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ALLOTYPES OF THE FOURTH COMPONENT OF COMPLEMENT IN KOREAN

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Summary The analysis of genetic polymorphism in C4 was performed on EDTA-plasma from 169 healthy unrelated Koreans. Plasma samples were subjected to high-voltage agarose gel electrophoresis followed by immunofixation. C4B allotypes were further detected by a hemolytic overlay method. The allele frequencies of C4A and C4B were as follows; for C4A, C4A*3=0.6099, C4A*4=0.1702, C4A*Q0=0.1525, C4A*2=0.0461, and C4A*R=0.0213; for C4B, C4B*1=0.6406, C4B*2=0.2740, C4B*5=0.0569, C4B*Q0=0.0178, and C4B*R=0.0107. C4A*3 and C4B*1 were among the most common alleles at each locus. C4A*6 was not detected in this study, but this allele is relatively common in both Caucasoid and Negroid populations. C4B*5 is a common allele in Asian, which is rare in Caucasoids and Negroids. C4B*5 appeared to be a characteristic allele of Oriental. In the C4A locus, five individuals with duplicated allotypes (three C4A 3,3+2, one C4A 4,3+2, and one C4A 3,3+3) were observed, and in the C4B locus, one individual with duplicated allotype (C4B 1,1+1) was detected.

INTRODUCTION

The human complement component 4 (C4), is a component protein of C3 convertase in the classical activation pathway. It is composed of three disulfide-linked chains, α chain of molecular weight (Mr) 93,000, β chain of Mr 78,000, and γ chain of Mr 20,000, but is synthesized as a single polypeptide of about Mr 209,000 containing 1,722–1,744 amino acids before being secreted (Schreiber and Müller-Eberhard, 1974; Tack *et al.*, 1981; Porter, 1985; Yu, 1991). The concentration of C4 is 27–32.03 mg/dl in the serum of normal human (Chiarelli *et al.*, 1988; Vergani,

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1987).

The C4 protein is encoded by two genetic loci, C4A and C4B, located between HLA-B and HLA-D in the major histocompatibility complex on the short arm of chromosome 6 (6p21.3) (Carroll *et al.*, 1984; Dunham *et al.*, 1987; Spence *et al.*, 1989). The two genes are arranged in tandem that are about 10 kb apart from each other and approximately 30 kb apart from the C2 and BF genes (Carroll *et al.*, 1984) and less than 2 kb apart from the 21-OH genes (Carroll *et al.*, 1985).

The various allotypes of C4 genes show genetic polymorphism in structural, functional, serological, and hemolytic characteristics. Human C4 structural polymorphism was first identified by Rosenfeld *et al.* (1969) using agarose gel electrophoresis. And then after a family study, it showed that the polymorphism of C4 was controlled by two closely linked genetic loci. The results were confirmed by improved electrophoresis after desialisation of plasma (O'Neill *et al.*, 1978; Awdeh and Alper, 1980). So far, at least 13 alleles of C4A and 16 alleles of C4B including a null allele at each locus have been identified among various populations (Mauff *et al.*, 1990). C4A and C4B genes have been found to be duplicated and to be transmitted to offspring in several different haplotypes (Raum *et al.*, 1984; Giles *et al.*, 1987). C4A and C4B can be distinguished by specific antisera, anti-Rogers and anti-Chido respectively, and reversed correlation have been found infrequently (Giles, 1990). Mauff *et al.* (1984) showed that the C4B protein has a higher hemolytic activity than C4A. These functional and structural differences have been determined by a sequence of four amino acids in the C4d region (Yu *et al.*, 1988).

The present study was performed to investigate the genetic polymorphism of C4A and C4B in Korean and the results were discussed in comparison with that of other populations.

SUBJECTS AND METHODS

The EDTA-plasma was collected from a total of 259 healthy Koreans including 109 unrelated subjects and 30 families each with 3 children living in Seoul. None of the couples of the 30 families were consanguineous, and therefore, they were considered 60 unrelated subjects making the total number 169. The allotyping of C4 was performed on samples pretreated with carboxypeptidase B and neuraminidase using a high-voltage agarose gel electrophoresis followed by immunofixation. C4B allotypes were further detected by a hemolytic overlay method (Mauff *et al.*, 1983).

The nomenclature used for the C4 allotypes was according to Mauff *et al.* (1990).

