GENETIC POLYMORPHISMS OF HUMAN COMPLEMENT COMPONENTS IN JAPANESE AND THEIR APPLICATION TO PARENTAGE TESTING

K.SUZUKI,H.MATSUMOTO(Dept. Legal Medicine)*,Y.MATSUO,K.SHODA, M.IWATA(Dept. Blood Transfusion, Osaka Medical School),** G.J.O'NEILL(Columbia University)

Genetic polymorphisms of several components of human complement have been successively elucidated by some investigators[1)~8)] and now the existence of genetic polymorphism of the complement receptor as well as of the other components is being demonstrated.

In the field of forensic science, many polymorphic markers in blood have been applied to parentage testings. The typing techniques would be required to be always reproducible and not so difficult to operate when the polymorphic markers are employed for parentage testings. Some components were detected by their function, but this technique required some special and unstable reagents. On the other hand, immnoblotting is easy to perform and detects proteins by their antigenecities. We demonstrate the result of the investigation on complement polymorphisms in Japanese using immunoblotting or other conventional techniques.

MATERIALS AND METHODS

All the samples were prepared as EDTA-plasma by centrifugation. For C4 typing, samples were treated with neuraminidase under continuous dialysis[9)]. Monoclonal antibodies which were kindly provided by Dr.G.J.O'Neill were employed to discriminate the epitopic difference between C4A and C4B proteins. The typing procedures are presenred in Table 1.. Immunoblotting was carried out by simple diffusion from gel to nitrocellulose without any electroblotting apparatus.

RESULTS AND DISCUSSION

Phenotype distribution and gene frequencies observed in this study are shown in Table 2 and 3. Some new variants among Japanese were detected in C2, C6, and C7 and a new BF variant was found in a paternity case.

The band pattern of a new C2 variant showed more anodic

* Prof. Hideo Matsumoto, Osaka Medical School, 2-7 Daigakumachi Takatsuki City, J-569 Osaka, Japan

** 2-7 Daigakumachi, Takatsuki City, J-569 Takatsuki City, Japan

Advances in Forensic Haemogenetics 1

Advances in Forensic Haemogenetics 1
Edited by B. Brinkmann and K. Henningsen
© Springer-Verlag Berlin Heidelberg 1986
Springer-Verlag Berlin Heidelberg 1986

migration than that of C2AT and was tentatively designated C2Ax(Fig.1). The other rare variants which have been found so far only in Japanese[10] and in Korean[11], C2AT and C2BH, were also observed at polymorphic frequencies.

Table 1. Electrophoretic and detecting procedures

C3 :	Agarose gel electrophoresis + protein staining
BF :	Immunofixation agarose gel electrophoresis(IAGE) + protein staining
C4 :	IAGE + protein staining
	AGE + immunoblotting using monoclonal antibody
	AGE + hemolytic detection
	SDS-gel electrophoresis
C2,C6,C7 :	Isoelectric focusing in polyacrylamide gel + immunoblotting

Among the 215 samples of this study, one rare type,BF F0.75, which has been reported so far in Japanese[12) 13)] was detected and another variant was newly observed(Fig.2) in a paternity case and its genetic transmission was confirmed. This new variant was named BFF0.25 considering its relative mobility compared with those of BFF0.75 and BFF[14)]. The significant association of C2*AT with BF*F was estimated using 2 x 2 association analysis by Fisher's exact test(p=0.0021). This association was already described by Tokunaga et.al. with another significant association of C2*BH with BF*F[12)].

