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Brief Communication

Family Study on the Polymorphisms of the Sixth and Seventh Components (C6 and C7) of Human Complement: Linkage and Haplotype Analyses

KATSUSHI TOKUNAGA,[1] GEORG DEWALD,[1] KEIICHI OMOTO,[1] AND TAKEO JUJI[2]

SUMMARY

Genetic polymorphisms of the sixth and seventh complement components (C6 and C7) have been studied in Japanese family material using polyacrylamide gel isoelectric focusing followed by immunoblotting. Three common and four rare alleles were observed at the locus for C6. Inheritance of the two rare C6 variants, M11 and B3, was first confirmed. Three common C7 allotypes were classified as C7 1, C7 2, and C7 4, respectively. Linkage analysis confirmed the close linkage between the loci for C6 and C7. The maximum lod score was 8.43 at $\theta = 0$ (95% confidence limits: $\theta = 0$ and $\theta = .07$). No significant linkage disequilibrium was found between C6 and C7 in directly determined haplotypes of unrelated parents.

INTRODUCTION

The sixth and the seventh components of complement (C6, C7) are both single-chain glycoproteins with a molecular weight of approximately 120,000. These components act sequentially in the assembly of the membrane attack complex, whose primary biological function is the destruction of invading organisms.

C6 has been shown to be polymorphic in all major ethnic groups [1]. There are two common (*C6*A* and *C6*B*) and a number of rare codominant alleles at a single autosomal locus. In the Japanese population, a third common allele, *C6*B2*, was observed with a frequency of .076; in addition, several "new" rare

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[1] Department of Anthropology, Faculty of Science, University of Tokyo, Hongo, Bunkyo-ku, Tokyo 113, Japan.

[2] Blood Transfusion Service, Tokyo University Hospital, Tokyo, Japan.

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alleles could be identified [2, 3]. Despite several attempts to establish the chromosome location of the C6 locus, a close linkage could not be detected for more than 40 genetic markers [4–6].

In 1978, Hobart et al. [7] described three inherited structural forms of C7 controlled by codominant alleles at an autosomal locus (*C7*1, C7*2,* and *C7*3*). Observing only 13 variant patterns among 1,228 samples from a Caucasian population, the authors reported the gene frequencies for both *C7*2* and *C7*3* to be less than .01. Because of the low frequency of the C7 variants, only two informative families could be identified for linkage analysis between C6 and C7; nevertheless, the odds in favor of close linkage between C6 and C7 were > 1,000:1. Nakamura et al. [8] reported a high degree of C7 polymorphism in Japanese. They described three common alleles (*C7*B, C7*M, C7*A*) and assumed that they correspond to *C7*1, C7*2,* and *C7*3* detected by Hobart et al. [7].

In our present study on C6 and C7 polymorphisms with extensive Japanese family material, we attempted to establish the close linkage between the structural loci for C6 and C7 and to analyze C6-C7 haplotypes for a possible linkage disequilibrium.

MATERIALS AND METHODS

Family Material

Plasma samples were obtained from a total of 135 Japanese families, including 3-generation families. The family material was comprised of 143 matings with 417 offspring.

C6 Typing

C6 allotypes were determined essentially as described [3] using a thin-layer polyacrylamide gel isoelectric focusing and an immunoblotting procedure.

C7 Typing

C7 allotypes were determined by techniques basically the same as those for C6 typing: 2.2% ampholine (pH 5–7) was incorporated with 10% glycerol in a thin-layer polyacrylamide gel (T = 5%, C = 3%). Plasma samples of 15 μl were applied with filter paper pieces at the anodal side of the gel.

Protein transfer by a "press blotting" was carried out as follows. After focusing, the acrylamide gel was overlayed with 0.5% agarose gel (1 mm thickness) in phosphate-buffered saline (PBS) at pH 7.2, and then with a nitrocellulose sheet presoaked in PBS. Two sheets of filter papers, paper towels (approximately 2 cm thickness), a glass plate, and a 2 kg weight were placed on the nitrocellulose filter for 1 hr.

