Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 7890–7892, December 1984
Genetics

Evidence for linkage between the loci coding for the binding protein for the fourth component of human complement (C4BP) and for the C3b/C4b receptor

(genetic linkage/linkage disequilibrium)

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Communicated by Paul E. Lacy, August 27, 1984

ABSTRACT **Three pedigrees informative for the segregation of genetic variants of the binding protein for the fourth component of complement (C4BP) and C3b/C4b receptor (C3bR) have been identified. There were 10 informative meioses with no recombinants, indicating a close linkage between the loci encoding C4BP and C3bR, *C4BP* and *C3bR* [maximum lod (logarithm of odds of linkage) score: 2.4 at recombinant fraction = 0.0]. In addition, in the four unrelated individuals who were doubly heterozygous (*C4BP*1*, *C4BP*2*, *C3bR*A*, *C3bR*B*), the infrequent allele *C4BP*2* segregated together with the uncommon allele *C3bR*B*, supporting the hypothesis of linkage between *C4BP* and *C3bR* and suggesting that linkage disequilibrium exists between these particular alleles. We conclude that the loci encoding C3bR and C4BP, two functionally related molecules, are linked.**

Genetic variants for many of the complement components have been found in humans and other species (reviewed in ref. 1). The study of the segregation of these variants has shown that some of the complement proteins that are functionally related are also genetically associated in linkage groups. Two such linkage groups have been found in humans: (*i*) the second and fourth components of complement (C2 and C4) and factor B (2–5) and (*ii*) the sixth and seventh components of complement (C6 and C7) (6, 7). The latter linkage group includes components with molecules of similar size that are part of the membrane attack complex. The former linkage group, located inside the major histocompatibility complex (MHC), includes complement components involved in the formation of both the classical- and the alternative-pathway C3 convertases, C4b2a and C3bBb, respectively. At least four proteins regulate the activity of these C3 convertases. Two are serum glycoproteins; factor H and C4-binding protein (C4BP), and two are membrane glycoproteins: the C3b/C4b-receptor (C3bR) and the decay-accelerating factor (DAF). Because of the similarities of the functional properties of these four molecules, the hypothesis that they may be controlled by a "new" cluster of linked loci is now proposed. Testing of this hypothesis by classical segregation analysis is made possible by the recently demonstrated genetic polymorphism of C4BP, factor H, and C3bR in humans (8–13). Two allelic variants of C4BP (8, 9) and three of factor H (10) have been identified by isoelectric focusing under completely denaturing conditions. Three distinct forms of C3bR (11–13) have been identified by NaDodSO$_4$/polyacrylamide gel electrophoresis on human erythrocytes and leukocytes that vary in molecular weight by relatively large amounts (160,000, 190,000, and 220,000). Neither *C4BP* nor *C3bR* are closely linked to the major histocompatibility complex (8, 14). We now present data indicating that the genes that code for C4BP and C3bR are closely linked in humans.

MATERIALS AND METHODS

Typing of C4BP. Phenotyping for *C4BP* was performed as described (9) by using fresh EDTA-containing serum or serum samples stored at −80°C. Samples were treated with neuraminidase before immunoprecipitation. Immunoprecipitates were analyzed by isoelectric focusing under completely denaturing conditions on vertical 4.5% polyacrylamide slab gels and stained with Coomassie blue for analysis.

Typing of the C3b/C4b Receptors. Phenotyping for *C3bR* was performed by using blood samples collected in citrate dextrose. Surface-labeling of erythrocytes by ^{125}I and purification of C3bR was performed as reported (11, 13). A mouse monoclonal IgG1 antibody (57F) against C3bR was utilized for immunoprecipitation (generous gift of Victor Nussenzweig, New York University). Immunoprecipitates were subjected to slab gel electrophoresis by the method of Laemmli with a 5% polyacrylamide gel and autoradiography.

