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HUMAN C7 POLYMORPHISM: CLASSIFICATION AND ASSOCIATION ANALYSIS WITH C6

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Summary Polymorphism of the seventh component of human complement (C7) was investigated in Japanese. Four common and one rare allotypes were observed with desialized samples. Besides three common alleles, *C7*1*, *C7*2* and *C7*4*, the existence of the hypomorphic variant, *C7*3*, was confirmed in Japanese with a polymorphic frequency. The recently described *C7*5* was found to correspond to *C7*3* by comparing with reference samples. Moreover, a rare variant, tentatively named C7 7, was considered to be new. The population samples were also typed for C6. A rare variant, designated M92, was newly found. No significant associations between C6 and C7 alleles were found.

INTRODUCTION

The sixth component (C6) and the seventh component (C7) of the complement system participate in the formation of the membrane attack complex against microorganisms. Genetic polymorphism of C7 was first discovered by Hobart *et al.* (1978) using polyacrylamide gel isoelectric focusing and a hemolytic detection. A common allele, *C7*1*, and two rare alleles, *C7*2* and *C7*3* at a single autosomal locus were recognized in Caucasians. In Japanese, three alleles were detected with frequencies more than 0.01, using polyacrylamide gel isoelectric focusing and an immunoblotting method (Nakamura *et al.*, 1984a). The three common allotypes were identified to be C7 1, C7 2 and C7 4, respectively (Tokunaga *et al.*, 1986). Recently, Nishimukai and Tamaki (1986) reported another variant, which showed more basic bands than C7 1 after treatment with neuraminidase, and designated C7 5. They observed a high frequency of *C7*5* (0.049) in a western Japanese population and suggested the lower protein concentration of *C7*5* products than those of the other alleles.

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Genetic polymorphism of C6 has been investigated in many ethnic groups. Two common alleles, *C6*A* and *C6*B*, and several rare variants at a single autosomal locus have been described in Caucasians using prolonged agarose gel electrophoresis or polyacrylamide gel isoelectric focusing followed by hemolytic overlay (Hobart *et al.*, 1975; Mauff *et al.*, 1980). Whitehouse and Putt (1983) applied an immunoblotting procedure, involving electrophoretic protein transfer, in order to obtain C6 patterns after isoelectric focusing. In Japanese, the third common allele, *C6*B2*, and many rare variants have been detected using isoelectric focusing in polyacrylamide gel or agarose gel followed by hemolytic overlay or immunoblotting (Tokunaga *et al.*, 1983, 1984; Nishimukai *et al.*, 1985).

C6 and C7 are single chain glycoproteins, and show functional and physicochemical similarities to each other (Podack *et al.*, 1976). The close linkage of the structural loci for C6 and C7 has been confirmed (Tokunaga *et al.*, 1986; Lachmann *et al.*, 1978), although the chromosomal location of the loci is still unknown (Olving *et al.*, 1979; Bender *et al.*, 1983).

Nakamura *et al.* (1984b) reported positive associations of *C6*B* with *C7*B* (=*C7*1*) and *C6*M* with *C7*B* from the population data. On the contrary, Tokunaga *et al.* (1986) could not find linkage disequilibrium between any C6 and C7 alleles in an extensive family study. Nishimukai and Tamaki (1986) also described no significant positive association in a population analysis.

The purpose of the present study is to investigate C7 polymorphism in an eastern Japanese population and to analyze possible associations between C6 and C7 alleles. Moreover, correspondence between C7 5 by Nishimukai and Tamaki (1986) and C7 3 by Hobart *et al.* (1978) is reported. Two newly observed variants are also described.

MATERIALS AND METHODS

A total of 351 ACD-plasma samples were obtained from healthy blood donors living in an eastern area of Japan, Ibaraki prefecture. Plasma samples were stored at −30°C for up to a few months before typing. For C7 typing, 217 samples out of 351 were treated with 5 U/ml neuraminidase (type V, Sigma, U.S.A.) at room temperature overnight, as described for C4 typing (Mauff *et al.*, 1983). All native plasma samples were phenotyped for C6.

