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GENETIC POLYMORPHISMS OF COMPLEMENT COMPONENTS C6 AND C7 IN KOREAN

Kyung Sook PARK,[1] Masami YANAGISAWA,[2] Katsushi TOKUNAGA,[2] and Keiichi OMOTO[2]

[1]*Department of Biology, Sung-Shin Women's University, Seoul 136, Korea*
[2]*Department of Anthropology, The University of Tokyo, Tokyo 113, Japan*

Summary Genetic polymorphisms of the complement components C6 and C7 were investigated in Korean living in Seoul using isoelectric focusing and immunoblotting. Three common and four rare C6 allotypes were observed. The allele frequencies estimated were as follows: *C6*A* 0.433, *C6*B* 0.523, *C6*B2* 0.039, and rare alleles (M91, M92, M11 and M2) 0.005. Three C7 variants, besides a common allele, were observed with polymorphic frequencies. Two rare C7 variants, considered to be new, were also observed. The allele frequencies estimated for *C7*1*, *C7*2*, *C7*3*, *C7*4*, and rare variants (tentatively named K1 and K2) were 0.843, 0.073, 0.034, 0.048 and 0.002, respectively. The C6 and C7 allele frequencies are similar to those in Japanese and Chinese. The association analysis between C6 and C7 showed a significant negative association between C6 B and C7 4 allotypes ($p < 0.02$).

INTRODUCTION

Genetically determined polymorphisms have been shown for most human complement proteins. Polymorphism of the sixth component (C6) was first described by Hobart *et al.* (1975). Two predominant alleles, C6*A and C6*B, and several rare alleles have been described in Caucasian populations (Hobart and Lachmann, 1976; Kühnl and Kreckel, 1980; Kunstmann *et al.*, 1980). The variants are designated according to their relative isoelectric points (Mauff *et al.*, 1980). The introduction of an immunoblotting method after isoelectric focusing in polyacrylamide gel enabled us to carry out extensive population surveys (Tokunaga *et al.*, 1984). It is interesting that *C6*B* is commoner than *C6*A* and that the third

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common allele, *C6*B2*, and a number of rare variants have been found in Japanese and in Mainland Chinese populations (Tokunaga *et al.*, 1983, 1984; Washio *et al.*, 1986; Zeng *et al.*, 1986).

Genetic variation of the seventh component of complement (C7) was first detected by Hobart *et al.* (1978). They reported a common allele, *C7*1*, and two rare variants, *C7*2* and *C7*3*, in a Caucasian population. Close linkage between C6 and C7 loci has been established (Hobart *et al.*, 1978; Tokunaga *et al.*, 1986). Isoelectric focusing with neuraminidase-treated plasma and subsequent immunoblotting have been established as a suitable method for C7 typing (Nakamura *et al.*, 1984a; Nishimukai and Tamaki, 1986; Washio *et al.*, 1986). Interestingly, besides the commonest allele, *C7*1*, at least three variants, *C7*2*, *C7*3* and *C7*4*, occur with polymorphic frequencies in Japanese and in Mainland Chinese populations (Washio *et al.*, 1986; Zeng *et al.*, 1986).

Previously, we have reported the genetic polymorphisms of the second complement component (C2) and factor B (BF) in Korean (Park *et al.*, 1985). The purpose of this study was to investigate the allelic distribution of the linkage group C6 and C7 in Korean. The result of an association analysis between C6 and C7 alleles is also presented.

MATERIALS AND METHODS

EDTA-plasma samples were obtained from 490 healthy blood donors living in Seoul, Korea. Typing of C6 and C7 was carried out using polyacrylamide gel isoelectric focusing and immunoblotting as described previously (Tokunaga *et al.*, 1984; Washio *et al.*, 1986). Briefly, isoelectric focusing (pH 5–8) was performed in a thin layer (0.5 mm thick) polyacrylamide gel. Native plasma or neuraminidase-treated plasma was applied for C6 or C7 typing, respectively. After focusing, proteins were transferred on to a polyvinyden fluoride filter (Durapore, Millipore) by press blotting, and then C6 or C7 bands were detected by a two step enzyme immunoassay.

RESULTS

C6 polymorphism

The C6 patterns observed in the present study are demonstrated in Fig. 1. Five common and four rare phenotypes were observed, in which seven allotypes were distinguishable. Three common allotypes, A, B and B2, and four rare allotypes, M2, M11, M91 and M92, were identified by direct comparison with our reference samples (Tokunaga *et al.*, 1983, 1984; Washio *et al.*, 1986). The distribution of C6 phenotypes and allele frequencies are shown in Table 1. The allele frequencies estimated for *C6*A*, *C6*B*, *C6*B2*, and the rare variants combined are 0.433, 0.523, 0.039 and 0.005, respectively. The observed numbers of the phenotypes were in good agreement with those expected on Hardy-Weinberg equilibrium.

