Linkage studies in hereditary angio-oedema

E. B. ROBSON, P. J. LACHMANN, M. J. HOBART, AND A. W. JOHNSTON

From the MRC Human Biochemical Genetics Unit, The Galton Laboratory, University College London; The MRC Group on Mechanisms in Tumour Immunity, The Medical School, Hills Road, Cambridge; and Woodend General Hospital, Aberdeen

SUMMARY The locus for hereditary angio-oedema must lie well outside the limits of the HLA complex. Linkage tests with 16 marker loci gave no hint of linkage. In particular, close linkage is excluded for C6, PGM₁, MNSs, Gm, Rh, Km, Hp, and ABO.

Hereditary angio-oedema (HAE) is a familial disease characterised by recurrent attacks of angio-oedema in the subcutaneous tissues, and sometimes in the bowel and in the respiratory tract. Attacks in the respiratory tract may produce fatal asphyxia. The disease is inherited as an autosomal dominant trait and is caused by deficiency of C1-inhibitor (α -neuraminoglycoprotein). The patients are heterozygous for the gene determining HAE and have generally in the region of one-sixth of the normal level of C1-inhibitor in their serum. In the majority (about 85%) of pedigrees the functional inhibitor deficiency is paralleled by deficiency of the protein measured antigenically. In about 15% of pedigrees a dysfunctional protein can be found by antigenic measurement.

C1-inhibitor acts by irreversible stoichiometric combination with a large number of esterases in the triggered enzyme systems of the blood plasma. Plasmin, kallikrein, and factors XIa and XIIa of the clotting system are inhibited as well as C1r and C1s. The low levels in heterozygotes appear to be sufficient to maintain homeostasis of the early complement components under normal conditions. Attacks appear to be triggered by the activation of one of the relevant enzymes (perhaps especially of plasmin) at an extravascular site where α 2-macroglobulin (another important inhibitor) is not present at high concentrations. Local depletion of C1-inhibitor results and this permits the uncontrolled activation of C1 with destruction of C4 and C2. The mediator producing the oedema is generated from C2 and it has been reported that this needs the presence of plasmin as well as of C1 and C4 (F. S. Rosen, 1977, personal communication).

Interest in the linkage relationships of the loci involved in the complement system was stimulated

by the discovery that Bf, Factor B of the alternative pathway, is linked to HLA (Allen, 1974). The relevance of such information in assessing the respective roles and evolutionary history of the numerous components of the complement system was apparent, and searches for linkage were subsequently undertaken with all the components where variation, quantitative or qualitative, could be shown in families. It was soon shown that some complement loci were HLA-linked: Bf (Allen, 1974); C2 (Fu *et al.*, 1974); C4 (Rittner *et al.*, 1975; Teisberg *et al.*, 1976). Other loci, including HAE, however, were not: C3 (Gedde-Dahl *et al.*, 1974); C6 (Mittal *et al.*, 1976; Hobart *et al.*, 1977; Olving *et al.*, 1977); C7 (Rittner *et al.*, 1976; Hobart *et al.*, 1978); C1r (Day *et al.*, 1975; Mittal *et al.*, 1976); and C1-inhibitor (Jackson *et al.*, 1976; Rittner, 1976; Ohela *et al.*, 1977; Tanimoto *et al.*, 1977; Blumenthal *et al.*, 1978). Many of these investigations were limited to a search for linkage with HLA, but in the cases where no evidence in favour of this particular linkage has been found, the relationships of the complement components, both among themselves and with unrelated genetical markers, become important. We report here on linkage investigations in two families of HAE which were extensively tested for red cell antigens, serum proteins, red cell enzymes, and the polymorphic complement systems C3 and C6, in addition to HLA.

Materials and methods

Family MRC 3396 is English and was discovered by one of the authors in the course of diagnostic laboratory investigations in a reference centre. Family MRC 1508 is Scottish and came under the care of one of the authors. The diagnosis of HAE was based on the criteria of history of attacks and

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the laboratory estimations of C $\bar{I}$ -inhibitor levels. All family members giving a history consistent with the disease showed less than 30% of the normal C $\bar{I}$ -inhibitor level. Two subjects in one of the families were asymptomatic (III.7 and III.11). III.7 was a boy of 11 years and III.11 was a boy of 3½ years.

The blood groups and the HLA types were determined by standard serological procedures, and the red cell enzymes typed by published techniques (Harris and Hopkinson, 1976). Complement typing for the polymorphic systems was carried out by the

method of Alper and Propp (1968) for C3 and by the method of Hobart *et al.* (1975) for C6.

In the linkage analysis the lod score method as tabulated by Maynard-Smith *et al.* (1961) was used. The scores are classified by the sex of the heterozygous parent, since it has been established for many linkage groups in man that the recombination fraction in females is greater than that in males. *HLA-A*, *HLA-B*, and *Bf* have been scored as a single locus *HLA*. Weighted scores were used for the *ABO*, *Rh*, and *MNSs* blood groups.