Phenotypings of C7 and C6 were performed using isoelectric focusing in polyacrylamide gel and immunoblotting procedure (Tokunaga *et al.*, 1984, 1986) with slight modifications as follows: Polyvinyden fluoride filter (Durapore, 0.22 μm, Millipore, U.S.A.) was used instead of the nitrocellulose filter because of its durability and an increasing efficiency for protein transfer. The hydrophobic filter was first treated with methyl-alcohol and then soaked in phosphate buffered saline (PBS). For the protein transfer by a 'press blotting,' the filter was directly layered on the polyacrylamide gel after focusing, followed by pressing with two filter papers, several

paper towels, and approximately 500 g weight for 30 min. The filter was easily separated from the gel in PBS.

Association analysis between C6 and C7 alleles was performed in 2×2 tables by chi-square statistics.

RESULTS

C7 polymorphism

The patterns of desialized C7 obtained from 217 samples are presented in Fig. 1. Nine different phenotypes were observed, in which four common and one rare allotypes were distinguished. Three common allotypes have been identified as C7 1, 2 and 4, respectively (Tokunaga *et al.*, 1986). The fourth common allotype, showing a weak major band more basic than that of C7 1 after neuraminidase-treatment, was previously named C7 5 by Nishimukai and Tamaki (1986). The variant samples found in this study and those from Caucasians were exchanged with Dr. Lachmann (MRC Group on Mechanisms in Tumour Immunity Unit, Cambridge). As the result of reference typing, the fourth common variant could not be distinguished from the hypomorphic variant, C7 3 (Hobart *et al.*, 1978) both in native and in neuraminidase-treated samples.

A rare variant, which showed the major band between those of C7 2 and C7 4, was different from any other variant. The variant was considered to be new, and tentatively named C7 7. The results of C7 phenotyping and allele frequencies are shown in Table 1. The allele frequencies estimated for *C7*1*, *C7*2*, *C7*3*, *C7*4* and *C7*7* are 0.813, 0.097, 0.037, 0.051 and 0.002, respectively. The deviation of

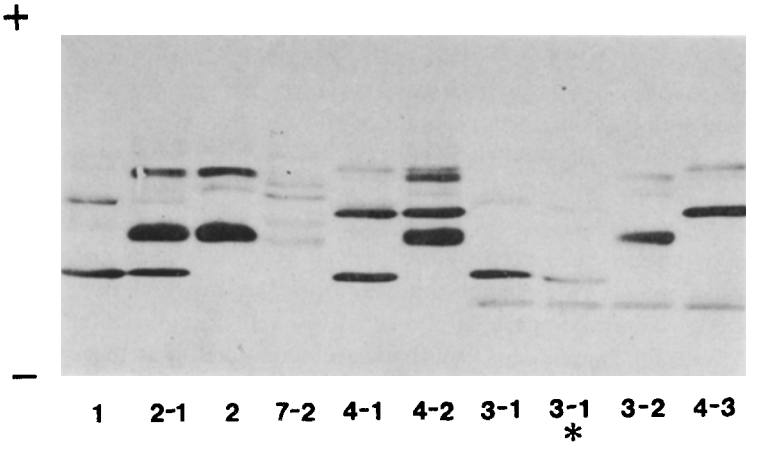

Fig. 1. Patterns of C7 phenotypes obtained from neuraminidase-treated samples by isoelectric focusing and immunoblotting. * Reference sample provided by Dr. P.J. Lachmann.

Table 1. Distribution of C7 phenotypes and allele frequencies.

Phenotypes	No. observed	%	No. expected	Allele frequencies
1	148	68.2	143.4	*C7*1*=0.813
2-1	26	12.0	34.2	*C7*2*=0.097
3-1	13	6.0	13.0	
4-1	18	8.3	18.0	*C7*3*=0.037
2	5	2.3	2.0	
3-2	2	0.9	1.6	*C7*4*=0.051
4-2	3	1.4	2.1	*C7*7*=0.002
Others[a]	2	0.9	2.7	
Total	217	100.0	217.0	1.000

[a] Others: C7 4-3, C7 7-2. $\chi^2=7.28$, d.f.$=5$, $0.20<p<0.30$.