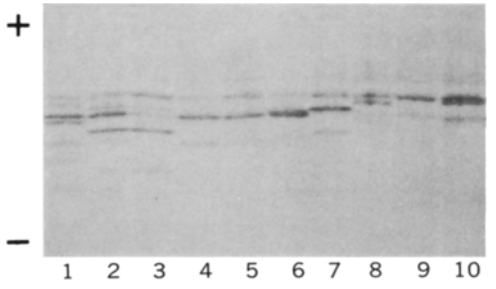

Fig. 1. Photograph showing C6 phenotypes. (1) BB1 control, (2) BB2, (3) AB2, (4) B, (5) AB, (6) M2B, (7) AM11, (8) AM91, (9) A, (10) AM92.

Table 1. Distribution of C6 phenotypes and allele frequencies.

Phenotypes	No. observed	%	No. expected	Allele frequencies
A	93	19.0	91.7	
AB	220	44.9	222.0	*C6*A*=0.433
B	134	27.3	134.3	
AB2	15	3.1	16.4	*C6*B*=0.523
BB2	23	4.7	19.9	
B2	0	0.0	0.7	*C6*B2*=0.039
AR[a]	3	0.6	2.2	
BR[a]	2	0.4	2.6	*C6*R*=0.005
Others	0	0.0	0.2	
Total	490	100.0	490.0	1.000

[a] Rare phenotypes: AM91 1, AM92 1, AM11 1, M2B 2. $\chi^2=2.06$, d.f.=5, $0.80<p<0.90$.

C7 polymorphism

The patterns of neuraminidase-treated C7 observed in the present study are demonstrated in Fig. 2. Four common and six rare phenotypes were observed, in which six allotypes were distinguishable. Four common allotypes have been identified as C7 1, 2, 3 and 4, respectively (Hobart *et al.*, 1978; Tokunaga *et al.*, 1986; Washio *et al.*, 1986). Two rare variants seem to be the new variants (Fig. 2b). One of them, tentatively named as K1, has a mobility slightly anodal to C7 1. The other, tentatively named as K2, has a mobility slightly cathodal to C7 4.

The distribution of C7 phenotypes and allele frequencies are shown in Table 2. The allele frequencies estimated for *C7*1*, *C7*2*, *C7*3*, *C7*4* and the rare variants combined are 0.843, 0.073, 0.034, 0.048 and 0.002, respectively. The observed numbers of the phenotypes were in agreement with Hardy-Weinberg expectation.

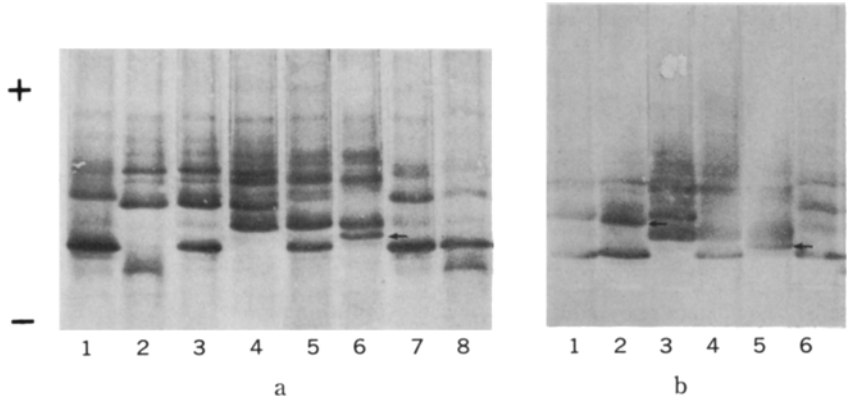

Fig. 2. Photographs showing C7 phenotypes. The arrow indicates the variant band, K1 and K2. a: (1) 1, (2) 4-3, (3) 4-1, (4) 4-2, (5) 2-1, (6) 2-K1, (7) 1, (8) 3-1. b: (1) 4-1, (2) 1-K2, (3) 4-2, (4) 2-1, (5) 2-K1, (6) 1.

Table 2. Distribution of C7 phenotypes and allele frequencies.

Phenotypes	No. observed	%	No. expected	Allele frequencies
1	347	70.8	348.1	
2-1	62	12.7	60.7	*C7*1*=0.843
3-1	31	6.3	27.8	
4-1	38	7.8	39.6	*C7*2*=0.073
3-2	0	0.0	2.4	
4-2	3	0.6	3.5	*C7*3*=0.034
4-3	2	0.4	1.6	
2	3	0.6	2.6	*C7*4*=0.048
3	0	0.0	0.6	
4	2	0.4	1.1	*C7*R*=0.002
Others[a]	2	0.4	2.0	
Total	490	100.0	490.0	1.000

[a] Rare phenotypes: 2-K1 1, 1-K2 1. $\chi^2=4.33$, d.f.=6, $0.60<p<0.70$.

