Jpn. J. Human Genet. **30**, 15–20, 1985

GENETIC POLYMORPHISM OF TRANSFERRIN IN EGYPTIANS: ANALYSIS BY TWO ELECTRO-FOCUSING METHODS WITH DESCRIPTION OF UNUSUAL B VARIANT

Ismail M. SEBETAN,[1] Kouichi HIRAIWA,[2] and Suguru AKAISHI[2]

[1]*Department of Forensic Medicine, Tohoku University School of Medicine, Sendai 980, Japan*
[2]*Department of Legal Medicine, Fukushima Medical College, Fukushima 960, Japan*

Summary Two different electrofocusing procedures were employed for investigating the genetic variants of the human transferrin (*Tf*) in a population sample of 161 unrelated Egyptians. Five common transferrin C subtypes, C1, C1C2, C1C3, C2 and C2C3, and four rare variants, two slow and two fast variants which included unusual double-banded B variant have been demonstrated. The estimated allele frequencies were Tf^{C1}= 0.764, Tf^{C2}=0.193, Tf^{C3}=0.031, and Tf^{D}=0.006. The combined frequencies for the Tf^{B} were 0.006. The observed and expected phenotypes provide a good fit to the Hardy-Weinberg equilibrium.

INTRODUCTION

Separation of the genetic variants of the human serum transferrin using electrophoresis in starch gel (Smithies, 1957), agarose gel (Teisberg, 1970) and polyacrylamide gel (Kirk *et al.*, 1978) have shown a common transferrin type, termed transferrin C. It has a frequency of about 98% or more in all populations so far investigated. Several transferrin variants have also been reported: B variants which have faster and D variants which have slower electrophoretic mobility than transferrin C (Giblett, 1969). With the different electrophoretic techniques, native sera were subjected to analysis without any pre-run treatment. The use of isoelectric focusing for analysis of the transferrin variants has revealed five common transferrin C suballeles (Kühnl and Spielmann, 1979; Constans *et al.*, 1980a). The most common suballeles, Tf^{C1} and Tf^{C2}, have been observed in all the tested populations (Thymann, 1978; Hoste, 1979; Stibler *et al.*, 1979; Kueppers and Harpel, 1980; Constans *et al.*, 1980a, b; Sebetan *et al.*, 1982), while the rest of the suballeles seems to occur in certain populations (Constans *et al.*, 1980a). The products of the five suballeles are in the order of C4, C1, C3, C5 and C2 from anode to cathode. On

Received October 15, 1984

the contrary to the electrophoretic methods, the method of electrofocusing using native sera directly for analysis showed difficulties and problems in reproducibility and sensitivity. Therefore several procedures in which sera are subjected to pre-treatment prior to electrofocusing have been described (Stibler *et al.*, 1979; Constans *et al.*, 1980a; Sebetan *et al.*, 1982; Weidinger *et al.*, 1980).

This study provides the first data on the transferrin polymorphism among Egyptians. An improved electrofocusing technique with description of unusual B variant is also presented.

MATERIALS AND METHODS

Samples. Blood samples were collected by venipuncture from 161 healthy unrelated Egyptians without adding anticoagulant. Sera were separated by centrifugation and transported within two days in ice bag by air, then stored frozen at $-20°C$ until analysis.

Pre-treatment of samples. Native sera were used directly with the electrophoretic methods, while prior to electrofocusing all sera were treated by the following two methods:

A) Ferric chloride. 5 μl of serum was added to 15 μl of 0.3% ferric chloride and the mixture was incubated at room temperature for 10 min before application to the gel.

B) Ferrous ammonium sulphate. 5 μl of serum was added to 25 μl of 0.25% ferrous ammonium sulphate $\times 6H_2O$ as described by Constans *et al.* (1980a), and the mixture was incubated in the refrigerator for 18 hr before analysis.

