GENETIC POLYMORPHISM IN THE SERUM TRANSFERRIN OF *RATTUS RATTUS**

KAZUO MORIWAKI, KIMIYUKI TSUCHIYA AND TOSIhide H. YOSIDA

National Institute of Genetics, Misima, Japan, 411.

Received March 11, 1969

EXTENSIVE studies have been carried out on serum transferrin polymorphisms of various vertebrates including man, and reviewed comprehensively by several workers (GIBLETT 1962; BEARN and CLEVE 1966; and LUSH 1966). In the order Rodentia, transferrin variations have so far been reported in only a few species: the laboratory mouse, *Mus musculus* (COHEN 1960; SHREFFLER 1960; KLEIN, ROOP and ROOP 1966), and three species of voles, *Microtus* (MAURER 1967).

The present study is concerned with the inheritance of serum transferrin polymorphisms in the black rat, *Rattus rattus*, the distribution of this polymorphism in Japanese populations of *Rattus rattus* and the relationship between this polymorphism and morphological variations of the largest chromosome in the species.

MATERIALS AND METHODS

A total of 168 wild black rats, *Rattus rattus*, were collected from eight localities in Japan; Niitsu City in Niigata Prefecture, Coizumi in Gunma Pref., Mizukaido City in Ibaragi Pref., Misima City in Sizuoka Pref., Tottori City in Tottori Pref., Tsushima island in Nagasaki Pref., Okinoerabu island in Kagoshima Pref. and Mikurajima island in Tokyo Metro. (see Figure 1). Laboratory stocks having three kinds of transferrin phenotypes were established from the Misima population. Transferrin patterns from Wistar rats were used as reference.

Blood specimens usually were obtained from the caudal vein by cutting the tip of the tail. Serum was separated from the clot by centrifugation two hours after bleeding.

Electrophoretic assay of the serum was carried out by the following procedure. The starch gel for electrophoresis was composed of 12.7% (w/w) hydrolyzed starch in glycine-borate-NaOH buffer (25 mM glycine, 5 mM boric acid and 50 mM NaOH, pH 8.6). The hydrolyzed starch was obtained by treating potato starch powder (Wako Chemicals) with 0.12 N HCl at 37°C for 135 min. Borate buffer (300 mM boric acid and 50 mM NaOH, pH 8.3) was used for the electrode chambers. The gel tray was placed horizontally in the refrigerator at 5°C and connected to a constant current power supply for 15 hrs at 3.6 ma/cm². The proteins on the gel were stained with a saturated solution of amido black 10 B in water-methanol-glacial acetic acid (5:5:1). Excess dye was removed by several washings with the dye solvent. The other details of the procedure are similar to those described by SMITHIES (1955).

The position of the serum transferrins was determined by employing autoradiography with ⁵⁹Fe as described by GILLET, HICKMAN and SMITHIES (1959). 0.3 ml of serum was mixed with 0.1 ml ⁵⁹FeCl₃ solution (Daiichi Pure Chemicals Co.) containing 2.3 µg Fe and 20 µc ⁵⁹Fe. Approximately 0.05 ml of this mixture was applied to a slot in the gel. After electrophoresis the gel was wrapped in Saran Wrap (Asahi-Dow Chemicals) and placed in contact with non-screen

* Contribution No. 712 from the National Institute of Genetics, Japan. Supported in part by a Grant-in-Aid for Scientific Expedition from the Ministry of Education of Japan (1968).

FIGURE 1.—A map of Japan showing the source of each sample. The subdivisions in the circle are proportional to the frequencies of the various transferrin phenotypes. The dots represent regions where only T/T chromosomal types were found (YOSIDA 1968).

type X-ray film (Konishiroku Photo Co.) for 2 days. The film was developed for 5 min at 20°C in Konidol-X developer (Konishiroku Photo Co.).

RESULTS

Electrophoresis: Starch gel electrophoresis of serum proteins revealed the existence of three types of transferrin patterns TfR, TfRN and TfN in the natural populations of *Rattus rattus* in Japan. The nomenclature used was consistent with one proposed by ASHTON *et al.* (1967). TfR was characterized by two fast moving bands, TfN by two slowly moving bands and in TfRN all four bands were evident. These bands migrated into the region between slow α -globulin and fast α -globulin. The electrophoretic patterns of these three types and that of *Rattus norvegicus* serum which was used as a standard are shown in Figure 2. Auto-

FIGURE 2.—Electrophoretic patterns of rat serum transferrins. The white dots designate bands which were heavily labelled by ^{59}Fe .

radiography with ^{59}Fe unequivocally demonstrated that the four bands in *Rattus rattus* are iron-binding proteins, transferrins.

