range 4–6.5. 0.01 M $FeCl_3$ was added to sera prior to analysis

approximately pH 5.9. In Fig. 2, the Tf phenotypes examined after addition of 0.01 M $FeCl_3$ are shown. The iron-saturated transferrins become more prominent in the region of the gel corresponding to a pH of approximately 5.3–5.6. A third common Tf component, Tf C3, can be located between C1 and C2 at pH 5.4. The iron-saturated Tf B components can be seen at pH 5.3, the iron-saturated Tf D components are visible at pH 5.6. Figure 3 demonstrates the Tf phenotypes as revealed by immunofixation with a monospecific transferrin antiserum. The complex banding pattern is due to the different states of iron-binding to the variant genetic Tf types (apo-Tf, Fe_1-Tf, Fe_2-Tf).

Figure 4 gives a schematic presentation of the complex banding pattern of the transferrins. The region of pH 5.9 relates to the apotransferrins. The iron-

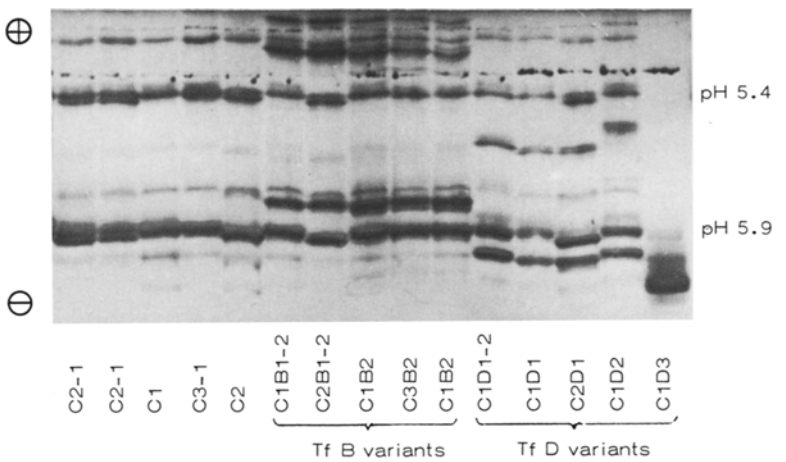

Fig. 3. Tf phenotypes after PAGIF at pH range 4–6.5 and subsequent immunofixation with monospecific anti-transferrin antiserum. 0.01 M $FeCl_3$ was added to sera prior to analysis

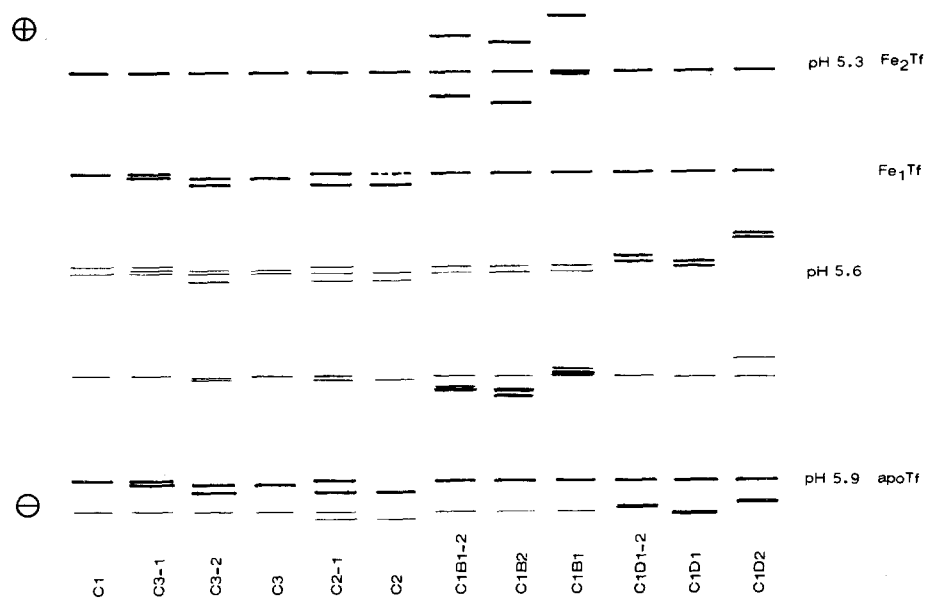

Fig. 4. Schematic presentation of transferrin phenotypes as obtained by PAGIF at pH 4–6.5. For TfC subtyping pH regions of 5.9 and of 5.4 have to be evaluated

saturated transferrins are located between pH 5.3 and 5.6. For classification of the common Tf subtypes the regions of 5.4 and 5.9 have to be evaluated.

Table 2 shows the results of family studies. The results are in agreement with an autosomal codominant mode of inheritance. The TfC subtypes are determined by three alleles, Tf^{C1}, Tf^{C2}, and Tf^{C3}.

Table 2.

A Distribution of Tf-phenotypes in 73 parents with a total of 73 children considering Tf^{C1} and Tf^{C2}

Parents	*n*	Children				
		C1	C2-1	C2	C1B2	C1B1-2
C1 × C1	44	44 (44)	—	—	—	—
C1 × C2-1	19	11 (9.5)	8 (9.5)	—	—	—
C1 × C2	1	—	1 (1)	—	—	—
C2-1 × C2-1	5	1 (1.25)	3 (2.5)	1 (1.25)	—	—
C2-1 × C2	1	—	1 (0.5)	0 (0.5)	—	—
C2 × C2	1	—	—	1 (1)	—	—
C1 × C1B2	1	1 (0.5)	—	—	0 (0.5)	—
C1 × C1B1-2	1	0 (0.5)	—	—	—	1 (0.5)
Total	73	57	13	2	0	1

B Distribution of Tf-phenotypes in 33 families with a total of 33 children considering Tf^{C1}, Tf^{C2}, and Tf^{C3}

Parents	*n*	Children					
		C1	C2-1	C2	C3-1	C3-2	C3
C1 × C1	14	14 (14)	—	—	—	—	—
C1 × C2-1	9	5 (4.5)	4 (4.5)	—	—	—	—
C1 × C3-1	3	1 (1.5)	—	—	2 (1.5)	—	—
C1 × C3-2	1	—	0 (0.5)	—	1 (0.5)	—	—
C2-1 × C2-1	4	1 (1)	2 (2)	1 (1)	—	—	—
C2-1 × C3-1	1	1 (0.25)	0 (0.25)	—	0 (0.25)	0 (0.25)	—
C2-1 × C3-2	1	—	1 (0.25)	0 (0.25)	0 (0.25)	0 (0.25)	—
Total	33	22	7	1	3	—	—

Expected values are given in brackets

With the distinction of Tf C subtypes this system appears to be useful, also, for application in cases of disputed paternity. We calculated the isolated theoretical exclusion rate for this system to be 18.66%.

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