Isoelectric focusing. Polyacrylamide gel electrofocusing was carried out as previously described (Sebetan *et al.*, 1982) except for the following alterations;

1. Thickness of the gel is reduced to 0.2 mm.
2. Riboflavin and UV light were used for the gel polymerization.
3. The cathodal electrode strip was saturated with 1 M aqueous solution of ethanolamine, and 1 M aqueous solution of phosphoric acid was used for the anodal electrode strip.
4. The power unit was adjusted to supply initial voltage of 300 V and maximum voltage of 1,400 V, and the total focusing time was about 5 hr.
5. Print immunofixation was performed using a cellulose acetate membrane soaked in five times diluted specific antisera (DAKO-immunoglobulins). The membrane was placed in contact with the surface of the gel for 2 min at room temperature, then removed and washed for overnight with saline, and stained with Amido black 10B.

Electrophoresis. Starch gel electrophoresis was done after the method of Smithies (1957), while the method of Teisberg (1970) was employed for agarose gel electrophoresis.

RESULTS AND DISCUSSION

The electrofocusing band pattern of the five common transferrin phenotypes observed after the two procedures are shown in Fig. 1, a and b. With the two applied methods, one major zone (typing zone) which showed very high staining intensity and two minor zones at the anodic and cathodic sides of the major zone were always observed. The identified zones reflect the different forms of iron binding transferrin (Hovanessian and Awdeh, 1975). The homozygote phenotypes exhibit a single band pattern; the anodal is termed Tf^{C1} and the cathodal Tf^{C2}, while Tf^{C3} was found to be electrofocused close to the cathodal side of Tf^{C1}. The heterozygotes are represented by double-banded pattern. The other common suballeles (Tf^{C4} and Tf^{C5}) were not detected in our population samples. Besides the five common subtypes, four variants were also encountered; two slow D variants which have identical electrophoretic mobility, and two fast B variants which correspond to different alleles. These variants are given in Fig. 2, a and b. The observed variants were compared with the transferrin variants which we previously reported in Japanese (Sebetan *et al.*, 1982). The obtained patterns indicate that these variants are not similar; the D variant detected in Egyptians was clearly cathodal to D Chinese, while the B variants observed in the Japanese have isoelectric points between those of the Egyptians. The most cathodal B variant found in Egyptians was the first example of a transferrin variant represented by double-banded pattern. The pattern of this variant was ascertained by print immunofixation on cellulose acetate membrane as shown in Fig. 2c. Treatment of the samples with 0.3% ferric chloride in conjunction with using ampholine pH 5–7 provides certain advantage if the pattern is compared with that observed when the ampholine pH 4–6.5 is used (Weidinger

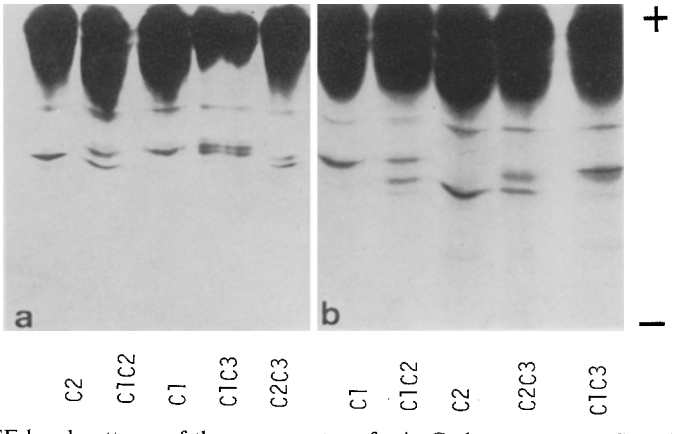

Fig. 1. IEF band patterns of the common transferrin C phenotypes. a, Samples treated with ferric chloride; b, samples treated with ferrous ammonium sulphate.

