Symposium at the 26th Annual Meeting of the Japan Society of Human Genetics, 1981: Genetic Markers in Blood

Genetic Markers

by Dr. Susumu Shibata

Erythrocyte blood group systems may be cited as representative prototypes of genetic markers, that have been studied extensively. The human leukocyte antigen (HLA) system, which is now studied with keen interest, is developing into one of the most important genetic markers. Both the blood groups and the HLA constitute critical factors for the success of blood transfusion and tissue transplantation. However, the majority of genetic markers, with exception of blood groups and HLA, are enzymes or other proteins of tissues and blood plasma which were discovered and identified largely by the development of electrophoretic and chromatographic techniques. They are highly evaluated tools for the recognition of human individuality and the study of human polymorphisms.

It is, of course, unavoidable that not all genetic markers can be discussed with equal emphasis and therefore we have adjusted the focus of the selection of subjects to those enzymes and other protein systems of blood serum and erythrocytes in which the members of the Japan Society of Human Genetics have been specially interested and have, so far, made notable contributions.

In this symposium we arranged the following 5 themes: (1) genetic markers of the complement system, (2) HLA (in relation to the function of lymphocytes), (3) pseudocholinesterase of serum and its variants, (4) some erythrocyte enzymes, and (5) a biochemical genetic survey of certain erythrocyte enzymes and other proteins in blood samples obtained from selected populations in Hiroshima and Nagasaki. Supplemental observation on genetic polymorphisms in Aichi Prefecture are also presented. We hope that this symposium will awaken the interest of those attending this meeting. Thank you.

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GENETIC POLYMORPHISMS OF COMPLEMENT SYSTEMS IN JAPANESE

Keiichi OMOTO and Katsushi TOKUNAGA

Department of Anthropology, University of Tokyo, Tokyo 113, Japan

Recently, genetic polymorphisms were detected by electrophoretic methods in a number of human complement proteins: C2, C3, C4, C5, C6, C7, C8, Factor B(BF), Factor D and C1-inhibitor (See Alper and Rosen, 1976; Lachmann and Hobart, 1978; and Hobart *et al.*, 1981 for C5 polymorphism). Some of these polymorphisms attracted special attention of investigators because of the discovery of associations between particular alleles and disseases, such as those between C3*F and rheumatoid arthritis (Farhud *et al.*, 1972) or BF*F1 and the early-onset insulin-dependent diabetes mellitus (IDDM) (Raum *et al.*, 1979; Kirk *et al.*, 1979) on the one hand, and because of the linkage between C2, C4 and BF loci and the MHC loci on the short arm of the chromosome No. 6, on the other. Thus far, most studies have been confined to the European racial groups. In this review, we present the data mainly obtained in our laboratory concerning the polymorphisms of BF and C2 in Japanese with special reference to the distribution of the variant types, linkage disequilibrium with alleles of HLA loci, and some evidences of disease associations. Also the present status of typing of C3 and C4 in Japan is briefly mentioned.

1. C3 polymorphism

Using fresh sera, this polymorphism is easily shown by protein stain after agarose gel electrophoresis. It is known that a "fast" variant of C3 has an appreciable frequency in Japanese: 12 out of 464 samples (Tokyo) in one study (Harada *et al.*, 1975) and 9 out of 1,092 samples (Kyoto) in another study (Nishimukai *et al.*, 1979) were found to be heterozygous for this variant. In our preliminary study (Tokyo), 8 samples among 525 were found to be heterozygous for a fast C3 variant. It is likely that this variant is not identical to the C3 F variant commonly found in Europeans. We recently examined C3 types of sera from 113 patients with rheumatoid arthritis and found no evidence for an elevated incidence of the variant C3 types (unpublished).

2. Factor B (BF) polymorphism

BF polymorphism is usually examined by an agarose gel electrophoresis followed by immunofixation (Alper *et al.*, 1972). In addition to a pair of common alleles (*BF*F* and *BF*S*), two relatively uncommon alleles *BF*F1* and *BF*S0.7* are

Table 1. Distribution of BF allele frequencies in various populations.