RESULTS

One hundred and eighty-four families have been allotyped for *C4BP*. Six of them were informative, five being of the mating type (*C4BP*1*, *C4BP*1* × *C4BP*1*, *C4BP*2*) and one of the mating type (*C4BP*1*, *C4BP*2* × *C4BP*1*, *C4BP*2*). Three of these six families were not available for further testing. The remaining three were allotyped for *C3bR*. Fig. 1, Fig. 2, and Table 1 summarize the typing results for both *C4BP* and *C3bR* in these three families.

In Family 1 the father is doubly heterozygous: *C4BP*1*, *C4BP*2*; *C3bR*A*, *C3bR*B*. The mother is homozygous to both loci (*C4BP*1*, *C4BP*1*; *C3bR*A*, *C3bR*A*). Three of the offspring inherited *C4BP*2* together with *C3bR*B* from the father, and the remaining two inherited *C4BP*1* together with *C3bR*A*. In Family 2 the father is again a double heterozygote (*C4BP*1*, *C4BP*2*; *C3bR*A*, *C3bR*B*). The mother is homozygous *C4BP*1*, *C4BP*1* and heterozygous *C3bR*A*, *C3bR*B*. In this family, five children were homozygous to the *C3bR* locus and, therefore, are informative. Three of them inherited *C4BP*1* together with *C3bR*A* from

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Abbreviations: C2–C7, second to seventh components of complement; C4BP, C4-binding protein; C3bR (also called CR1), C3b/C4b receptor; *C4BP* and *C3bR*, loci coding for C4-binding protein and the C3b/C4b receptor, respectively.

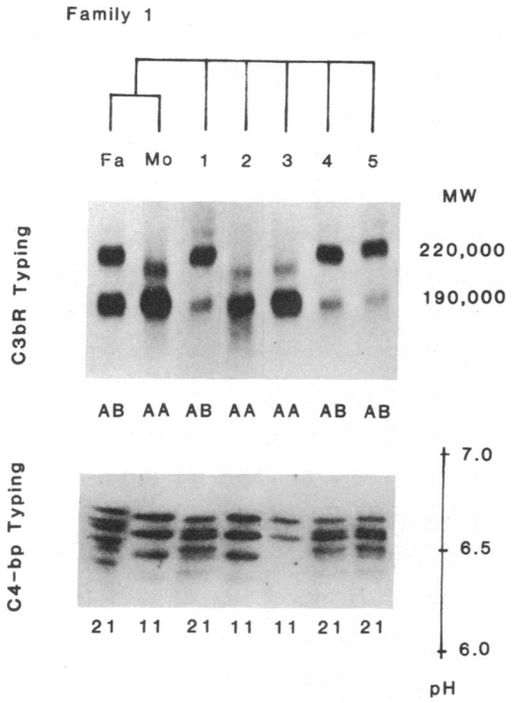

FIG. 1. C4BP and C3bR were allotyped in Family 1 (MW = M_r). Phenotypes for *C3bR* (upper panel) and *C4BP* (lower panel) were assigned for father (Fa), mother (Mo), and siblings (1–5).

the father; the remaining 2 inherited *C4BP*2* together with *C3bR*B*. The four remaining offspring in this family were *C3bR*A*, *C3bR*B* heterozygous, and no linkage information can be derived from them. In Family 3, both parents were double heterozygotes (*C4BP*1*, *C4BP*2*; *C3bR*A*, *C3bR*B*). The only child available thus far (*C4BP*1*, *C4BP*1*; *C3bR*A*, *C3bR*A*) suggests that *C4BP*2* must segregate together with *C3bR*B* from both parents, if these genes are indeed linked.