Association analysis between C6 and C7

The results of the association analysis between C6 and C7 are summarized in Table 3. A significantly negative association was observed between C6 B and C7 4 allotypes ($\chi^2=6.47$, $p<0.02$).

Table 3. Association analysis between C6 and C7.

Combinations[a]	+/+	+/−	−/+	−/−	p
C6A-C7 1	325	6	154	5	NS[b]
C6A-C7 2	50	281	19	140	NS
C6A-C7 3	20	311	13	146	NS
C6A-C7 4	33	298	12	147	NS
C6B-C7 1	373	6	106	5	NS
C6B-C7 2	54	325	15	96	NS
C6B-C7 3	28	351	5	106	NS
C6B-C7 4	28	351	17	94	<0.02
C6B2-C7 1	38	0	442	10	NS

[a] Only the combinations in which the incidence of the +/+ individuals exceeding 0.01 are presented. [b] NS, not significant.

DISCUSSION

The C6 allele frequencies reported in the East Asian populations are listed in Table 4 (Nishimukai *et al.*, 1985; Tokunaga *et al.*, 1983, 1984; Zeng *et al.*, 1986) together with those in the Caucasian population (Kunstmann *et al.*, 1980). The allele frequencies obtained in the present study are similar to those in the neighboring populations. It is confirmed that the East Asian population has characteristics of

Table 4. C6 allele frequencies in East Asian and Caucasian populations.

Populations	No. samples	C6 alleles				Authors
		A	B	B2	Others	
Korean						
Seoul	490	0.433	0.523	0.039	0.005	Present study
Japanese						
Northeastern	495	0.423	0.510	0.062	0.005	Tokunaga *et al.*, 1984
Tokyo	288	0.427	0.483	0.076	0.014	Tokunaga *et al.*, 1983
Western	135	0.467	0.481	0.037	0.015	Nishimukai *et al.*, 1985
Chinese						
Beijing	155	0.416	0.532	0.042	0.010	Zeng *et al.*, 1986
Guangzhou	255	0.445	0.518	0.033	0.004	Zeng *et al.*, 1986
German						
Köln	709	0.601	0.388	0.003	0.008	Kunstmann *et al.*, 1980

Table 5. C7 allele frequencies in East Asian and Caucasian populations.

Populations	No. samples	C7 alleles					Authors
		1	2	3(=5)	4	Others	
Korean							
Seoul	490	0.843	0.073	0.034	0.048	0.002	Present study
Japanese							
Eastern	217	0.813	0.097	0.037	0.051	0.002	Washio *et al.*, 1986
Western	183	0.809	0.104	0.049	0.038	—	Nishimukai and Tamaki, 1986
Chinese							
Beijing	152	0.865	0.069	0.020	0.043	0.003	Zeng *et al.*, 1986
Guangzhou	255	0.884	0.075	0.031	0.010	—	Zeng *et al.*, 1986
Caucasian (UK)	1,228	0.995	0.002	0.004	—	—	Hobart *et al.*, 1978

a higher frequency of *C6*B* than *C6*A*, the occurrence of the third common allele, *C6*B2*, and the existence of a number of rare alleles.

Table 5 shows the C7 allele frequencies reported in the East Asian populations (Nishimukai and Tamaki, 1986; Washio *et al.*, 1986; Zeng *et al.*, 1986) as well as in the Caucasian population (Hobart *et al.*, 1978). The present data on Korea are similar to those in the neighboring populations. The East Asian population may be characterized by the relatively high frequencies of *C7*2*, *C7*3* and *C7*4*.

Accordingly, both of the proteins coded by the closely linked complement genes, C6 and C7, show a higher degree of polymorphism in East Asian than in Caucasian populations. Thus, C6 and C7 systems are particularly valuable markers for the study of human populations in Asia.

A significantly nagative association between C6 and C7 allotypes was observed in the present study. However, all the previous studies on an extensive family material (Tokunaga *et al.*, 1986) and on several population materials (Nishimukai and Tamaki, 1986; Washio *et al.*, 1986; Zeng *et al.*, 1986) have failed to show any linkage disequilibrium or association, except for the report by Nakamura *et al.* (1984b), in which a significantly positive association between C6 B and C7 B (=C7 1) was described. Further studies are required to confirm the possible association between C6 and C7 alleles.

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