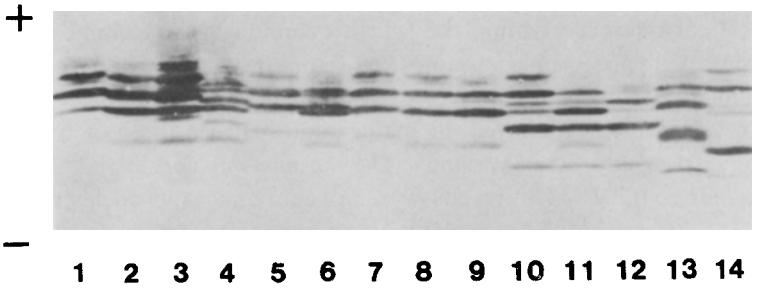

Fig. 2. Patterns of C6 phenotypes obtained by isoelectric focusing and immunoblotting. (1) C6 A, (2) C6 M92B, (3) C6 M91B, (4) C6 M1B, (5) C6 AM11, (6) C6 M11B, (7) C6 AM2, (8) C6 AB, (9) C6 B, (10) C6 AB2, (11) C6 BB2, (12) C6 B2, (13) C6 BB3, (14) C6 AB4. (3), (4) and (13) are reference samples.

the observed numbers of phenotypes from those expected on the Hardy-Weinberg equilibrium is statistically non-significant ($\chi^2=7.28$, d.f.$=5$, $0.20<p<0.30$).

C6 polymorphism

The C6 patterns observed in the present study are demonstrated in Fig. 2. Five common and six rare phenotypes were observed, in which seven allotypes were distinguished. Three common allotypes, A, B and B2, and three rare allotypes, M11, M2 and B4, were identified, by direct comparison with reference samples (Tokunaga *et al.*, 1983, 1984). Another variant shows the bands very close to C6 A bands, but slightly more basic than C6 A. Because the variant bands were more acidic than M91 bands (formerly 91, Tokunaga *et al.*, 1984) and was considered to be different from any variant previously described, it was designated M92. Distribution of C6 phenotypes and allele frequencies are shown in Table 2. The allele

Table 2. Distribution of C6 phenotypes and allele frequencies.

Phenotypes	No. observed	%	No. expected	Allele frequencies
A	64	18.2	71.1	*C6*A*=0.450
AB	164	46.7	150.8	
B	75	21.4	79.9	*C6*B*=0.477
AB2	20	5.7	20.2	
BB2	19	5.4	21.4	*C6*B2*=0.064
B2	3	0.9	1.4	
AR[a]	4	1.1	2.8	*C6*R*=0.009
BR[a]	2	0.6	3.0	
Others	0	0.0	0.4	
Total	351	100.0	351.0	1.000

[a] Incidence of rare phenotypes: C6 AM2 2, C6 AM11 1, C6 AB4 1, C6 M11B 1, C6 M92B 1. $\chi^2=4.96$, d.f.=5, $0.30<p<0.50$.

Table 3. Association analysis between C6 and C7 alleles.

Combinations[a]	+/+	+/−	−/+	−/−	χ^2	p
*C6*A−C7*1*	148	10	57	2	0.710	NS[b]
*C6*A−C7*2*	28	130	9	50	0.185	NS
*C6*A−C7*3*	10	148	6	53	0.928	NS
*C6*A−C7*4*	16	142	6	53	0.000	NS
*C6*B−C7*1*	157	11	48	1	1.475	NS
*C6*B−C7*2*	31	137	6	43	1.034	NS
*C6*B−C7*3*	13	155	3	46	0.145	NS
*C6*B−C7*4*	20	148	2	47	0.200	NS
*C6*B2−C7*1*	20	1	185	11	0.026	NS
*C6*B2−C7*2*	4	17	33	163	0.066	NS
*C6*R−C7*1*	5	0	203	9	0.169	NS

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

frequencies estimated for *C6*A*, *C6*B*, *C6*B2* and the rare variants are 0.450, 0.477, 0.064 and 0.009, respectively. The observed numbers of the phenotypes are distributed in accordance with Hardy-Weinberg law ($\chi^2=4.96$, d.f.=5, $0.30<p<0.50$).