The nitrocellulose sheet was immersed in 3% bovine serum albumin in PBS (BSA/PBS) overnight. After washing in PBS, the sheet was placed on a glass plate. A goat anti-human C7 serum (Cappel) diluted 1:400 with BSA/PBS was overspread on the sheet (3 ml/100 cm^2), then covered by a sealing film (Nesco film, Nippon Shoji) for even distribution and to avoid drying up of antiserum. After incubation for 30 min at 37°C and washing in PBS, a peroxidase-conjugated rabbit anti-goat immunoglobulin antiserum (DAKO) diluted 1:1,000 with BSA/PBS was then overspread on the sheet and covered by a sealing film. After incubation and washing, peroxidase activity was developed in 50 ml 0.01 M phosphate buffer (pH 7.2) containing 5 mg *o*-dianisidine and 10 μl 30% H_2O_2. The color reaction was terminated with distilled water. The sheet was dried and stored in the dark.

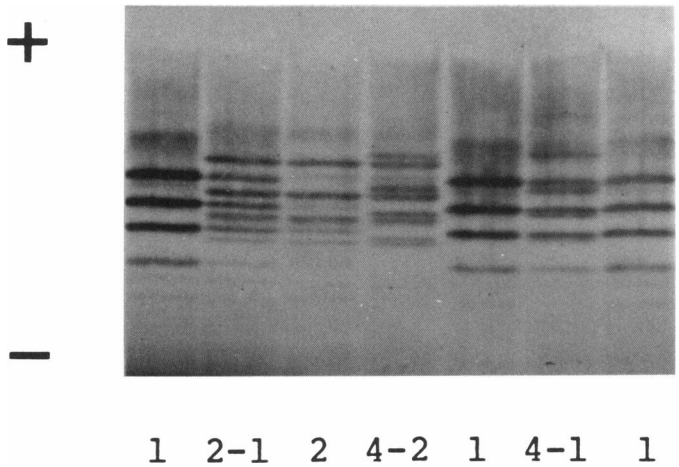

FIG. 1.—Patterns of C7 phenotypes obtained by isoelectric focusing and immunoblotting

Linkage and Association Analysis

Lod scores were calculated according to the method by Morton [9]. Linkage disequilibrium parameters (delta value, D) were calculated according to the definition D = hf(obs) − g(a) × g(b), where hf(obs) is the observed haplotype frequency and g(a) and g(b) are the gene frequencies of the alleles involved.

RESULTS

Figure 1 shows C7 patterns obtained by isoelectric focusing and subsequent immunoblotting. Three common and three rare phenotypes were observed in which three allotypes could be identified. Samples with these allotypes were sent to Drs. M. J. Hobart and P. J. Lachmann, Cambridge, for direct comparison. The three allotypes correspond to C7 1, C7 2, and C7 4, respectively, C7 4 being a "new" allotype that previously had been found in a Chinese student (Lachmann, personal communication). Comparing our C7 patterns and their frequencies with those published by Nakamura et al. [8], we concluded that *C7*1* corresponds to *C7*B*, *C7*2* to *C7*M*, and *C7*4* to *C7*A*.

Table 1 shows the C6 and C7 allele frequencies in 278 unrelated Japanese parents genotyped by family studies. Allele frequencies of C6 were close to those in our previous studies [2, 3]. Inheritance of the two previously observed rare C6 variants M11 and B3 [3, 10] was first confirmed: in one family the C6 M11 variant was observed in the mother and in one out of three children; in another family, the C6 B3 variant could be detected in both children of a mother with the phenotype of C6 AB3. In the C6 as well as in the C7 system, the observed phenotype frequencies were close to those expected on the assumption of a Hardy-Weinberg equilibrium (data not shown).

Table 2 shows the lod scores for C6 and C7 calculated from the family data. Forty-three informative children were obtained from 143 matings. The maximum of combined lod scores was 8.43 at a recombination fraction $\theta = 0$. The

TABLE 1

FREQUENCIES OF C6 AND C7 ALLELES IN UNRELATED PARENTS (278 INDIVIDUALS)

Alleles	Frequencies
*C6*A*	.446
*C6*B*	.466
*C6*B2*	.081
*C6**Rares*	.007
*C7*1*	.875
*C7*2*	.087
*C7*4*	.038

* *C6*A3*, *C6*M1*, *C6*M11*, and *C6*B3*.

95% confidence limits were $\theta = 0$ and $\theta = .07$. These results support the tight linkage between *C6* and *C7*. No difference was observed between data in males and females.

