GENETIC POLYMORPHISMS OF COMPLEMENT
C6 AND C7 IN TWO CHINESE POPULATIONS

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Summary   Genetic polymorphisms of the complement components C6 and C7 in two Chinese populations (Beijing and Guangzhou) were investigated, using isoelectric focusing and immunoblotting technique.   Three common and four rare allotypes of C6 were observed, and one of the rare allotypes was considered to be a new variant.   Ten different patterns of C7 observed were classified into three homozygous and seven heterozygous phenotypes, indicating occurrence of four common and a new variant alleles.   The new variants of C6 and C7 detected were designated as C6 B21 and C7 6, respectively.   There was no statistically significant difference for C6 and C7 allele frequencies between the Beijing and the Guangzhou populations except for *C7*4*.   The *C7*4* frequency was shown to be significantly higher in Beijing than in Guangzhou.   No significant positive association between C6 and C7 alleles was found.

INTRODUCTION

Genetic variants involving charge differences have been described for more than half of the complement components.   Genetic polymorphism of the sixth component of human complement (C6) was first detected by Hobart *et al.* (1975). Subsequent investigations on C6 in several populations have shown a high degree of allelic variation particularly among Asian-Pacific populations (Kunstmann *et al.*, 1980; Olving *et al.*, 1980; Ranford *et al.*, 1982; Tokunaga *et al.*, 1983, 1984).   However, no study was reported in mainland Chinese up to now.

The close linkage between C6 and C7 loci has been confirmed (Hobart *et al.*, 1978; Tokunaga *et al.*, 1986).   A genetic study of structural variants of the seventh component of human complement (C7) was first carried out by Hobart *et al.* (1978),

using polyacrylamide gel isoelectric focusing followed by functional detection.   It was reported that the inheritance of the observed patterns of C7 are determined by three codominant alleles, C7*1, C7*2, and C7*3, at a single autosomal locus.   The investigations of C7 polymorphism in Japanese have been performed using poly-acrylamide gel isoelectric focusing followed by immoblotting techniques, involving electrophoretic transfer (Nakamura et al., 1984a), or press blotting (Tokunaga et al., 1986).   No study has been reported on this system in Chinese.

Nakamura et al. (1984a) suggested that the three common allotypes of C7 named C7 B, C7 M, and C7 A correspond to C7 1, C7 2, and C7 3 described by Hobart et al. (1978), respectively.   However, the third allotype has been found to be identical to the C7 4 by Lachmann and Hobart (see Tokunaga et al., 1986). Recently, Nishimukai and Tamaki (1986) described the new cathodal variant in Japanese, named C7 5, recognized in neuraminidase-treated samples.

The present study is the first report on the polymorphisms of complement C6 and C7 in China.   The C6 and C7 polymorphisms were investigated using poly-acrylamide gel isoelectric focusing followed by immunoblotting in two populations (Beijing and Guangzhou) of mainland China.   The result of an association analysis between C6 and C7 alleles was also described.

MATERIALS AND METHODS

Blood samples were obtained from normal unrelated Chinese (Han nationality) adults.   Among these, 155 blood samples were from Beijing and 255 samples were from Guangzhou.   The samples were airshipped to Tokyo and then were stored at $-30°C$ prior to examination.

For C6 typing, slab gels ($200 \times 130 \times 0.5$ mm) were prepared using 2.8 ml of acrylamide solution 29.1% (w/v), 2.8 ml of bis-acrylamide solution 0.9% (w/v), 0.5 g taurin, 0.9 ml of Ampholine pH 5–8 (LKB), 10 μl of TEMED, and 10 ml of distilled water.   The gel was photopolymerized with 0.4 ml of riboflavin solution at room temperature.   Isoelectric focusing and immunoblotting were performed according to Tokunaga et al. (1984).

