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HAPLOTYPE ANALYSIS OF THE LINKAGE GROUP HLA-A:HLA-B:C4 IN JAPANESE: EVIDENCE FOR THE C4 LOCUS BEING BETWEEN THE HLA-A AND HLA-B LOCI

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Summary 176 HLA-A:HLA-B:C4 haplotypes of the Japanese population as deduced by family analysis are described. Four combinations between the HLA and C4 alleles, namely, *B12-C4*^{S}, *Bw54-C4*^{F}, *Bw52-C4*^{F} and *Aw33-C4*^{S}, are shown to be in significantly positive linkage disequilibrium. This finding suggests close proximity between the HLA-B and C4 loci. In a family with HLA-A:HLA-B recombinant, the evidence is presented indicating that the C4 allele travels with the HLA-A allele. Therefore, it is considered that the C4 locus is probably situated between the HLA-A and HLA-B loci.

INTRODUCTION

Genetic polymorphism of the fourth component of human complement (C4) was first described by Teisberg *et al.* (1976) using an agarose gel electrophoresis followed by immunofixation. Subsequently, Teisberg *et al.* (1977) and Mauff *et al.* (1978) working on C4 polymorphism in European populations confirmed that C4 polymorphism is controlled by codominant alleles at an autosomal locus. Linkage between the HLA system and the genes controlling the synthesis of human C4 was first described by Rittner *et al.* (1975). Teisberg *et al.* (1976, 1977) also described the linkage between the HLA and C4 systems, suggesting that the C4 locus was situated very close to the HLA-B locus of the MHC region.

On the other hand, O'Neill *et al.* (1978a, 1978b) described the electrophoretic polymorphism of C4 in EDTA plasma and presented a new hypothesis which sug-

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gested that two different genetic loci control the electrophoretic patterns of C4. These authors assume that one locus controls the presence (F) or absence (f^0) of the anodal bands and the other locus controls the presence (S) or absence (s^0) of the cathodal bands, and both loci are closely linked to the HLA-B locus.

Recently, Tokunaga *et al.* (1979) studied, using both agarose and polyacrylamide gel electrophoresis followed by immunofixation, the genetic polymorphism of C4 in the Japanese population. Based on population and family data, they confirmed the codominant mode of transmission of the two alleles —$C4^F$ and $C4^S$— at a single locus. As mentioned above, it has been shown that the C4 locus is close to the HLA-B locus, but it is not yet clear whether the C4 locus is located on the HLA-A side or HLA-D side of the HLA-B locus. In this respect, it is interesting that Rittner *et al.* (1977) described a family with HLA-B:HLA-D recombinant in which the C4 allele travelled with the D allele, indicating that the C4 locus is close to the HLA-D locus.

In the present study, we are concerned with 176 HLA-A:HLA-B:C4 haplotypes in the Japanese population determined by family analysis and with an assessment of the linkage disequilibrium between the two gene combinations. Moreover, in a family with HLA-A:HLA-B recombinant, the evidence indicating that the C4 allele travels with HLA-A allele is presented.

MATERIALS AND METHODS

A total of 96 healthy, unrelated individuals (pooled parents of 95 children) from the central part of Japan (Chiba Pref.) were tested for HLA and C4 phenotypes and the haplotypes assessed by family studies. 88 individuals out of 96 tested were informative for three factor (HLA-A:HLA-B:C4) haplotypes. HLA-A,B antigens were typed in peripheral blood lymphocytes using the microcytotoxicity test (Terasaki and McClelland, 1964). HLA-DR and HLA-C typings were performed on a part of the family materials. C4 typing was performed by the method described by Tokunaga *et al.* (1979) using a slab polyacrylamide gel electrophoresis followed by immunofixation. The calculation of gene frequencies, haplotype frequencies, the D value of linkage disequilibrium and an evaluation of that D value were performed as previously described by Horai *et al.* (1979).

RESULTS AND DISCUSSION

A total of 176 HLA-A:HLA-B:C4 haplotypes as deduced from 88 healthy unrelated Japanese are taken as being representative of the Japanese population. These haplotypes consist of a part of those which were previously reported by Horai *et al.* (1979) as to HLA-A:HLA-B:Bf haplotype analysis. In this material, the gene frequencies for C4 calculated by direct gene counting were $C4^F = 0.5625$ and $C4^S = 0.4375$, respectively. These are in agreement with those for other Japanese

Table 1. HLA:C4 haplotype frequencies (p) and the corresponding delta values (D) obtained from 176 haplotypes. Significance of D was tested by Fisher's exact method based on 2×2 contingency tables (*P<0.05, **P<0.01).