Thus, as summarized in Table 1, 10 out of the 15 offspring

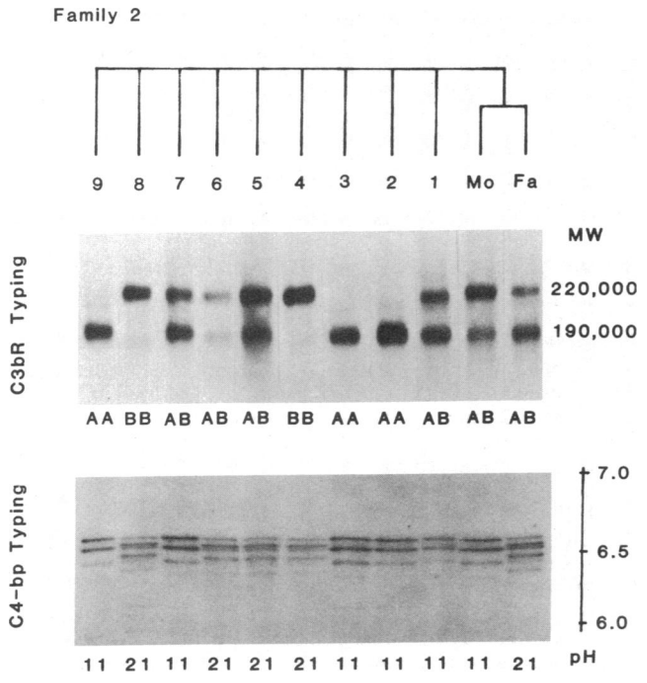

FIG. 2. Results (as in Fig. 1) for family 2; father (Fa), mother (Mo), and siblings (1–9).

Table 1. Analysis of linkage between *C4BP* and *C3bR* in three selected families

Family	Genotypes *C4BP*	Genotypes *C3bR*	Informative meioses* (NR:R)		lod score†
1 Father	*1,2*	*A,B*			
Mother	*1,1*	*A,A*			
Sibling 1‡	*1,2*	*A,B*			
2‡	*1,1*	*A,A*			
3‡	*1,1*	*A,A*			
4‡	*1,2*	*A,B*			
5‡	*1,2*	*A,B*			
			Subtotal	5:0	1.2
2 Father	*1,2*	*A,B*			
Mother	*1,1*	*A,B*			
Sibling 1	*1,1*	*A,B*			
2‡	*1,1*	*A,A*			
3‡	*1,1*	*A,A*			
4‡	*1,2*	*B,B*			
5	*1,2*	*A,B*			
6	*1,2*	*A,B*			
7	*1,1*	*A,B*			
8‡	*1,2*	*B,B*			
9‡	*1,1*	*A,A*			
			Subtotal	5:0	1.2
3 Father	*1,2*	*A,B*			
Mother	*1,2*	*A,B*			
Sibling 1	*1,1*	*A,A*			
			Subtotal	0	0
			Total	10:0	2.4

*NR, nonrecombinant; R, recombinant.
†lod analysis calculated at a recombination fraction of 0.0.
‡Informative siblings for lod analysis.

in these families were informative for the calculation of the lod (logarithm of odds of linkage) score value. This value was estimated as 2.4 at a recombination fraction of 0.0. (i.e., the odds on linkage are 256 to 1). In order to definitively establish the linkage between two loci, a lod score value of 3 is conventionally required. However, in the case of *C4BP* and *C3bR*, another observation strongly supports the suggested close linkage: in each of the four unrelated *C4BP* heterozygotes thus far encountered, *C4BP*2* appears to segregate together with *C3bR*B*. Because the gene frequency of *C3bR*B* is only 0.160, the equilibrium expectation would have been a more frequent association of *C4BP*2* with *C3bR*A* (83%). Thus, the probability of finding that the four *C4BP*2* alleles segregate together with *C3bR*B* would be 0.16^4 (7×10^{-4}) on the basis of the null hypothesis of genetic independence.

DISCUSSION

In this study we provide initial evidence for linkage between the loci coding for C4BP and C3bR in humans. Variation of C4BP is uncommon (<3% of normal population), and only three families informative for both loci were available for study. Nevertheless, these three families were informative in two ways. First, the lod score analysis suggested linkage between the loci for C4BP and C3bR, with no observed recombinants. Second, in the four unrelated individuals with the infrequent *C4BP* allele *C4BP*2*, it was found that this allele had or must have segregated together with the less common *C3bR* allele *C3bR*B*, suggesting the existence of linkage disequilibrium between these two alleles. Our pedigrees were not sufficient to evaluate linkage between the loci for factor H and C3bR or factor H and C4BP.