Populations	N	BF Alleles S	F	F1	S1 (S0.7)	Others[a]	Authors
Caucasoid							
USA Caucasian	158	.709	.278	—	.013	—	Alper *et al.*, 1972
West German	1,245	.808	.174	.008	.009	1	Mauff *et al.*, 1975
French	247	.757	.219	.010	.014	—	Hauptmann *et al.*, 1976
Norwegian	300	.817	.172	.005	.007	—	Teisberg & Olaisen 1977
Swiss	654	.805	.176	.010	.009	—	Scherz *et al.*, 1977
Italian	62	.718	.250	.008	.024	—	Scherz *et al.*, 1977
Dane	318	.780	.203	.008	.009	—	Mortensen & Lamm 1981
Spaniard	330	.658	.266	.052	.022	—	Rodriguez-Córdoba *et al.*, 1981
French Basque	201	.550	.296	.015	.139	—	Ohayon *et al.*, 1980
Australian Caucasian	470	.797	.187	.010	.006	—	Stewart *et al.*, 1979
Non-Caucasoid							
Lapps	197	.888	.112	0	0	0	Teisberg & Olaisen 1977
USA Oriental	86	.890	.110	0	0	0	Alper *et al.*, 1972
Japanese	360	.824	.176	0	0	0	Horai 1976
Japanese	487	.801	.198	0	0	1	Tokunaga *et al.*, 1981a
USA Negroid	127	.437	.512	.051	0	0	Alper *et al.*, 1972
South African Negroes	944	.282	.655	.034	.025	7	Mauff *et al.*, 1976
S.A. Indian	90	.645	.322	0	.033	0	Mauff *et al.*, 1976

[a] Observed number of variant phenotypes.

known in European populations. *BF*F1* determines the BF component migrating faster than BF F and its frequency is known to be higher in southern Europe than in northern Europe (Table 1). It is this allele that shows an association with IDDM (Raum *et al.*, 1979; Kirk *et al.*, 1979).

Horai (1976) was the first to report BF allele frequency in Japanese, but no variant phenotype other than the three common phenotypes (S, SF and F) were found among 360 healthy donors in Nara Prefecture. Recently, we detected a BF variant similar but not identical to BF F1 type (Tokunaga *et al.*, 1981a). It migrates slightly slower than BF F1 and the conversion product Bb after zymosan treatment shows a distinct band (Fig. 1). The variant allele was denoted *BF*FT* (*F Tokyo*). In a sample from 65 IDDM patients of Tokyo Women's Medical College, two samples were found to be heterozygous for this variant: one BF FFT and the other BF SFT. In a control sample of 491 unrelated healthy individuals, the variant was encountered only once. Thus, the incidence of the BF FT positive phenotypes was shown to be significantly elevated in IDDM patient group ($p<0.05$) (Tokunaga