Haplotype HLA	C4	p	D	Haplotype HLA	C4	P	D
A2	: F	.1364	−.0074	B12	: F	.0170	−.0309**
	: S	.1193	.0074		: S	.0682	.0309**
A3	: F	.0057	.0025	B13	: S	.0057	.0032
Aw24	: F	.2955	.0238	B15	: F	.0455	.0071
	: S	.1875	−.0238		: S	.0227	−.0071
A10	: F	.0455	.0007	Bw16	: F	.0227	−.0092
	: S	.0341	−.0007		: S	.0341	.0092
A11	: F	.0568	.0057	Bw22	: F	.0170	.0010
	: S	.0341	−.0057		: S	.0114	−.0010
Aw31	: F	.0114	−.0078	B27	: F	.0057	.0025
	: S	.0227	.0078	Bw35	: F	.0682	.0138
Aw33	: F	.0057	−.0199*		: S	.0284	−.0138
	: S	.0398	.0199*	B40	: F	.1136	−.0142
Aw34	: F	.0057	.0025		: S	.1136	.0142
				Bw46	: F	.0170	−.0053
B5	: F	.1193	.0234		: S	.0227	.0053
	: S	.0511	−.0234	Bw48	: F	.0057	−.0039
Bw51	: F	.0568	.0025		: S	.0114	.0039
	: S	.0398	−.0025	Bw54	: F	.0966	.0263*
Bw52	: F	.0511	.0192*		: S	.0284	−.0263*
	: S	.0057	−.0192*	B1b1	: F	.0227	−.0060
B7	: F	.0227	.0004		: S	.0284	.0060
	: S	.0170	−.0004				

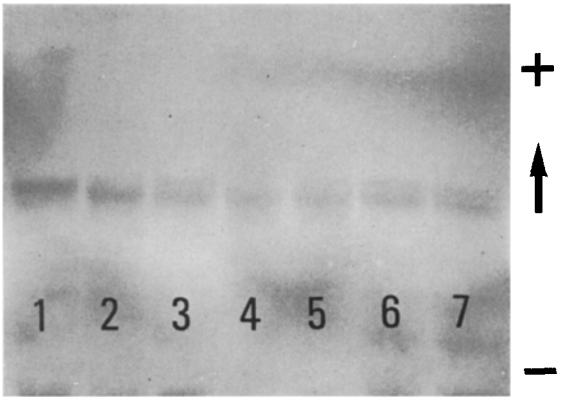

Fig. 1. Photograph showing the C4 patterns of the family Wat. samples. A vertical slab polyacrylamide gel electrophoresis using Tris/EDTA/Borate discontinuous buffer system was carried out followed by immunofixation with a monospecific anti-C4 antiserum. 1. control: C4 F, 2. father(F): C4 S, 3. mother(M): C4 FS, 4. child I(CI): C4 S, 5. child II(CII): C4 S, 6. mother's brother(MB): C4 FS, 7. mother's sister(MS): C4 FS.