Previously, two serum proteins and three integral membrane proteins have been identified that regulate activities of

Table 2. Human C3b and C4b binding proteins

Protein	Location	Ligand	M_r	Genetics: Polymorphic variants	Genetics: Linkage
Factor H	Serum	C3b	160,000	3	?
C4BP	Serum	C4b > C3b	590,000*	2	C3bR
C3bR	Erythrocytes and leukocytes	C3b > C4b	160,000 190,000 220,000	3	C4BP
DAF	Erythrocytes	C4b2a > C3bBb	70,000	?	?
gp45–70	Leukocytes	C3b > C4b	45,000–70,000	?	?

*C4BP dissociates into M_r 70,000 subunits under reducing conditions.

the classical- and alternative-pathway convertases (Table 2). The first three (factor H, C4BP, and C3bR) exhibit polymorphism and share functional properties. Factor H is a serum glycoprotein that regulates decay of the alternative-pathway C3 convertase (C3bBb) by dissociating factor Bb from C3b (15). It also serves as a cofactor for I-mediated cleavage of the α-chain of C3b (16). C4BP, a serum glycoprotein, inhibits the activity of the classical-pathway C3 convertase (C4bC2a) both by displacing C2a from C4b and by promoting cleavage of C4b by I (17, 18). However, C4BP shares regulatory properties with factor H, since it can also bind C3b and promote cleavage of C3b by I (19, 20). Finally, C3bR is an integral membrane glycoprotein found on erythrocytes and most leukocytes (21). C3bR binds C4b and C3b, and its functional capacities encompass those of C4BP and factor H. C3bR promotes the decay of both classical- and alternative-pathway convertases and serves as a cofactor for I-mediated cleavage of C4b and C3b (21, 22).

In addition to C4BP, factor H, and C3bR, two other C3b/C4b-binding proteins have been described. DAF (decay accelerating factor) and gp45–70 (human glycoprotein with M_rs between 45,000 and 70,000) are integral membrane glycoproteins (Table 2). DAF binds to the classical- and alternative-pathway convertases, C4bC2a and C3bBb, and in so doing, dissociates C2a or factor Bb from the enzyme complex rendering it inactive (23, 24). DAF has a 10-fold greater binding activity for the classical- than for the alternative-pathway convertase (24). It is found on erythrocytes, platelets, and probably leukocytes. gp45–70 is a more recently described group of functionally similar molecules on human leukocytes (25). gp45–70 binds C3b better than C4b and has been isolated by affinity chromatography using these ligands (25). In preliminary studies, we have found that it dissociates the classical-pathway convertase and also serves as cofactor for I-mediated cleavage of C3b. Of interest, a similar-sized molecule is the major C3b binding protein of rabbit alveolar macrophages (26, 27).

The C3b/C4b binding proteins listed in Table 2 are functionally similar in that they bind C3b and/or C4b and dissociate enzyme complexes containing these proteins. This report provides evidence for a genetic linkage between two of these regulatory proteins, C4BP and C3bR, possibly resulting from gene duplication analogous to that suspected for C2 and factor B (28). Further studies are required to determine whether factor H is linked to C4BP and C3bR, whether DAF and/or gp45–70 are polymorphic and, if so, whether they are genetically linked to any of these molecules.

The authors thank Ms. Alice Varipapa, Lorraine Whiteley, and Peggy Hart for excellent manuscript preparation. This work was supported in part by National Institutes of Health (HL 09011), the Lottie Caroline Hardy Trust Fund, and a grant from the Monsanto Corporation.