The two transferrin bands of *Rattus norvegicus* (Wistar strain) were previously described by BEATON, SELBY and WRIGHT (1961), and analyzed chemically by GORDON and LOUIS (1963). An extension of the present electrophoretic survey to *Rattus norvegicus* did not reveal serum transferrin variants in any of the inbred strains examined, which included Wistar, Wistar King A, Wistar King S, Long Evans, Castle's Black, Buffalo, Wayne's pink-eyed yellow hooded, Fischer, CW-1, NIG III, NIG IV, YOS, and Toma, nor in the fifty or so wild animals from several localities in Japan.

Progeny tests of the transferrin variants: The results of matings involving all six combinations of the three transferrin patterns observed are summarized in Table 1. These results are consistent with a genetic theory based on a pair of codominant autosomal alleles. There was good agreement between observed and expected numbers. The possibility of sex-linkage was eliminated when in a mating of TfN females and TfR males all 17 offspring were TfRN.

TABLE 1
Progeny test of serum transferrin variants in Rattus rattus

Parental phenotype	Number of litters	Progenies			Total
		TfR	TfRN	TfN	
TfR $\times$ TfR	56	245	0	0	245
TfR $\times$ TfN	6	0	24	0	24
TfN $\times$ TfN	4	0	0	23	23
TfR $\times$ TfRN	41	83	91	0	174
TfN $\times$ TfRN	4	0	10	7	17
TfRN $\times$ TfRN	25	36	59	30	125

TABLE 2

Distribution of transferrin phenotypes in wild populations of Rattus rattus in Japan

Localities	Phenotypes			Total
	TfR	TfRN	TfN	
Niitsu	11 (10.5)	11 (12.0)	4 (3.5)	26
Ooizumi	35 (35.3)	7 (6.4)	0 (0.3)	42
Mizukaido	5 (4.9)	6 (6.2)	2 (1.9)	13
Misima	20 (19.7)	3 (3.2)	0 (0.1)	23
Tottori	21 (21.1)	3 (2.8)	0 (0.1)	24
Tsushima	11	0	0	11
Okinoerabu	14 (13.1)	6 (7.8)	2 (1.1)	22
Mikurajima	7	0	0	7

Parentheses represent expected values based on the Hardy-Weinberg hypothesis.

The locus has tentatively been designated *Tf* with the allele responsible for the faster bands being *Tf^R* and its counterpart *Tf^N*.

Local differences in the frequencies of transferrin phenotypes: Frequencies of the transferrin phenotypes in various localities are illustrated by circular diagrams on the map (Figure 1) and the details are summarized in Table 2. The frequencies of the three phenotypes in each of the localities do not differ significantly from Hardy-Weinberg expectations. A test for homogeneity, after the method of NEEL and SCHULL (1954), revealed that the six populations with both alleles differed significantly from one another with respect to the frequencies of those alleles.

Association between Tf and chromosome No. 1: In *Rattus rattus* the largest chromosome (No. 1) has been found to be polymorphic (YOSIDA *et al.* 1965). Three cytologic situations have been observed. Some rats have a telocentric homomorphic pair of No. 1 chromosome (T/T), others a pair consisting of a telocentric and subtelocentric (T/S) and still others a subtelocentric homomorphic pair (S/S). The possibility that the *Tf* locus is on chromosome No. 1 was explored when a female of chromosomal constitution S/T and homozygous for allele *Tf^N* was crossed with a T/T, *Tf^R/Tf^R* male. This resulted in some progeny heterozygous at the *Tf* locus (*Tf^R/Tf^N*) and with both types of chromosomes (T/S). Matings of these "doubly heterozygous" rats resulted in six phenotypes. Observed numbers of each segregant did not differ significantly from the numerical expectations based on two independent loci with the equivalent of two co-dominant alleles at each. The appearance of more than three phenotypes is sufficient to discard the hypothesis that the *Tf* locus is located on chromosome No. 1.