Association analysis between C6 and C7

Analysis on the possible associations between C6 and C7 alleles was performed in 217 samples (Table 3). No significant associations were found.

DISCUSSION

Recently, Nishimukai and Tamaki (1986) proposed a new allotype C7 5, using agarose gel isoelectric focusing followed by immunobotting with desialized samples. They also presented pedigrees indicating the inheritance of the C7 5. In the present study, we confirmed that the C7 5 reported by Nishimukai and Tamaki corresponds to the C7 3 described by Hobart *et al.* (1978). The same conclusion was also obtained by Drs. M.J. Hobart and P.J. Lachmann (Cambridge, UK, personal communication). Interestingly, *C7*3* exists at a polymorphic frequency in Japanese, in spite of the finding that the protein concentration of C7 3 may be lower than those of the other allotypes (Hobart *et al.*, 1978; Nishimukai and Tamaki, 1986). We consider that samples should be desialized prior to C7 typing by immunoblotting. Without neuraminidase-treatment, C7 3 bands may be difficult to distinguish from the minor bands of the other allotypes or non-specific bands in the immunoblotting detection.

Moreover, a rare variant, tentatively named C7 7, was newly found. Recently another rare variant was found in a Chinese population and was tentatively named C7 6 (Zeng *et al.*, 1986). The C7 frequencies observed in the present material from eastern Japan were statistically not significantly different from those in western part of Japan (Nishimukai *et al.*, 1986).

The allele frequencies of C6 estimated in the present study are similar to those of the other Japanese populations (Tokunaga *et al.*, 1983, 1984; Nishimukai *et al.*, 1985; Nakamura *et al.*, 1984b). We confirmed that *C6*B* is more common than *C6*A* and that *C6*B2* is the third common allele in Japanese. A rare variant, *C6*M92*, was newly found. The variant was also found in a family samples from Tokyo, which have been collected for the other project. The pedigree shows the inheritance of *C6*M92* as well as *C7*3* (Fig. 3).

The present study showed no significant association between C6 and C7 alleles,

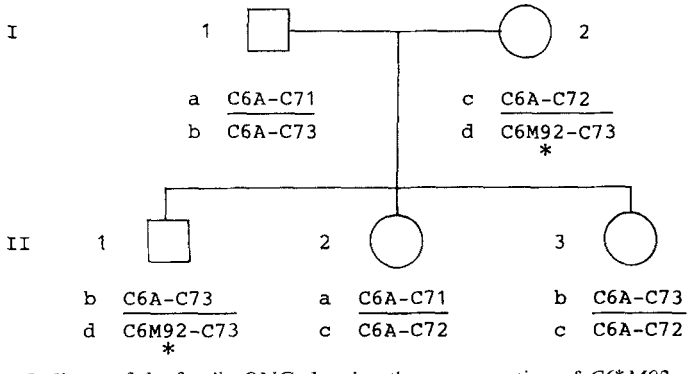

Fig. 3. Pedigree of the family ONG showing the cosegregation of *C6*M92* and *C7*3*.

including *C7*3*. The result in addition to the previous reports (Tokunaga *et al.*, 1986; Nishimukai and Tamaki, 1986) support that there is no linkage disequilibrium between C6 and C7 loci.

Extensive studies on genetic polymorphism of C7 in various human populations would be expected, because the frequencies of *C7*2*, *C7*3* and *C7*4* are high in Japanese, while they are low in Caucasians. It is interesting that in Japanese, both C6 and C7 show higher degree of polymorphism than in Caucasians.