For C7 typing, the acrylamide gel was prepared in the similar manner as for C6. Plasma samples were treated with 5 U/ml neuraminidase (Sigma Chemical Co., St. Louis) overnight at room temperature prior to isoelectric focusing.   Immunoblotting was carried out in the similar manner as for C6 (Tokunaga et al., 1986).   A goat anti-human C7 serum (Cappel Lab. Inc., West Chester) diluted 1 : 400 with 3% bovine serum albumin in phosphate-buffered saline (BSA/PBS) was used as the first antibody and a peroxidase-conjugated anti-goat immunoglobulins (Dako Patts a/s, Glostrup, Denmark) diluted 1 : 1,000 with BSA/PBS was used as the second antiserum.

Association analysis was performed by Chi-square test with Yates' correction.

RESULTS

The photograph of the nitrocellulose membrane showing the patterns of the various C6 phenotypes is shown in Fig. 1. At least ten different phenotypes were observed. The diagram of C6 allotypes identified in the present study is presented in Fig. 2. Among these allotypes six were identified as A, B, B2, B3, A21, and M91 (formerly 91), by direct comparison with Japanese control specimens (Tokunaga et al., 1983, 1984). A rare variant showed the bands slightly more acidic than those of B3, so it was designated as C6 B21 according to the International Nomenclature System (Mauff et al., 1980).

The distributions of C6 phenotypes and allele frequencies in two Chinese populations are presented in Table 1. The allele frequencies of C6 calculated from 155 individuals of Beijing population and 255 individuals of Guangzhou population were as follows: for Beijing population, $C6*A=0.4161$, $C6*B=0.5323$, $C6*B2=$

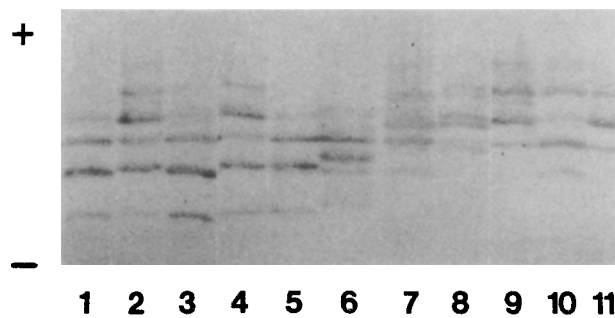Fig. 1. Photograph showing C6 phenotypes demonstrated using isoelectric focusing and immunoblotting. (1) C6 BB3, (2) C6 AB21, (3) C6 BB3, (4) C6 AB21, (5) C6 BB21, (6) C6 BB2, (7) C6 M91B (control), (8) C6 AM91, (9) C6 A21A, (10) C6 A21B (control), (11) C6 A.
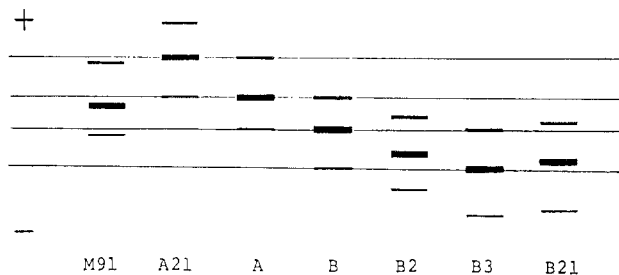

Fig. 2. Diagram of C6 allotypes identified in the present study. Only one major band, one acidic and one basic minor bands are shown for each allotype.

Table 1. Distribution of C6 phenotype and allele frequencies.