C6 and C7 were transferred from the both surfaces of a slab gel to nitrocellulose sheet; one surface for C6 blot

Table 2. Phenotype distribution and gene frequencies of BF, C2, C6 and C7

BF	C2	C6	C7
S = 154	C = 191	A = 43	1 = 164
FS = 53	BC = 13	AB = 97	2-1 = 31
F = 7	ATC = 8	B = 51	4-1 = 15
F0.75S = 1	BHC = 2	AB2 = 11	2 = 2
215	AxC = 1	BB2 = 8	3-1 = 2
	215	AR = 2	4 = 1
		BR = 2	215
		R = 1	
		215	
		R:rare allotypes (M2 , B11)	
BF*S =0.8419	C2*C =0.9442	C6*A =0.4558	C7*1=0.8744
BF*F =0.1558	C2*B =0.0302	C6*B =0.4860	C7*2=0.0814
BF*F0.75=0.0023	C2*AT=0.0186	C6*B2=0.0488	C7*4=0.0395
χ^2=0.829 1d.f.	C2*BH=0.0047	C6*R =0.0094	C7*3=0.0047
0.25<p<0.50	C2*Ax=0.0023	χ^2=0.893 4d.f.	χ^2=0.745 2d.f.
	χ^2=0.753 1d.f.	0.90<p<0.95	0.50<p<0.75
	0.25<p<0.50		

Advances in Forensic Haemogenetics 1
(c) Springer-Verlag Berlin Heidelberg 1986

(Fig.3) and the other for C7 blot(Fig.4). C6 showed appreciable polymorphism in Japanese as well as in other ethnic groups. It was confirmed that C6 polymorphism was controlled by three common alleles, C6*A, C6*B, and C6*B2 in Japanese as previously reported[15)~17)]. Moreover, two rare variants were confirmed, one was identified as M2 by direct comparison and the other was designated B11 on the suggestion by Dr.K.Tokunaga. The genetic polymorphism of C7 was controlled by three common alleles, C7*1, C7*2, and C7*4 in Japanese[18)]. A rare type which seemed to be C7 3 was found in two samples of this study and in one sample of a patient of Buerger's disease. Any significant association between C6 and C7 alleles could not be observed in this study, however, further investigation must be carried out to determine which alleles would be strongly associated each other.

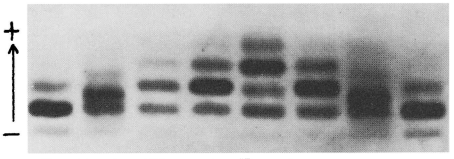


Fig.2. Immunofixation patterns of BF allotypes

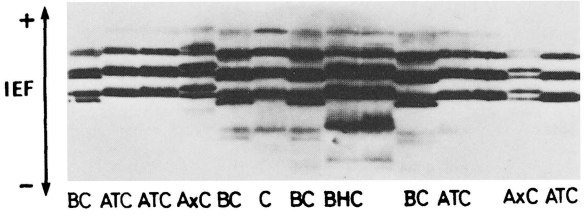


Fig. 1 IEF patterns of C2 revealed by immunoblotting Ax is a new variant and tentatively designated Reference typing was performed by Dr.TOKUNAGA

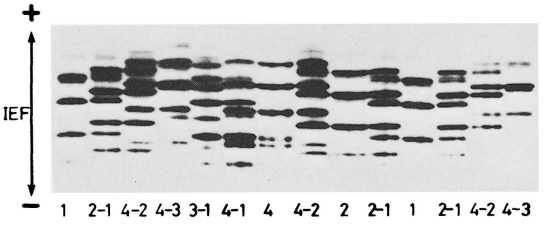


Fig.4 IEF patterns of C7 revealed by immunoblotting

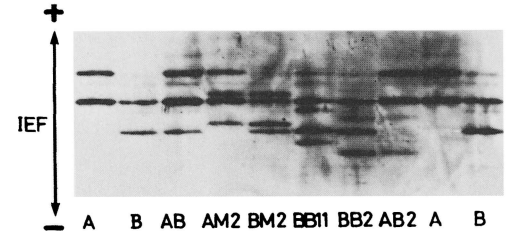


Fig. 3 IEF patterns of C6 revealed by immunoblotting Reference typing of M2 and B11 was performed by Dr.TOKUNAGA

Any other variants except C3S were not observed in this study although s few rare variants, S0.2, S0,25, F0.6, F0.65, and F0.8 were demonstrated among 1692 samples in Japanese[19)].