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REFERENCES

Bender, K., Bissbort, S., Mayerová, A., Mauff, G., and Wienker, T.F. 1983. C6 linkage studies. *J. Immunogenet.* **10**: 61–67.

Hobart, M.J., Lachmann, P.J., and Alper, C.A. 1975. Polymorphism of human C6. In *22nd Colloquim Brugge 1974, Protides of the Biological Fluids*, Pergamon Press, Oxford, pp. 575–580.

Hobart, M.J., Joysey, V., and Lachmann, P.J. 1978. Inherited structural variation and linkage relationships of C7. *J. Immunogenet.* **5**: 157–163.

Lachmann, P.J., Hobart, M.J., and Woo, P. 1978. Combined genetic deficiency of C6 and C7 in man. *Clin. Exp. Immunol.* **33**: 193–203.

Mauff, G., Alper, Ch.A., Hobart, M., Kühnl, P., Kunstmann, G., Meo, T., Olving, J.H., and Rittner, Ch., 1980. Statement on the nomenclature of human C6 polymorphism. *Immunobiology* **158**: 139–144.

Mauff, G., Alper, Ch.A., Awdeh, Z., Batchelor, J.R., Bertrams. J., Bruun-Petersen, G., Dawkins, R.L., Démant, P., Edwards, J., Grosse-Wilde, H., Hauptmann, G., Klouda, P., Lamm, L., Mollenhauser, E., Nerl, C., Olaisen, B., O'neill, G., Rittner, Ch., Roos, M.H., Skanes, V., Teisberg, P., and Wells, L. 1983. Statement on the nomenclature of human C4 allotypes. *Immunobiology* **164**: 184–191.

Nakamura, S., Ooue, O., and Abe, K. 1984a. Genetic polymorphism of the seventh component of complement in a Japanese population. *Hum. Genet.* **66**: 279–281.

Nakamua, S., Ooue, O., Akiyama, K., and Abe, K. 1984b. Genetic polymorphism of complement C6 and haplotype analysis between C6 and C7 in a Japanese population. *Hum. Genet.* **68**: 138–141.

Nishimukai, H., Kitamura, H., and Tamaki, Y. 1985. C6 polymorphism in Japanese: typing by agarose gel isoelectric focusing-immunofixation. *Hum. Hered.* **35**: 30–33.

Nishimukai, H. and Tamaki, Y. 1986. Genetic polymorphism of the seventh component of complement: A new variant. *Vox. Sang.* **51**: 60–62.

Olving, J.H., Olaisen, B., Gedde-Dahl, T., Jr., and Teisberg, P. 1979. Genetic linkage relations of the sixth component of complement (C6). *Hum. Genet.* **46**: 181–192.

Podack, E.R., Kolb, W.P., and Müller-Eberhard, H.J. 1976. Purification of the sixth and seventh component of human complement without loss of hemolytic activity. *J. Immunol.* **116**: 263–269.

Tokunaga, K., Yukiyama, Y., and Omoto, K. 1983. Polymorphism of the complement component C6 in Japanese. *J. Immunogenet.* **10**: 419–424.

Tokunaga, K., Yamamura, N., and Omoto, K. 1984. An immunoblotting technique for complement C6 typing: three new variants. *Jpn. J. Human Genet.* **29**: 415–419.

Tokunaga, K., Dewald, G., Omoto, K., and Juji, T. 1986. Family study on the polymorphisms of the sixth and seventh components (C6 and C7) of human complement: linkage and haplotype analyses. *Am. J. Hum. Genet.* **39**: 414–419.

Whitehouse, D.B. and Putt, W. 1983. Immunological detection of the sixth complement component (C6) following flat bed polyacrylamide gel isoelectric focusing and electrophoretic transfer to nitrocellulose filters. *Ann. Hum. Genet.* **47**: 1–8.

Zeng, Z., Tokunaga, K., Omoto, K., and Du, C. 1986. Genetic polymorphisms of complement C6 and C7 in two Chinese populations. *Jpn. J. Human Genet.* **31**: 263–271.