RESULTS

The genetic polymorphism of C4 was observed in 169 unrelated healthy Koreans. Figure 1a demonstrates the C4 immunofixation and Fig. 1b demonstrates

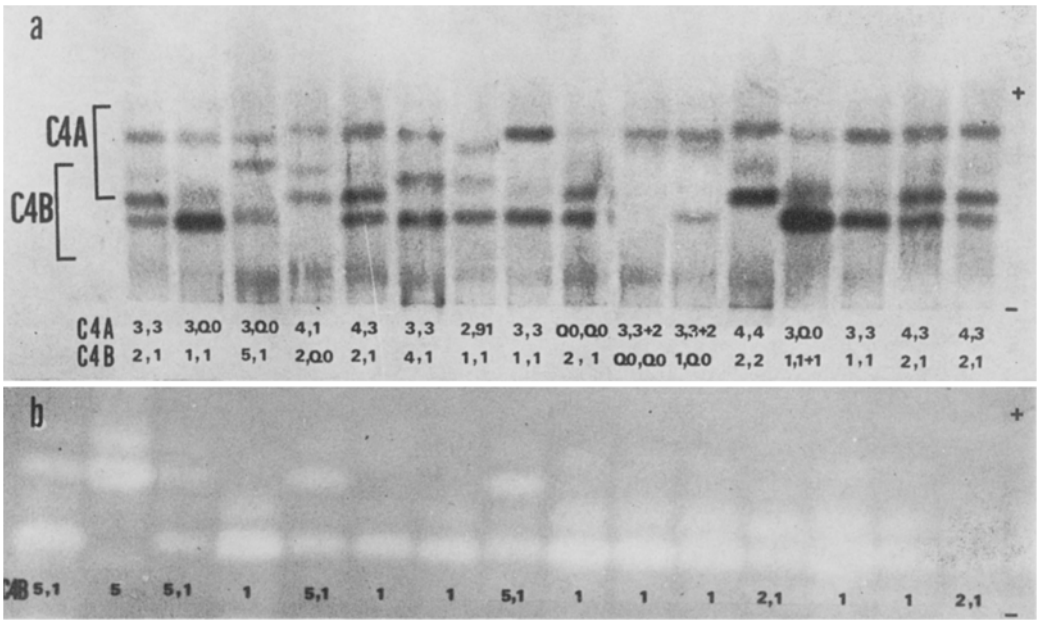

Fig. 1. a, C4A and C4B allotypes detected by immunofixation. b, C4B allotypes detected by hemolytic assay.

hemolytic patterns of the various phenotypes observed in this study. The hemolytic activities of C4B variants were higher than the C4A. The distribution of C4A and C4B allotypes and allele frequencies is shown in Table 1. Four common (A*Q0, A*2, A*3, and A*4) and two rare (A*1 and A*91) alleles and two duplicated haplotypes (A*3+3 and A*3+2) at the C4A locus and four common (B*Q0, B*1, B*2, and B*5) and two rare (B*4 and B*13) alleles and one duplicated haplotype (B*1+1) at the C4B locus were identified. Three individuals with duplicated C4A 3,3+2 (1.8%), one with C4A 4,3+2 (0.6%), one C4A with 3,3+3 (0.6%), and one C4B 1,1+1 (0.6%) were found. C4 phenotypes of individuals with duplicated haplotype were as follows; two individuals with C4A 3,3+2 C4B 1,1, one with C4A 3,3+2 C4B 1,Q0, one with C4A 3,3+3 C4B 1, Q0, one with C4A 4,3+2 C4B 2,Q0, and one with C4A 3,Q0 C4B 1,1+1.

DISCUSSION

Eight alleles (CA*Q0, *1, *2, *3, *4, *91, *3+2, *3+3) at the C4A locus and seven alleles (C4B*Q0, *1, *2, *4, *5, *13, *1+1) at the C4B locus were observed in Koreans. The C4 shows a high degree of polymorphism and several alleles show remarkable differences in various populations. In Table 2, the reported C4 allele frequencies in various ethnic groups are presented. The distribution

Table 1. C4 phenotype and allele frequencies in Korean.

Phenotype		n	%	Allele frequencies (%)
C4A	2	53	31.4	A*3=0.6099
	3, Q0	31	16.3	A*4=0.1702
	3, 3	27	15.9	A*Q0=0.1525
	4, 3	15	8.9	A*2=0.0461
	4, Q0	12	7.1	Others=0.0213
	3, 2	8	4.7	
	4	8	4.7	
	4, 4	4	2.4	
	4, 1	3	1.8	
	3, 3+2	3	1.8	
	3. 1	1	0.6	
	2, 91	1	0.6	
	4, 91	1	0.6	
	3, 3+3	1	0.6	
	4, 3+2	1	0.6	
		169	100.0	
C4B	1	50	29.6	B*1=0.6406
	2, 1	49	28.9	B*2=0.2740
	1, 1	32	18.9	B*5=0.0569
	2, 2	10	5.9	B*Q0=0.0178
	5, 1	9	5.3	Others=0.0107
	2	4	2.4	
	5	4	2.4	
	1, Q0	4	2.4	
	5, 2	3	1.8	
	4, 1	1	0.6	
	2, Q0	1	0.6	
	13, 13	1	0.6	
	1, 1+1	1	0.6	
		169	100.0	