As for C4(Fig.5,6), some aberrant types were observed in this study. Hemolytically inactive C4B allotype, B4, was

Advances in Forensic Haemogenetics 1
(c) Springer-Verlag Berlin Heidelberg 1986

found in a family with insulin-dependant diabetes mellitus and another example which showed mobility close to C4A3 was suspected to be C4B product by immunoblotting with a monoclonal antibody specific to the epitope of C4B protein and α-chain typing. The latter was detected through blood typing for a family in which a child was born with chromosomal abnormality(partial deletion of 3q) and is under further investigation. Gene duplication at both C4 loci is not so rare event. Duplicated genes at C4B locus were found to be transmitted through three generation in one family and through two generation in the other in this study

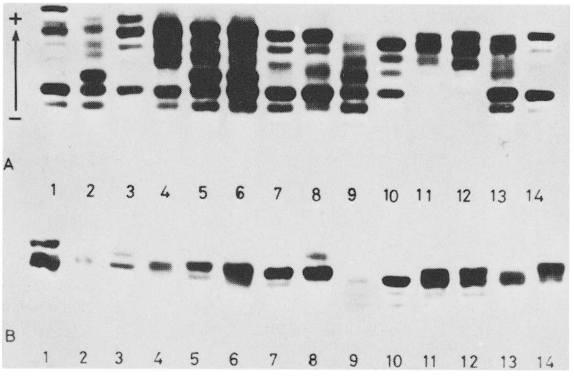


Fig.5.A.Immunofixation patterns of all the C4A allotypes in this study. B.Blot with a monoclonal antibody specific to C4A epitope. 1)A6,3;2)A5,4;3)A5,3;4)A4,3;5)A4;6)A4,3;7)A3;8)A3;9)A3,2;10)A2;11)A3,2;12)A3,13?;13)A3,2;14)A3. 1),9),10)were kind gift from Dr.O'Neill.

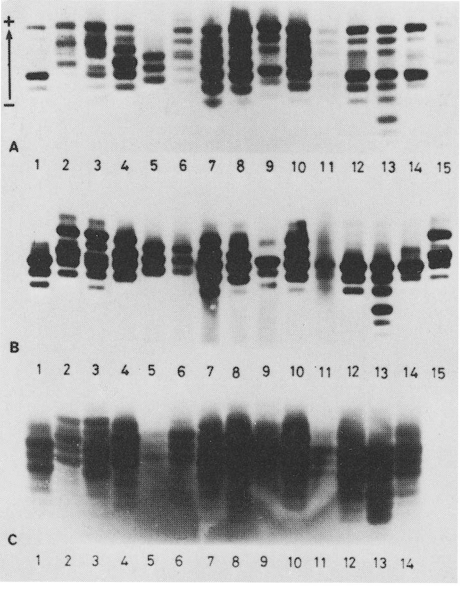
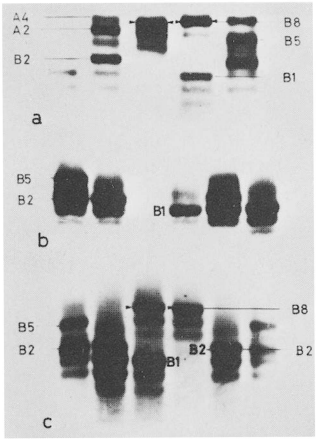


Fig.6.A.Immunofixation patterns of all the C4B allotypes in this study. B. Blot with a monoclonal antibody specific to C4B epitope. C.Hemolytic detection. 1)B1;2)B5,2;3)B5,1;4)B4,21,2;5)B31;6)B29;7)B21,1;8)B2,1;9)B12;10)B21,11;11)B11;12)B1;13)B1,96;14)B1;15)B5,2. 3),5),6) were kind gift from Dr.O'Neill.