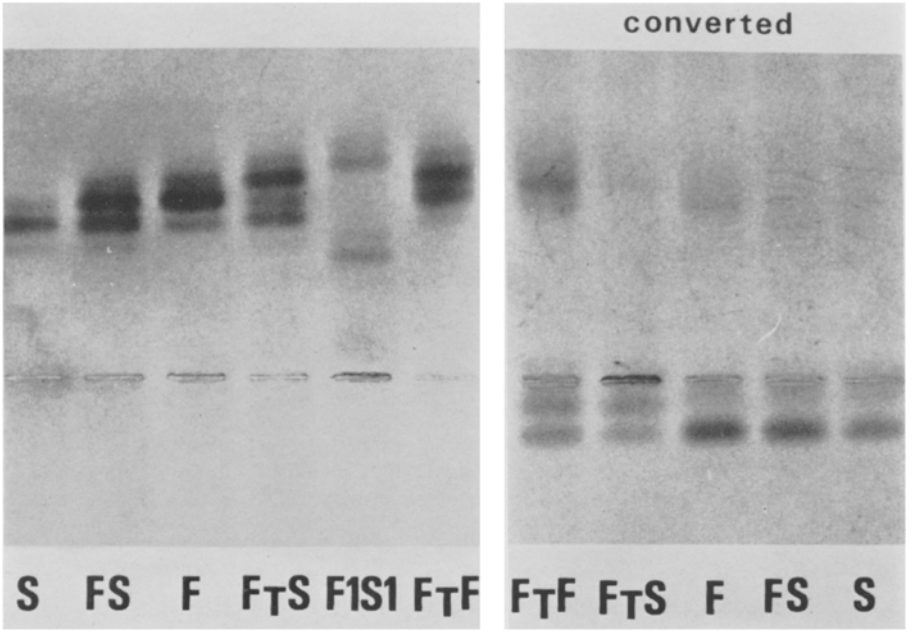

Fig. 1. Photographs showing agarose gel electrophoretic patterns of various BF phenotypes. To the right, the conversion fragments after zymosan treatment are shown.

Table 2. BF types observed among 108 IDDM patients in Japan. Data of controls is taken from Tokunaga *et al.*, 1981b.

	Patients (n=108)	Controls (n=496)	p
BF S+	103 (95.4%)	473 (95.4%)	NS
BF F+	33 (30.6%)	176 (35.5%)	NS
BF FT+	3 (2.8%)	1 (0.2%)	<0.025[a]

[a] RR=14.1

et al., 1981a). More recently, BF typing was carried out on 43 IDDM patients of Toranomon Hospital, Tokyo, revealing a further BF FT positive individual (unpublished data). This resulted in the total incidence of 2.8% BF FT positive phenotypes in IDDM patients in contrast to 0.2% in controls (Table 2). It is interesting that association of a rare BF variant and IDDM is found in different racial groups (Southern European and Japanese) but the variant BF alleles involved in this association are not identical between two racial groups. BF typing was also carried out in a sample from 123 patients of rheumatoid arthritis. BF FTS phenotype was observed once, showing no statistically significant increase of the variant.

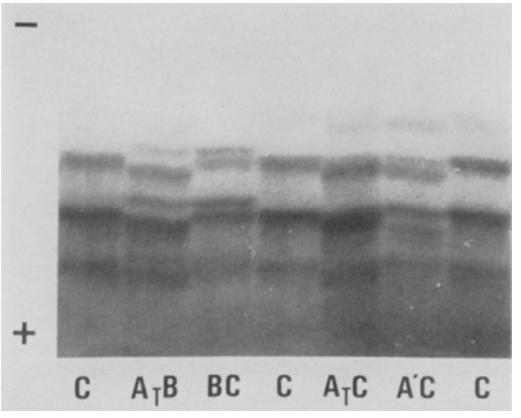

Fig. 2. Photograph showing the isoelectrofocusing patterns of various C2 phenotypes found in Japanese (Tokunaga *et al.*, 1981b). Note that the bands corresponding to C2 AT show relatively more intense hemolytic activity.

We also studied associations between BF alleles and allele of HLA A, HLA B and HLA C (Tokunaga *et al.*, 1982). The following associations were found to have significantly positive delta values and considered to be in linkage disequilibrium: *BF*F−HLA Aw33* ($p<0.0001$), *BF*F−HLA B17* ($p<0.025$), *BF*F−HLA B15* ($p<0.025$), *BF*F−HLA Bw44* ($p<0.0001$) and *BF*F−HLA Cw3* ($p<0.05$). Among these associations, only the *BF*F−HLA Bw44* is known to be present in general European populations.

3. C2 polymorphism

Polymorphism of C2 was detected in our laboratory by isoelectrofocusing (pH 5–7 or 5–8) in polyacrylamide gel followed by visualization of C2 hemolytic bands with sensitized sheep erythrocytes in agarose overlay.