A comparison of the geographic distribution of Chromosome No. 1 and trans-

TABLE 3

Distribution of transferrin and chromosome No. 1 types in three Rattus rattus populations

Localities	Transferrin phenotypes			Chromosome 1			Total
	TfR	TfRN	TfN	T/T	T/S	S/S	
Ooizumi	34 (0.872)	5 (0.128)	0 (0)	17 (0.436)	17 (0.436)	5 (0.128)	39
Mizukaido	5 (0.385)	6 (0.461)	2 (0.154)	6 (0.462)	6 (0.462)	1 (0.076)	13
Okinoerabu	14 (0.636)	6 (0.273)	2 (0.091)	18 (0.818)	4 (0.182)	0 (0)	22

Parenttheses represent the frequencies.

ferrin polymorphisms. As previously reported (Yosida *et al.* 1967), the overall frequencies of T/T, T/S and S/S chromosomal types in natural populations of *Rattus rattus* in Japan were 0.848, 0.148 and 0.004, respectively. The pooled frequencies of the three transferrin phenotypes were rather similar: TfR = 0.738, TfRN = 0.214 and TfN = 0.048. In an effort to detect association between the two polymorphic systems three populations were examined more closely (Table 3). All of the animals in these populations were typed for both transferrins and chromosomes. The observed frequencies of the various phenotypes were then compared with those expected if no association existed between the two systems. The results indicate the absence of any obvious association between the transferrin patterns and the chromosome No. 1 morphology.

DISCUSSION

The examination of wild black rats (*Rattus rattus*) from a number of localities in Japan revealed the existence of electrophoretically detectable variants of the serum transferrins. Three phenotypes were recognized and these appear to be controlled by two codominant alleles, Tf^R and Tf^N .

The overall frequencies of the two alleles are 0.845 and 0.155 for Tf^R and Tf^N , respectively. In six of the eight populations sampled a polymorphism was observed with the frequency of Tf^R ranging from 0.615 to 0.938. In the remaining two only Tf^R was present. These last two were samples from small islands and since these islands are separated geographically by a considerable distance the possibility that an identical selective force is involved in the loss of Tf^N is remote. A more likely explanation invokes genetic drift, although admittedly there is no direct evidence. In the samples collected on Honshu (mainland of Japan) both alleles are present with Tf^R being obviously favoured. Whether the polymorphism detected is stable or transient cannot be determined from the present data. The significant heterogeneity observed, when populations were compared, suggests that the polymorphism is transient. Nevertheless, the possibility of selective pressures differing from locality to locality and the occurrence of drift could account for these variations even with a stable polymorphism.

A comparison of the distributions of the transferrin variants and the chromosomal polymorphisms is rather interesting. Breeding and population data have, of course, shown that these two systems behave independently. The distribution of the No. 1 chromosomal polymorphism has been described in considerable detail by YOSIDA (1968). Rats having three types of chromosome No. 1, T/T, T/S, and S/S, were collected along the east and southeast coast of Honshu, whereas from Hokkaido and the northwest coast of Honshu only T/T individuals were obtained (Figure 1).

A similar examination of the transferrin data does not show any geographic patterns of either allelic or zygotic frequencies. One possible explanation of these findings lies in history. In ancient times the rats originating in southeast Asia migrated to the east and southeast coast of Honshu through China and Korea. Rats in these geographic regions have the three types both of chromosomes (YOSIDA, unpublished data) and transferrins (MORIWAKI, unpublished data). In the past the habitat of the rats was probably restricted to the east and southeast coast of Honshu by the mountains in the center. It may be assumed that the northwestern part was populated by descendants of a few small populations with T/T chromosomes and three types of transferrins. Such a situation would be ideal for the occurrence of genetic drift. Even if this hypothesis explains the frequency distribution of the chromosomal types, the explanation for the distribution of the transferrin alleles is different.

Two electrophoretic bands of transferrin in Rattus rattus: Both TfR and TfN phenotypes can be separated electrophoretically into two bands with the system used. The separation of the two bands appears the same in both phenotypes. Similar situations have been reported in zebu cattle (ASHTON 1969), in the vole (MAUER 1967) and *Rattus norvegicus* (GORDON and LOUIS 1963). The latter has received considerable attention. According to GORDON and LOUIS there is no marked difference between the two components in the light-absorption spectrum, molecular weight and amino acid composition. These authors also discount sialic acid as being responsible for the differential mobility in the two components since they are equally affected on treatment with neuramidase. It is, however, quite possible that all of the sialic acid was not removed from the transferrin molecule by the neuramidase treatment. For instance, PARKER and BEARN (1961) were able to reduce purified human transferrins to a single band by complete digestion with neuramidase. A similar situation could be expected in the two bands of TfR or TfN in *Rattus rattus*.