Those markers which showed appreciable polymorphism seems to be useful when they are applied to parentage testing. Exclusion ratio is therefore one criterion for selecting effi-ient cient markers. The ratios were calculated as follows, 11.6% for BF, 5.2% for C2, 22.0% for C6, 12.5% for C7, 14.5% for C4A, and 21.6% for C4B,

Advances in Forensic Haemogenetics 1
(c) Springer-Verlag Berlin Heidelberg 1986

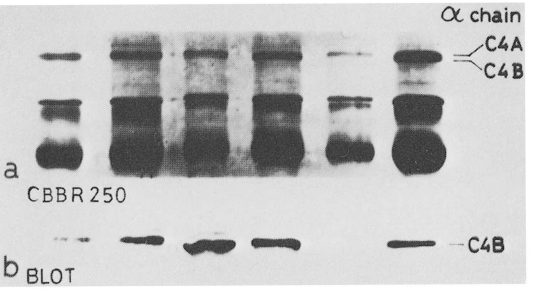
respectively. These five markers except C3 have been employed for parentage testing over 50 cases in our laboratory without any difficulties in typing.



Hemolytically inactive C4B allotype(suspected), which was tentatively named C4B8. a.IAGE pattern b.hemolytic detection c. immunoblotting with a monoclonal antibody specific to C4B epitope

Table 3. Phenotype distribution of C4A and C4B and their gene frequencies.

phenotypes				gene frequencies	
A locus	no.	B locus	no.		
5,4	1	5	29	A^*_*5 = 0.0012	
4	38	5,21	2	A^*_*4 = 0.1580	
4,3	73	5,2	23	A^*_*3 = 0.7127	
4,2	2	5,1	26	A^*_*2 = 0.0727	
3	267	4,1	2	A Qo= 0.0554	
3,2	61	21,1	3		1.0000
Qo	1	2	45	B^*_*5 = 0.0855	
	443	2,1	97	B^*_*2 = 0.2113	
χ^2=50.4(df=4)		12	4	B^*_*1 = 0.5115	
		11	2	B^*_*R = 0.0208	
		1	195	B Qo= 0.1709	
		1,96	1		1.0000
		Qo	11		
			443		
		χ^2=24.1(df=6)			



SDS-gel electrophoresis of α chain of inactive C4B8. From left to right, mother(A4,2B2,0),child (A4,2B8,0),father(A4,3B8,1),controls(C4A null, B null, A4,3B5,2)

REFERENCES

1)Alper,C.A.et.al. J.Exp.Med.135,68-80(1972)
2)Alper,C.A.et.al. J.Exp.Med.144,1111-1115(1976)
3)Azen,E.A.et.al. Science 162,905-907(1968)
4)Alper,C.A.et.al. J.Clin.Invest.47,2181-2191(1968)
5)O'Neill,G.J.et.al. P.N.A.S.USA 75,5165-5169(1978)
6)Mauff,G.et.al. Immunobiology 164,184-191(1983)
7)Hobart,M.J.et.al. in Protides of the Biological Fluids. Pergamon Press.N.Y. 12,575(1975)
8)Hobart,M.J.et.al. J.Immunogenet.5,157-163(1978)
9)Awdeh,Z.l.et.al. P.N.A.S.USA 77,3576-3580(1980)
10)Tokunaga,K.et.al. Hum.Genet.58,213-216(1981)
11)Park,K.S.et.al. Jpn.J.Hum.Genet.30,9-14(1985)
12)Tokunaga,K.et.al.Hum.Genet.60,42-45(1982)
13)Nishimukai,H. J.Kyoto Pref.Univ.Med.91,1177-1202(1982)
14)Mauff,G.et.al. Z.Immun.-Forsh.154,115-120(1978)
15)Tokunaga,K.et.al. J.Immunogenet.10,419-424(1983)
16)Nishimukai,H.et.al. Hum.Hered.35,30-33(1985)
17)Suzuki,K.et.al. Act.Crim.Japon.51,87-91(1985)
18)Nakamura,S.et.al. Hum.Genet.66,279-281(1984)
19)Nishimukai,H.et.al. Hum.Hered.35,69-72(1985)

Advances in Forensic Haemogenetics 1
(c) Springer-Verlag Berlin Heidelberg 1986