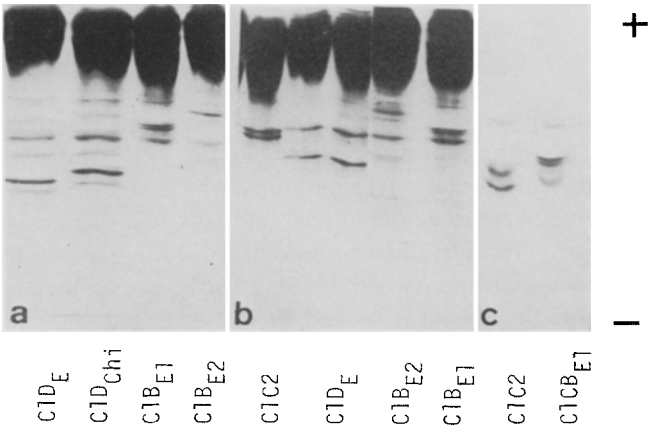

Fig. 2. IEF band patterns of the variant transferrin phenotypes. a, Samples treated with ferric chloride; b, samples treated with ferrous ammonium sulphate; c, immunofixation pattern of the unusual B variant. (E, Egypt; Chi, Chinese)

Table 1. Transferrin phenotypes in Egyptians.

Phenotypes	Number observed	Frequency observed (%)	Number expected	Allele frequencies
C1	89	55.28	93.9	$Tf^{C1}=0.764$
C1C2	55	34.16	47.5	$Tf^{C2}=0.193$
C1C3	9	5.60	7.6	$Tf^{C3}=0.031$
C2	3	1.9	5.9	$Tf^{B}=0.006$
C2C3	1	0.6	1.9	$Tf^{D}=0.006$
C3	0	0	0.2	
C1B	2	1.2	1.5	
C1D	2	1.2	1.5	
C2B	0	0	0.4	
C2D	0	0	0.4	
C3B	0	0	0.06	
C3D	0	0	0.06	
B	0	0	0.01	
D	0	0	0.01	
BD	0	0	0.01	
	161	99.94	160.95	

et al., 1980). In the other procedure which was originally described by Constans *et al.* (1980a), we observed high resolution with reproducible pattern, but the treatment of sera with ferric chloride described in this study offers a very rapid typing,

since it needs 10 min only as compared with 18 hr if the method of Constans *et al.* (1980a) will be used, in addition to the excellent resolution and reproducibility.

The distribution of the observed phenotypes is given in Table 1. The homozygote phenotype Tf C3 was not encountered and the variants were found as heterozygote of Tf C1. The computed gene frequencies were $Tf^{C1}=0.764$, $Tf^{C2}=0.193$, $Tf^{C3}=0.031$, $Tf^{D}=0.006$. The combined frequencies for the Tf^{B} were 0.006. The observed and expected phenotype distribution fits the Hardy-Weinberg equilibrium ($\chi_0^2=5.033$, $0.75<p<0.90$, d.f.$=10$).

Comparison of the newly reported gene frequencies of the transferrin subtypes among different populations is given in Table 2. Egyptians and Europeans (Germans and French) are more or less similar, while marked differences could be observed between Egyptians and Japanese or Pygmy population, especially regarding the absence of the Tf^{C3} suballele. It should be pointed out that the same population sample we have reported in Japanese (Sebetan *et al.*, 1982) has been reinvestigated using the two methods employed in this study, but absence of Tf^{C3} was confirmed. Tf^{C3} has not been reported in white Americans and some European populations (Thymann, 1978; Hoste, 1979; Stibler *et al.*, 1979; Kueppers and Harpel, 1980), but we believe that the reason is most probably attributed to overlooking of Tf^{C3}, because unsuitable techniques were applied.

The new system will increase the isolated probability of proving non-paternity among Egyptians to 18% as compared with 1% by the electrophoretic methods.

Table 2. Comparison of the reported gene frequencies of the Tf phenotypes among different populations.