Population	Phenotypes	Obs. No.	%	Exp. No.	Allele frequencies	
Beijing	A	35	22.58	26.84	$C6*A:$	0.4161 ± 0.0280
	AB	54	34.84	68.66		
	B	50	32.26	43.92	$C6*B:$	0.5323 ± 0.0283
	AB2	3	1.94	5.40		
	BB2	10	6.45	6.91	$C6*B2:$	0.0419 ± 0.0114
	B2	0	0	0.27		
	AR*	2	1.29	1.25	$C6*R:$	0.0097 ± 0.0056
	BR*	1	0.64	1.60		
	B2R*	0	0	0.13		
	R*	0	0	0.01		
	Total	155	100	154.99	$\chi^2=9.9861$, d.f.$=6$, p>0.10	
	* Rare phenotypes:	AM91	1			
		A21A	1			
		BB3	1			
Guangzhou	A	54	21.18	50.52	$C6*A:$	0.4451 ± 0.0220
	AB	108	42.35	117.50		
	B	74	29.02	68.32	$C6*B:$	0.5176 ± 0.0221
	AB2	10	3.92	7.56		
	BB2	7	2.75	8.79	$C6*B2:$	0.0333 ± 0.0079
	B2	0	0	0.28		
	AR*	1	0.39	0.89	$C6*R:$	0.0039 ± 0.0028
	BR*	1	0.39	1.03		
	B2R*	0	0	0.07		
	R*	0	0	0.003		
	Total	255	100	254.97	$\chi^2=2.9995$, d.f.$=6$, p>0.80	
	* Rare phenotypes:	AB21	1			
		BB21	1			

0.0419, $C6*R=0.0097$; for Guangzhou population, $C6*A=0.4451$, $C6*B=0.5176$, $C6*B2=0.0333$, $C6*R=0.0039$. The differences between the observed numbers and the expected numbers from Hardy-Weinberg equilibrium are not statistically significant in both populations. It was shown that there are at least three common alleles of C6 in Chinese as in Japanese: $C6*A$, $C6*B$ and $C6*B2$.

Ten different patterns of C7 were observed in the present study (Fig. 3). Among these patterns, nine different C7 phenotypes which consisted of three homozygous
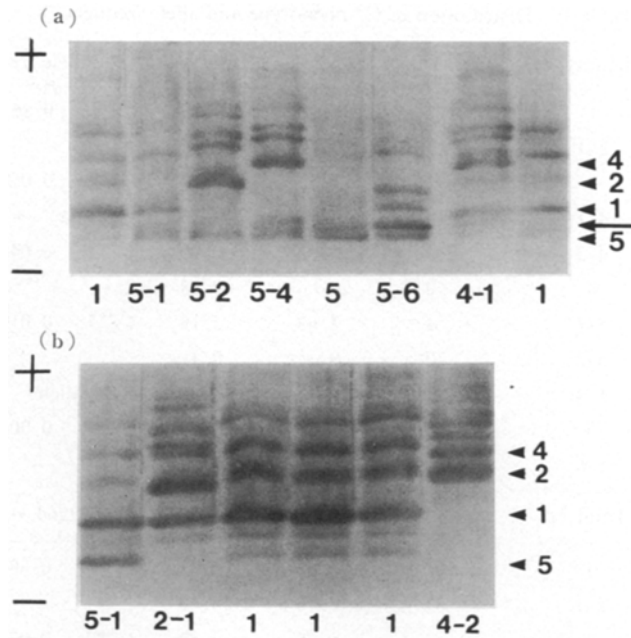
Fig. 3 (a, b).   Photographs showing the different patterns of C7 phenotypes in the neu-
    raminidase-treated samples, demonstrated using polyacrylamide gel isoelectric
    focusing immunoblotting technique.

and six heterozygous patterns were identified as C7 1, C7 2, C7 5, C7 2-1, C7 4-1,
C7 4-2, C7 5-1, C7 5-2 and C7 5-4.   The apparently new variant showing an inter-
mediate band between the C7 1 and C7 5 was found once in Beijing population.
It was tentatively designated as C7 6.

The distributions of the phenotypes and allele frequencies of C7 in the two
Chinese populations are given in Table 2.   The allele frequencies of C7 calculated
from 152 individuals of Beijing population and 255 individuals of Guangzhou popu-
lation were as follows: for Beijing population, $C7*1$=0.8651, $C7*2$=0.0691, $C7*4$=
0.0428, $C7*5$=0.0197, $C7*6$=0.0033; for Guangzhou population, $C7*1$=0.8843,
$C7*2$=0.0745, $C7*4$=0.0098, $C7*5$=0.0314.   The observed numbers of the pheno-
types were in good agreement with Hardy-Weinberg expectation in both popula-
tions.   It was shown that the less common alleles, $C7*2$, $C7*4$ and $C7*5$, have
polymorphic frequencies in Chinese.