Fig. Pedigrees of two families with HAE.

Table Linkage data on C $\bar{I}$ inhibitor

Locus	Sex of heterozygote	No of heterozygotes	No of offspring	3-generation data rc*	2- and 3-generation data nrc†	Lod scores for recombination fractions of				
						0.05	0.10	0.20	0.30	0.40
HLA	M	1	2	2	0	-2.00	-1.40	-0.80	-0.44	-0.19
	F	3	10	3	.	-2.63	-1.56	-0.64	-0.24	-0.05
P	M	1	2	.	.	+0.10	+0.08	+0.05	+0.02	+0.01
C6	M	1	2	.	.	+0.26	+0.21	+0.13	+0.06	+0.02
	F	2	7	2	2	-2.16	-1.33	-0.58	-0.23	-0.05
C3	F	2	4	1	3	-0.16	+0.17	+0.21	+0.22	+0.14
Rh	M	6	11	4	5	-4.48	-2.80	-1.28	-0.54	-0.16
	F	3	10	2	2	-1.25	-0.63	-0.16	0.00	+0.04
PGM ₁	M	1	5	.	.	-1.44	-0.89	-0.39	-0.15	-0.03
	F	1	2	.	.	-0.72	-0.44	-0.19	-0.08	-0.02
ACP ₁	M	2	3	0	1	-0.44	-0.19	+0.01	+0.07	+0.06
	F	1	2	.	.	-0.72	-0.44	-0.19	-0.08	-0.02
MNSs	M	4	11	.	.	-2.00	-0.67	+0.11	+0.15	+0.10
	F	3	9	1	3	-0.63	-0.16	+0.15	+0.20	+0.08
ABO	M	4	10	3	2	-2.63	-1.56	-0.66	-0.28	-0.10
	F	4	11	6	0	-7.17	-4.87	-2.66	-1.43	-0.61
EsD	F	2	3	2	1	-1.72	-1.14	-0.59	-0.30	-0.11
Hp	M	3	10	3	0	-5.16	-3.43	-1.77	-0.89	-0.34
	F	3	8	1	1	-2.88	-1.77	-0.78	-0.30	-0.07
ADA	M	2	9	0	3	-0.35	+0.09	+0.36	+0.35	+0.22
Do	M	1	3	.	.	-0.72	-0.44	-0.19	-0.08	-0.02
Gm	M	2	8	1	1	-0.63	-0.16	+0.13	+0.15	+0.06
	F	4	11	5	2	-5.88	-3.87	-1.97	-0.97	-0.36
Km	M	2	8	2	0	-4.16	-2.73	-1.38	-0.67	-0.25
	F	1	2	1	1	-0.72	-0.44	-0.19	-0.08	-0.02
Gc	F	1	2	.	.	+0.26	+0.21	+0.13	+0.06	+0.02
Secretor	F	1	5	.	.	-0.19	+0.02	+0.12	+0.09	+0.03

*, recombinant; †, non-recombinant

Results

The pedigrees are given in the Fig. The pattern of inheritance of HAE is clearly autosomal dominant, as in all other published families. The two families were considered together for the linkage analysis since they were of the same common subtype of HAE. The lod scores are given in the Table. Taking a lod score of -2.7 (= odds of 500:1 against linkage) and assuming that the female recombination fraction is 1.7 times that of the male, we can exclude linkage between the C1-inhibitor locus and marker loci at the following male recombination fractions: C6 1%; PGM₁ 2%; MNSs 3%; HLA 8%; Gm 9%; Rh 10%; Km 11%; Hp 16%, and ABO 16%. The positive lod scores for P, Sec, and Gc are based on trivial data. When many markers are tested there are inevitably some small positive lod scores at the larger recombination fractions, entirely the result of chance, and consequently we do not consider the small positive scores with ADA and MNSs to be useful hints at the moment.

Discussion

Our results confirm that HAE must lie well outside the limits of the HLA complex. Neither is it closely linked to the complement locus C6 which is known not to be linked to HLA. There are no clear hints about the assignment of HAE to any other known linkage group, single marker, or chromosome. The only other report of a general linkage investigation is that of Jackson *et al.* (1976). They too were able to exclude the possibility of close linkage with ABO and also to exclude an additional locus, that of transferrin (Tf). It has already been shown that there are at least three different chromosomal regions involved in the complement system, and HAE seems likely to involve a fourth site. Since there are still at least 14 other polypeptide chains to be accounted for, the number of sites still to be identified may be quite large. Their chromosomal assignment and relationship to the rest of the genome will be a formidable task.

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Requests for reprints to Dr E. B. Robson, Department of Genetics and Biometry, University College London, Wolfson House, 4 Stephenson Way, London NW1 2HE.