Table 3 shows the frequencies of C6-C7 haplotypes of unrelated parents determined directly in our genotyped families. In one family, haplotypes could not be determined from segregation patterns. Eight different haplotypes were observed with frequencies more than .005. No significant associations between *C6* and *C7* alleles were found except for a weak association of *C6*B* with *C7*4* ($\chi^2 = 3.55$, $.05 < P < .10$).

DISCUSSION

A possible genetic relationship between C6 and C7 has been suggested on the basis of both physicochemical and functional similarities [11]. Lachmann et al. [12] reported a case of a combined deficiency of C6 and C7 that was transmitted as a single genetic characteristic within the family. Hobart et al. [7] demonstrated a genetic linkage between *C6* and *C7* by studying two informative families.

Our present study extends the results of Hobart et al. [7] and establishes now a very close linkage between the structural loci for human C6 and C7. The calculation of lod scores revealed a maximum lod score of 8.43 at $\theta = 0$, corresponding to a likelihood ratio of $1:2.7 \times 10^8$. The close linkage supports the assumption that *C6* and *C7* are the products of tandemly duplicated genes.

TABLE 2

LOD SCORES FOR LINKAGE BETWEEN C6 AND C7 LOCI

Informative parent	No. matings	Informative offspring	Lod scores at various recombination fractions (θ)					
			0	.05	.10	.20	.30	.40
M	7	21	4.21	3.75	3.26	2.24	1.21	0.36
F	8	22	4.21	3.73	3.23	2.19	1.17	0.34
M + F	15	43	8.43	7.48	6.49	4.43	2.39	0.70

TABLE 3

ASSOCIATION ANALYSIS BETWEEN C6 AND C7 ALLELES (554 HAPLOTYPES)

Haplotypes	Frequencies	D($\times 10^3$)	*P*
*C6*A-C7*1*	.390	...	...
*C6*A-C7*2*	.045	...	...
*C6*A-C7*4*	.011	−6	<.15
*C6*B-C7*1*	.406	...	...
*C6*B-C7*2*	.034	...	...
*C6*B-C7*4*	.025	7	<.10
*C6*B2-C7*1*	.072	...	...
*C6*B2-C7*2*	.007	...	...
Rares* ...	.009	...	...

* *C6*B2-C7*4*, *C6*A3-C7*1*, *C6*M1-C7*1*, *C6*M11-C7*1*, and *C6*B3-C7*1*.

At least three gene clusters coding for functionally related complement components have been described so far. C2, C4, and factor B, which form the C3 convertases of the classical and alternative pathways of complement activation, are coded by a gene cluster located inside the major histocompatibility complex (MHC). Recently, Rodriguez de Cordoba et al. [13] demonstrated that in humans the genes for three regulatory proteins of C3 activation, namely, C4 binding protein, factor H, and C3b/C4b receptor (CR1), are tightly linked to each other, but neither to the *MHC* nor to *C3*. The C6-C7 linkage group is the third case. Not only in man, but also in dogs [14] and in common marmoset [15], have *C6* and *C7* been shown to be closely linked.

Three allotypes of C7 observed in the present study have been compared directly with the reference samples from Caucasians. Two of these correspond to C7 1 and C7 2 as suggested by Nakamura et al. [8]. On the other hand, the third allotype is different from C7 3 but identical to C7 4 found by Hobart and Lachmann (personal communication). Recently, Nishimukai and Tamaki [16] described a new allotype C7 5 in Japanese, which can be recognized with neuraminidase-treated samples. We also examined the desialyzed samples from eastern Japanese and found *C7*5* with a frequency of .033 (unpublished data). In the present study with untreated samples, C7 5 is included in C7 1.

Nakamura et al. [17] studied C6 and C7 polymorphisms in 475 unrelated Japanese. Their calculation of C6-C7 haplotype frequencies from the population data revealed a significant positive linkage disequilibrium for the haplotypes *C6*B-C7*B* (i.e., *C6*B-C7*1*) and *C6*M-C7*B*. In our study, however, the haplotype frequencies were determined by direct counting in genotyped families, and no significant associations between C6 and C7 alleles were found. Nishimukai and Tamaki [16] also observed no significant associations from a Japanese population material. It is interesting to examine other Mongolian populations for C6 and C7 polymorphisms and for a possible C6-C7 association.

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