Table 3 shows the result of association analysis between C6 and C7 allotypes.
No significant positive association was found in Beijing and Guangzhou populations.

Z. ZENG, K. TOKUNAGA, K. OMOTO, and C. DU

Table 2. Distribution of C7 phenotype and allele frequencies.

Population	Phenotypes	Obs. No.	%	Exp. No.	Allele frequencies	
Beijing	1	117	76.97	113.76	C7*1:	0.8651±0.0196
	2-1	15	9.87	18.18		
	2	2	1.32	0.73	C7*2:	0.0691±0.0145
	4-1	10	6.58	11.26		
	4-2	2	1.32	0.90	C7*4:	0.0428±0.0116
	4	0	0	0.28		
	5-1	4	2.63	5.18	C7*5:	0.0197±0.0080
	5-2	0	0	0.41		
	5-4	1	0.66	0.26	Rare allele:	
	5	0	0	0.06	C7*6:	0.0033±0.0033
	5-6	1	0.66	0.02		
	Total	152	100.01	151.04	$\chi^2=7.2264$, d.f.=6, p>0.30	
Guangzhou	1	202	79.22	199.41	C7*1:	0.8843±0.0142
	2-1	31	12.16	33.60		
	2	2	0.78	1.42	C7*2:	0.0745±0.0116
	4-1	5	1.96	4.42		
	4-2	0	0	0.37	C7*4:	0.0098±0.0044
	4	0	0	0.02		
	5-1	11	4.31	14.16	C7*5:	0.0314±0.0077
	5-2	3	1.18	1.19		
	5-4	0	0	0.16		
	5	1	0.39	0.25		
	Total	255	100.00	255.00	$\chi^2=6.8061$, d.f.=6, p>0.30	

Rare phenotype was not calculated in Chi-square.

DISCUSSION

A comparison of the C6 allele frequencies between Beijing and Guangzhou populations showed no statistically significant difference (Table 4).

In Table 5, the C6 allele frequencies in various populations thus far reported are presented. It appears that the gene frequency of C6*B in Chinese is higher than that in the other populations. Interestingly, the most common allele of C6 in Chinese is C6*B, as in Japanese and Micronesian populations (Tokunaga et al., 1983, 1984; Ranford et al., 1982). In contrast, it has been shown that C6*A is

Table 3. Association analysis between C6 and C7 among unrelated Chinese.

			Correlation pattern				χ^2	p
			+/+	+/−	−/+	−/−		
C6 A-C7	1	B	87	5	59	1	1.360	N.S.
		G	171	2	78	4	1.930	N.S.
C6 A-C7	2	B	15	77	4	56	2.266	N.S.
		G	22	151	14	68	0.871	N.S.
C6 A-C7	4	B	9	83	4	56	0.140	N.S.
		G	5	168	0	82	1.148	N.S.
C6 A-C7	5	B	3	88	4	57	0.421	N.S.
		G	8	165	7	75	1.538	N.S.
C6 B-C7	1	B	109	4	37	2	0.001	N.S.
		G	186	4	63	2	0.001	N.S.
C6 B-C7	2	B	13	100	6	33	0.399	N.S.
		G	25	165	11	54	0.566	N.S.
C6 B-C7	4	B	10	103	3	36	0.012	N.S.
		G	2	188	3	62	1.613	N.S.
C6 B-C7	5	B	5	103	1	38	0.001	N.S.
		G	12	178	3	62	0.039	N.S.
C6 B2-C7	1	B	13	0	133	6	0.001	N.S.
		G	17	0	232	6	0.027	N.S.
C6 B2-C7	2	B	2	11	17	122	0.012	N.S.
		G	2	15	34	204	0.005	N.S.
C6 B2-C7	4	B	0	13	13	126	0.403	N.S.
		G	1	16	4	234	0.091	N.S.
C6 B2-C7	5	B	0	13	6	133	0.001	N.S.
		G	1	16	14	224	0.285	N.S.