The difference between TfR and TfN transferrins can probably simply be explained by amino acid substitution. This hypothesis, however, is yet to be verified.

The authors are especially grateful to DR. M. L. PETRAS of the University of Windsor for his valuable suggestions and criticisms in the preparation of the manuscript. Special thanks are also due to Miss HARUMI SAKATA for her technical assistance.

SUMMARY

An electrophoretic survey of a serum transferrin polymorphism using starch

gel in natural populations of the black rat, *Rattus rattus* in Japan revealed three phenotypes TfR, TfRN and TfN. Progeny tests suggest that these three phenotypes are controlled by two alleles at a single autosomal locus. Though the frequency of the Tf^R allele in the wild was obviously greater than that of Tf^N , laboratory test crosses between heterozygotes have not conclusively demonstrated inferiority of the Tf^N allele. An examination of 8 populations revealed the presence of both alleles in six and a homogeneity test suggested significant dissimilarity in the frequencies of the two alleles. Any strong relationships between the transferrin locus and either chromosome No. 1 or the sex chromosome were ruled out. Finally the distributions of the chromosomal and transferrin polymorphisms appear to be the result of different mechanisms.

LITERATURE CITED

- ASHTON, G. C., D. G. GILMOUR, C. A. KIDDY, and F. K. KRISTJANSSON, 1967 Proposals on nomenclature of protein polymorphisms in farm livestock. *Genetics* **56**: 353-362.
- BEARN, A. G., and H. CLEVE, 1966 Genetic variations in the serum proteins. pp. 1321-1342. In: *The Metabolic Basis of Inherited Disease*, 2nd Ed. Edited by J. B. STANBURY, J. B. WYNGARDSEN and D. S. FREDRICKSON. McGraw Hill, New York.
- BEATON, G. H., A. E. SELBY, and A. M. WRIGHT, 1961 Starch gel electrophoresis of rat serum proteins I. Procedure and designation of components. *J. Biol. Chem.* **236**: 2001-2004.
- COHEN, B. L., 1960 Genetics of plasma transferrins in the mouse. *Genet. Res.* **1**: 431-438.
- GIBLETT, E. R., C. G. HICKMAN, and O. SMITHIES, 1959 Serum transferrins. *Nature* **183**: 1589-1590.
- GIBLETT, E. R., 1962 The plasma transferrins. *Progr. Medic. Genet.* **2**: 34-63.
- GORDON, A. H., and L. N. LOUIS, 1963 Preparation and properties of rat transferrin. *Biochem. J.* **88**: 409-414.
- KLEIN, P. A., B. L. ROOP, and W. A. ROOP, 1966 Starch gel electrophoretic patterns of murine transferrin. *Nature* **212**: 1376-1377.
- LUSH, I. E., 1966 *The Biochemical Genetics of Vertebrates Except Man*. North-Holland Publishing Co., Amsterdam.
- MAURER, F. W., 1967 Heritability of the plasma transferrin protein in three species of *Microtus*. *Nature* **215**: 95-96.
- NEEL, J. V., and W. J. SCHULL, 1954 *Human Heredity*. The University of Chicago Press, Chicago.
- PARKER, W. C., and A. G. BEARN, 1961 Alterations in sialic acid content of human transferrin. *Science* **133**: 1014-1016.
- SHREFFLER, D. C., 1960 Genetic control of serum transferrin type in mice. *Proc. Natl. Acad. Sci. U.S.A.* **46**: 1378-1384.
- SMITHIES, O., 1955 Zone electrophoresis in starch gels: Group variations in the serum proteins of normal human adults. *Biochem. J.* **61**: 628-641.
- YOSIDA, T. H., 1968 Local differences in frequencies of No. 1 chromosomal polymorphism of *Rattus rattus*. *Ann. Rept. Natl. Inst. Genet. Japan* **18**: 51-53.
- YOSIDA, T. H., A. NAKAMURA, and T. FUKAYA, 1965 Chromosomal polymorphism in *Rattus rattus* (L.) collected in Kusudomari and Misima. *Chromosoma* **16**: 70-78.
- YOSIDA, T. H., Y. MORIGUCHI, Y. S. KANG, and K. SHIMAKURA, 1967 Population survey of No. 1 chromosome polymorphism of black rats (*Rattus rattus*) collected in Japan and Korea. *Ann. Rept. Natl. Inst. Genet. Japan* **17**: 61-62.