1. Alper, C. A. (1981) in *The Role of the Major Histocompatibility Complex in Immunobiology*, ed. Dorf, M. E. (Garland, New York), p. 173.
2. Alper, C. A. (1976) *J. Exp. Med.* **144,** 1111–1115.
3. Teisberg, P., Olaisen, B., Jonassen, R., Gedde-Dahl, T., Jr., & Thorsby, E. (1976) *Nature (London)* **264,** 253–254.
4. Meo, T., Atkinson, J., Bernoco, M., Bernoco, D. & Ceppellini, R. (1976) *Eur. J. Immunol.* **6,** 916–919.
5. Allen, F. H., Jr. (1974) *Vox Sang.* **27,** 382–384.
6. Hobart, M. J., Joysey, V. & Lachmann, P. J. (1978) *J. Immunogenet.* **5,** 157–163.
7. Lachmann, P. J., Hobart, M. J. & Woo, P. (1978) *Clin. Exp. Immunol.* **33,** 193–203.
8. Rodriguez de Cordoba, S., Ferreira, A., Nussenzweig, V. & Rubinstein, P. (1983) *J. Immunol.* **131,** 1565–1569.
9. Rodriguez de Cordoba, S., Rubinstein, P. & Ferreira, A. (1984) *J. Immunol. Methods* **69,** 165–172.
10. Rodriguez de Cordoba, S. & Rubinstein, P. (1984) *J. Immunol.* **132,** 1906–1908.
11. Dykman, T. R., Cole, J. L., Iida, K. & Atkinson, J. P. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 1698–1702.
12. Wong, W. W., Wilson, J. G. & Fearon, D. T. (1983) *J. Clin. Invest.* **72,** 685–690.
13. Dykman, T. R., Hatch, J. A. & Atkinson, J. P. (1984) *J. Exp. Med.* **159,** 691–703.
14. Hatch, J. A., Atkinson, J. P., Suarez, B. K. & Dykman, T. R. (1984) *J. Immunol.* **132,** 2168–2169.
15. Whaley, K. & Ruddy, S. (1976) *J. Exp. Med.* **144,** 1147–1163.
16. Pangburn, M. K., Schreiber, R. D. & Muller-Eberhard, H. J. (1977) *J. Exp. Med.* **146,** 257–270.
17. Gigli, I., Fujita, T. & Nussenzweig, V. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 6596–6600.
18. Scharfstein, J. A., Ferreira, A., Gigli, I. & Nussenzweig, V. (1978) *J. Exp. Med.* **148,** 207–222.
19. Fujita, T. & Nussenzweig, V. (1979) *J. Exp. Med.* **150,** 267–276.
20. Fujita, T., Gigli, I. & Nussenzweig, V. (1978) *J. Exp. Med.* **148,** 1044–1051.
21. Fearon, D. T. (1979) *Proc. Natl. Acad. Sci. USA* **76,** 5867–5871.
22. Iida, K. & Nussenzweig, V. (1981) *J. Exp. Med.* **153,** 1138–1150.
23. Nicholson-Weller, A., Burge, J. & Austen, K. F. (1981) *J. Immunol.* **127,** 2035–2039.
24. Nicholson-Weller, A., Burge, J., Fearon, D. T., Weller, P. F. & Austen, K. F. (1982) *J. Immunol.* **129,** 184–188.
25. Cole, J. L., Housley, G. A., Jr., Dykman, T. R., McDermott, R. P. & Atkinson, J. P. (1985) *Proc. Natl. Acad. Sci. USA,* in press.
26. Schneider, R. J., Kulczycki, A., Jr., Law, S. K. & Atkinson, J. P. (1981) *Nature (London)* **290,** 789–792.
27. Dixit, R., Schneider, R., Law, S. K., Kulczycki, A., Jr., & Atkinson, J. P. (1981) *J. Biol. Chem.* **257,** 1595–1597.
28. Shreffler, D. C. (1982) in *Histocompatibility Antigens: Structure and Function*, Receptors and Recognition, eds. Parham, P. & Strominger, J. (Chapman & Hall, London), Vol. 14, Ser. B, p. 189.