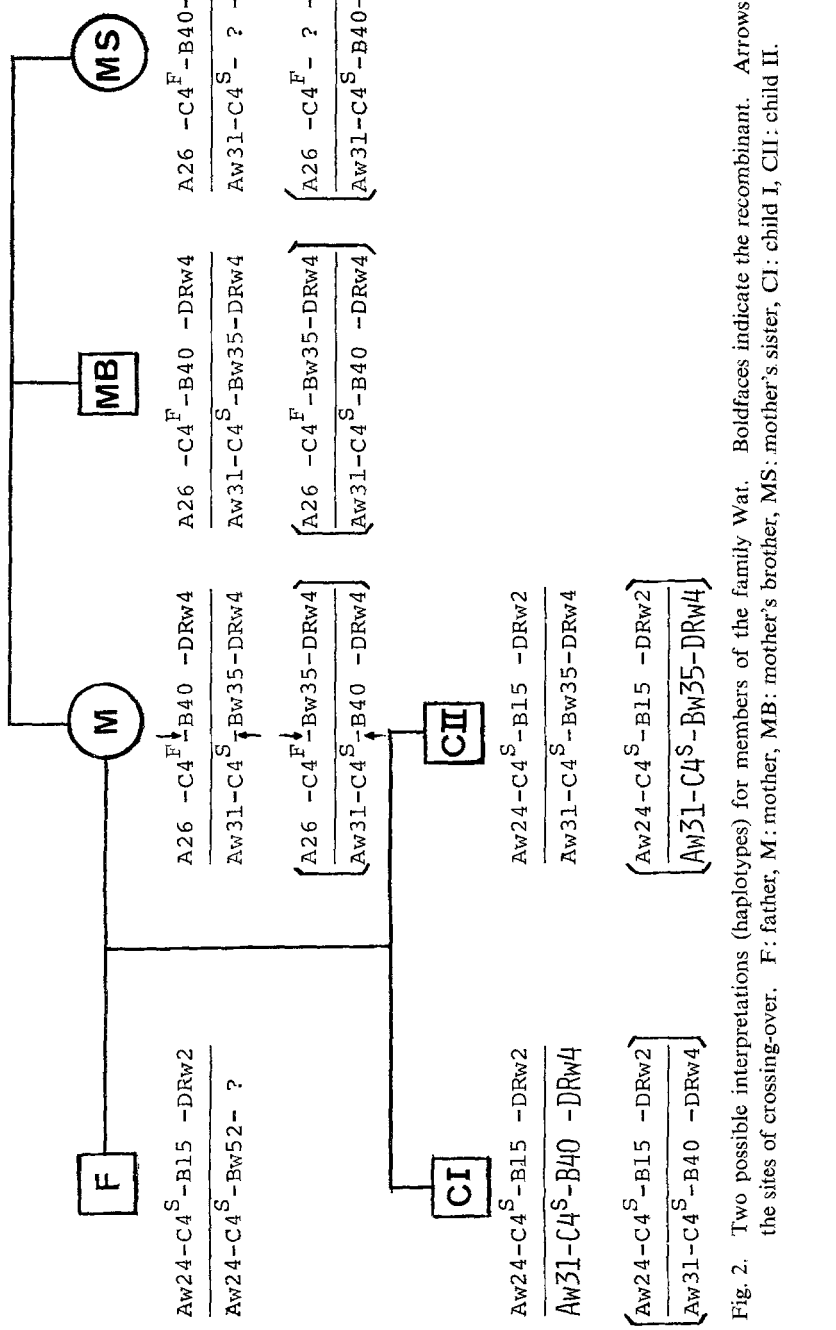

Fig. 2. Two possible interpretations (haplotypes) for members of the family Wat. Boldfaces indicate the recombinant. Arrows point to the sites of crossing-over. F: father, M: mother, MB: mother's brother, MS: mother's sister, CI: child I, CII: child II.

Table 2. Results of typing on HLA-A, HLA-B, HLA-C, HLA-DR and C4 systems of members of the family Wat. F: father, M: mother, MB: mother's brother, MS: mother's sister, CI: child I, CII: child II.

F	Aw24,	—,	B15,	Bw52,	Cw3,	—,	DRw2,	—,	C4S
M	A26,	Aw31,	Bw35,	B40,	Cw3,	—,	DRw4,	—,	C4FS
MB	A26,	Aw31,	Bw35,	B40,	Cw3,	—,	DRw4,	—,	C4FS
MS	A26,	Aw31,	B40,	—,	Cw3,	—,	DRw4,	—,	C4FS
CI	Aw24,	Aw31,	B15,	B40,	Cw3,	—,	DRw2,	DRw4,	C4S
CII	Aw24,	Aw31,	B15,	Bw35,	Cw3,	—,	DRw2,	DRw4,	C4S

(Tokyo) as previously reported by Tokunaga *et al.* (1979). The observed haplotype frequencies and their D values in two factor (HLA-A,B:C4) combinations are listed in Table 1. The significance of D was tested by the exact method of Fisher based on the 2×2 contingency table. As shown in Table 1, significantly positive D values are as follows: *B12-C4*^{S} ($P<0.01$), *Bw54-C4*^{F} ($P<0.05$), *Bw52-C4*^{F} ($P<0.05$), and Aw33-C4^{S} ($P<0.05$). This finding suggests close proximity between the HLA-B and C4 loci, since three significant combinations between the HLA-B and C4 were observed, while there was one between the HLA-A and C4 genes. The significant combination between HLA-A and C4 genes (*Aw33-C4*^{S}) may be due to the linkage disequilibrium between the HLA-A and HLA-B genes (*Aw33-B12*) in the Japanese population as previously indicated by Horai *et al.* (1979).

In the family materials, one family (Wat.) shows a cross-over between the HLA-A and B loci. The results of HLA-A,B,C,DR typing and C4 typing of the Wat. family are given in Table 2. The C4 patterns of plasma of the family members are shown in Fig. 1. All the typings were confirmed by two blood samples taken at separate times. Figure 2 is the pedigree and the haplotypes assessed for this family. Unfortunately, the mother's haplotypes could not be determined even by the typing of her siblings, and two possible interpretations of haplotypes are given in Fig. 2. For one of the possible maternal haplotypes (shown on the upper side), the cross-over is likely to have occurred in the child CI who obtained from his mother the haplotype *Aw31-C4*^{S} together with the *B40*, in contrast to his brother (CII). On the other hand, if the other possible maternal haplotype (shown on the lower side in parenthesis) is the case, the cross-over occurred in the child CII who obtained from his mother the haplotype *Aw31-C4*^{S} together with the *Bw35*, in contrast to his brother (CI). It is not clear which case is the real recombinant, but undoubtedly the *C4*^{S} gene travels with the *Aw31* gene, and the cross-over occurred between the C4 and HLA-B genes. Therefore, it is likely that the C4 locus is located on the HLA-A side of the HLA-B locus. On the basis of the association data presented in this report we have also confirmed that the C4 locus is situated very close to the HLA-B locus as previously reported by Teisberg *et al.* (1976, 1977) and O'Neill *et al.* (1978b). It is postulated, therefore, that the C4 locus is situated between the HLA-

A and HLA-B loci. As the HLA-C typing was not informative in this family, it is not clear whether the C4 locus is located on the HLA-A side or HLA-B side of the HLA-C locus.

It is, from our data, difficult to explain the results reported by Rittner *et al.* (1977), indicating that the C4 locus is close to the HLA-D locus. The possibility of a double cross-over in this small chromosomal region may not be excluded. Therefore, further studies of various recombinant families in the HLA region which are informative for C4 polymorphism are needed to draw conclusion as to the exact location of this locus.

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