of C4 phenotypes in Koreans is similar to that found in Japanese and Chinese. The C4A*3 and C4B*1 are the most common alleles at each locus in various populations, as was also found in this study. C4A*6 is a relatively common allele in Caucasoid and Negroid populations, which is not found in the Korean, Japanese, and Chinese. The C4B*5 is a common allele in Asians, which is rare in Caucasoids and Negroids. The C4B*5 appears to be a characteristic allele of the Orientals.

Table 2. Distribution of C4A and C4B allele frequencies in various populations.

Population / Allele		Korean (n=169)	Korean (n=99)	Japanese (n=341)	Southern Chinese (n=61)	American Caucasian (n=623)	American Caucasian (n=63)	American Black (n=35)	Finn (n=254)	Tunisian (n=169)
C4A*	Q0	0.153	0.079	0.067	0.115	0.190	0.095	0.071	0.113	0.089
	1	0.014				0.010		0.057		0.053
	2	0.046		0.106	0.041	0.055	0.016	0.042	0.081	0.124
	3	0.610	0.734	0.686	0.605	0.631	0.722	0.742	0.754	0.716
	4	0.170	0.222	0.132	0.199	0.075	0.071	0.014	0.007	
	5		0.005			0.002				
	6					0.035	0.079	0.057	0.016	0.015
	Others	0.007	0.015	0.009		0.002	0.008	0.014		
C4B*	Q0	0.018	0.155	0.158	0.147	0.104	0.111	0.071	0.175	0.003
	1	0.640	0.526	0.587	0.423	0.743	0.760	0.714	0.657	0.124
	2	0.274	0.300	0.167	0.405	0.199	0.087	0.142	0.153	0.766
	3		0.005			0.021	0.024	0.071	0.016	0.098
	4	0.004				0.004				
	5	0.057		0.088	0.016	0.005				
	6		0.052				0.008			
	Others	0.007	0.015		0.008	0.004	0.008			0.012
Investigators		Present study	Kim et al., 1986	Tokunaga et al., 1985	Hawkins et al., 1988	Alper et al., 1983	Howard et al., 1986	Howard et al., 1986	Partanen & Koskimies, 1986	Ayed & Gorgi, 1990

Null alleles of C4A or C4B occurs at frequencies of 0.003–0.190 in various normal populations. Braun *et al.* (1990) reported that some of the C4A null alleles were associated with deletion of genes and others were due to nonexpressed genes. Schneider *et al.* (1986) suggested that gene deletion of C4 loci was associated with deletions of 21-OHA and 21-OHB genes. But, Yamada *et al.* (1990) reported that the deletion of C4A gene was not found in Japanese patients with SLE (0%), or in healthy controls (0.6%).

Six different rare C4 phenotypes with duplicated form were detected. Raum *et al.* (1984) and Giles *et al.* (1987) found a duplication of the C4A locus on the haplotype C4A 3 C4A 2 C4B Q0. The present study shows that duplication of one C4 locus is accompanied with the null allele of the other C4 locus in three individuals (one with C4A 3,3+2 C4B 1,Q0, one with C4A 4,3+3 C4B 2,Q0, and one with C4A 3,Q0 C4B 1,1+1). This result suggests that a C4A gene has been

converted to a C4B gene, and reverse. Yu and Campbell (1987) reported that the C4B locus of the HLA haplotype B44 DRw6 C4A 3 C4B Q0 was not actually a C4B null allele, but probably had been encoded by another C4A 3 allotype. Also, Partanen and Campbell (1989) and Tokunaga *et al.* (1991) showed that the both C4B loci of C4A 3+2 C4B Q0 and C4A 3+3 C4B Q0 haplotypes where duplicated form of C4A allele and C4B null allele correspond to C4A gene instead of C4B gene.

C4 variants and null alleles of this study were based on electrophoretic mobility of the C4 protein. This data did not include the analysis on the DNA level for the observed null alleles and duplicated variants. Recently, restriction fragment length polymorphism (RFLP) has been proved to be useful in the investigation of the basis of C4 genetic variants including the null alleles (Mimori *et al.*, 1990; Braun *et al.*, 1990; Schneider, 1990).

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