Relatively few studies on the distribution of C2 polymorphic types have been reported (Table 3). In Japanese, at least four alleles at the C2 locus seem to occur: *C2*C*, *C2*B*, *C2*AT* and *C2*A'* (Fig. 2 and Table 4). *C2*AT* and *C2*A'* are the variants not hitherto known in European populations (Tokunaga *et al.*, 1981b). The results of simultaneous typing of HLA-A, -B, and -C specificities indicated the presence of significant associations of *C2*AT* with *HLA-B15* ($p<0.0001$) and with *HLA-A26* ($p<0.01$), and of *C2*B* with *HLA-Bw61* ($p<0.025$). None of these allelic combinations seem to be in linkage disequilibrium in European populations, in contrast to the fact that some allelic combinations between BF and HLA-B indicated linkage disequilibrium common to both European and Japanese populations. This finding may suggest that the BF locus is more closely linked to HLA-B locus than the C2 locus.

No difference in the C2 phenotypic distribution was observed between samples

Table 3. Distribution of C2 allele frequencies in various populations.

	N	C2 Alleles C	B	"A"[a]	Authors
USA Caucasian	75	.97	.02	AC 1	Alper, 1976
European	274	.962	.038	0	Meo *et al.*, 1977
Norwegian	122	.97	.03	0	Olaisen *et al.*, 1978
West German	289	.965	.035	0	Dewald & Rittner, 1979
Australian Caucasian	188	.965	.035	0	Kirk *et al.*, 1980
USA Oriental	43	.97	.03	0	Alper, 1976
Japanese	229	.937	.017	0.046	Tokunaga *et al.*, 1980
Japanese	521	.939	.022	AT .034 A′ .006	Tokunaga *et al.*, 1981b
USA Negroid	30	.97	.03	0	Alper, 1976

[a] "A" refers to the "acidic" variants.

Table 4. Distribution of C2 phenotypes and allele frequencies in Japanese (Tokunaga *et al.*, 1981b).

Phenotype	Obs. No.	%	Exp. No.	χ^2
C	459	88.1	458.97	0.000
ATC	32	6.1	32.85	0.022
BC	22	4.2	21.59	0.008
A′C	6	1.2	5.63	0.024
ATB	1	0.2	0.77	0.069
AT	1	0.2	0.59	0.285
B	0	0	0.25	0.254
ATA′	0	0	0.20	0.202
A′B	0	0	0.13	0.132
A′	0	0	0.02	0.017
Total	521	100.0	521.00	1.013

Allele frequencies: $C2^C = 0.939 \pm 0.007$
$C2^{AT} = 0.034 \pm 0.006$
$C2^B = 0.022 \pm 0.005$
$C2^{A'} = 0.006 \pm 0.002$

of IDDM patients and controls. However, in an investigation of C2 types in 120 samples of rheumatoid arthritis patients, a statistically significant deficit of C2 AT positive phenotype was observed ($p < 0.025$) (unpublished data). In this respect, it is interesting that the C2 AT positive phenotype seems to have an increased C2 hemolytic activity compared to those of other phenotypes (Tokunaga *et al.*, 1981b).

There are three theoretical possibilities for an association between the variant complement allele and the particular disease: 1) dysfunction of the variant complement protein, 2) linkage disequilibrium with disease susceptibility gene(s) in MHC region, and 3) epistatic effects. The first possibility seems to be preferred in the recent review by Rittner and Bertrams (1981). At present, however, there seems to be no positive evidence for one of these possibilities so far as the electrophoretic variant of the human complement is concerned.

4. C4 polymorphism

Tokunaga *et al.* (1979) reported the population data of C4 polymorphism in Japanese following the two allele model. In view of the rapidly increasing literatures supporting the more recent two locus model of C4 first proposed by O'Neill *et al.* (1978), it seems to be premature to use this system as an established genetic marker.

In concluding, two polymorphic systems of the complement, BF and C2, are of particular importance at present as genetic markers for studies of linkage with HLA loci, disease associations and population genetics in Japanese.

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