B, Beijing; G, Guangzhou; N.S., Not significant.

the most common allele in Caucasians (Hobart *et al.*, 1976; Kunstmann *et al.*, 1980; Olving *et al.*, 1980).

The gene frequency of *C6*B2* in Chinese, is a little lower than those in Japanese and in Micronesian but higher than those in the other populations, especially, in Caucasians. The allele *C6*B3* was suggested to be the fourth common allele in a western Japanese population (Nishimukai *et al.*, 1985). Nevertheless, it seems to be rare in Chinese populations as in eastern Japanese (Tokunaga *et al.*, 1983, 1984; Nakamura *et al.*, 1984b).

A comparison of the C7 allele frequencies (Table 4) indicated that there are no

Table 4. Comparisons of C6 and C7 allele frequencies between two Chinese populations.

Population	N	$C6*A$	$C6*B$	$C6*B2$	Others
Beijing	155	0.4161	0.5323	0.0419	0.0097
Guangzhou	255	0.4451	0.5176	0.0333	0.0039
		$\chi^2=0.660$	$\chi^2=0.167$	$\chi^2=0.405$	$\chi^2=1.070$
		d.f.=1	d.f.=1	d.f.=1	d.f.=1
		$p>0.30$	$p>0.50$	$p>0.50$	$p>0.30$
	N	$C7*1$	$C7*2$	$C7*4$	$C7*5$
Beijing	152	0.8651	0.0691	0.0428	0.0197
Guangzhou	255	0.8843	0.0745	0.0098	0.0314
		$\chi^2=0.651$	$\chi^2=0.082$	$\chi^2=9.606$	$\chi^2=0.992$
		d.f.=1	d.f.=1	d.f.=1	d.f.=1
		$p>0.30$	$p>0.70$	$p<0.005$	$p>0.30$

Table 5. C6 allele frequencies in various populations.

Population	No.	C6 allele				Authors
		A	B	B2	Others	
China						
Beijing	155	.416	.532	.042	.010	Present study
Guangzhou	255	.445	.518	.033	.004	
Japan	135–495	.423–.467	.481–.510	.037–.076	.005–.015	Tokunaga et al., 1983, 1984; Nishimukai et al., 1985
W. Pacific						
Micronesia	186	.446	.452	.067	.035	Ranford et al., 1982
Polynesia	245	.629	.359	.006	.006	
Melanesia	186	.693	.307	.000	.000	
Germany	709	.601	.388	.003	.008	Kunstmann et al., 1980
Norway						
Lapps	167	.533	.467	.000	.000	Olving et al., 1980
Caucasian	1623	.587	.409	.004		Olving et al., 1980

statistically significant differences for the allele frequencies of $C7*1$, $C7*2$ and $C7*5$ between Beijing and Guangzhou populations, while for the allele frequency of $C7*4$, there is highly significant difference between the two populations ($p<0.005$). The result suggests that this allele has a geographically restricted distribution in the

northern Chinese population and spread to the southern Chinese population by gene flow.   It is interesting to examine other Chinese populations in this respect.

Nakamura and his colleagues described a result of an association analysis between C6 and C7 in a Japanese population.   They suggested that two combinations between C6 and C7 alleles are in positive linkage disequilibrium (Nakamura *et al.*, 1984b).   However, Tokunaga *et al.* (1986) found no evidence of linkage disequilibrium between C6 and C7 alleles in a haplotype study with 135 genotyped families.   Nishimukai and Tamaki (1986) also found no significant association in a Japanese population.   In this study, again no association between C6 and C7 alleles was found.

Note Added in Proof: Recently, the correspondence of the C7 5 (Nishimukai and Tamaki, 1986) with the C7 3 reported by Hobart *et al.* (1978) has been confirmed (see Washio *et al.*, *Jpn J. Human Genet.* in press).

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