Population	N	Allele frequencies				References
		Tf^{C1}	Tf^{C2}	Tf^{C3}	$Tf^{variants}$	
Germans	252	0.795	0.155	0.042	0.008	Kühnl and Spielmann (1979)
French	250	0.788	0.132	0.053	0.027	Constans *et al.* (1980a)
Danish	132	0.8144	0.1856	—	—	Thymann (1978)
Belgium	253	0.784	0.206	—	0.01	Hoste (1979)
Swedes						Stibler *et al.* (1979)
Stockholm	100	0.905	0.090	—	0.005	
Umea	100	0.840	0.155	—	0.005	
Swedish Lapps	100	0.830	0.165	—	0.005	
USA						Kueppers and Harpel (1980)
White	149	0.802	0.188	—	0.01	
Black	166	0.843	0.111	—	0.045	
Pygmy	337	0.820	0.027	—	0.153	Constans *et al.* (1980b)
Japanese	300	0.773	0.212	—	0.015	Sebetan *et al.* (1982)
Egyptians	161	0.764	0.193	0.31	0.012	This study

Acknowledgement The authors are grateful to Mr. Kazuo Hayasaka for his skillful photographs.

REFERENCES

Constans, J., Kühnl, P., Viau, M., and Spielmann, W. 1980a. A new procedure for determination of transferrrin C (Tf^{C}) subtypes by isoelectric focusing. Existence of two additional allele, Tf^{C4} and Tf^{C5}. *Hum. Genet.* **55**: 111–114.

Constans, J., Viau, M., Jaeger, G., and Palisson, M.J. 1980b. Gc, Tf, Hp subtype and α_1-antitrypsin polymorphisms in a Pygmy Bi-Aka sample. Phenotype association between Tf^{D1} and Gc^{1A1} (Gc^{Ab}) variants. *Hum. Hered.* **31**: 129–137.

Giblett, E.R. 1969. *Genetic Markers in Human Blood*, Blackwell, Oxford and Edinburgh, pp. 126–159.

Hoste, B. 1979. Group specific component (Gc) and transferrin (Tf) subtypes ascertained by isoelectric focusing. *Hum. Genet.* **50**: 75–79.

Hovanessian, A. and Awdeh, Z.L. 1975. In *Progress of Isoelectric Focusing and Isotachophoresis*, Righetti, P.G., ed., North-Holland-Amsterdam/Oxford-New York, pp. 205–211.

Kirk, R.L., Matsumoto, H., and Katayama, K. 1978. Transferrin variants in Korea and Japan. *Jpn. J. Human Genet.* **23**: 1–7.

Kueppers, F. and Harpel, B.M. 1980. Transferrin C subtypes in US Blacks and Whites. *Hum. Hered.* **30**: 376–382.

Kühnl, P. and Spielmann, W. 1979. A third common allele in the transferrin system, Tf^{C3}, detected by isoelectric focusing. *Hum. Genet.* **50**: 193–198.

Sebetan, I.M., Oshida, S., and Akaishi, S. 1982. Transferrin (Tf) polymorphism: an analysis by isoelectric focusing. *Forensic Sci. Int.* **19**: 281–286.

Smithies, O. 1957. Variations in human serum β-globulin. *Nature* **180**: 1482–1483.

Stibler, H., Beckman, G., and Sikström, C. 1979. Subtypes of transferrin C. *Hum. Hered.* **29**: 320–324.

Teisberg, P. 1970. High voltage agarose electrophoresis in study of C3 polymorphism. *Vox Sang.* **19**: 47–56.

Thymann, M. 1978. Identification of a new serum protein polymorphism as transferrin. *Hum. Genet.* **43**: 225–229.

Weidinger, S., Schwarzfischer, F., and Cleve, H. 1980. Classification of transferrin (Tf) subtypes by isoelectric focusing. *Z. Rechtsmed.* **85**: 255–261.