Volume 217, number 1, 65–68 FEB 04778 June 1987

Heterogeneity in the structural basis of the human complement C4A null allele (C4A*Q0) as revealed by *Hin*dIII restriction fragment length polymorphism analysis

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Received 23 March 1987

The highly polymorphic fourth component of human complement (C4) is usually encoded by two genes, C4A and C4B, adjacent to the 21-hydroxylase (21-OH) genes, 21-OHA and 21-OHB, and is also remarkable in the high frequency of the 'null' alleles, C4A*Q0 and C4B*Q0. The molecular basis for the C4A*Q0 allele was studied in 26 families through restriction fragment length polymorphism (RFLP) analysis with C4 and 21-OH cDNA probes after digestion of the DNA with the endonuclease HindIII. The individuals expressing the extended haplotype HLA-A1 (of A2) Cw7 B8 C2C BfS C4AQ0B1 DR3 have a large deletion taking off the C4A and 21-OHA genes.

Complement component C4; 21-Hydroxylase; Null allele; Restriction fragment length polymorphism

1. INTRODUCTION

The class III genes of the major histocompatibility complex (MHC) code for three complement proteins, C2, C4, and factor B (Bf), and the steroid 21-hydroxylase (21-OH) enzymes. C4 and 21-OH are coded by two separate but closely linked loci, C4A, C4B, and 21-OHA, 21-OHB. The map of this region on the short arm of the sixth human chromosome has been established: the 21-OH genes lie 3′ of both the C4A and C4B genes, and the C2 and Bf genes lie 5′ to these genes [1].

The fourth component of human complement (C4) is characterized by its extensive genetic polymorphism at both loci and the high frequency of the 'null' alleles, C4A*Q0 and C4B*Q0 [2–4].

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Restriction fragment length polymorphism (RFLP) analysis may be useful to understand the molecular basis of these null alleles. Therefore, we have applied the technique of Southern blot analysis [5] using a full-length C4 cDNA and a 21-OH probe to examine the genomic DNA of individuals carrying a C4A*Q0 allele. We report here that the C4A*Q0 allele appearing in the extended HLA-A1 Cw7 B8 C2C BfS C4AQ0B1 DR3 haplotype is due to a deletion of about 30 kb, including the C4A and 21-OHA genes.

2. MATERIALS AND METHODS

2.1. *Family material*

The 26 families included in this study were selected from the material of normal families investigated within the 'HLA-Provinces Françaises' project [6] and the Ninth International Histocompatibility Workshop [7]. The members of these families have been typed for HLA-A, B, C, DR,

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00145793/87/$3.50

Bf, and C2 according to recommended methods [8,9].

2.2. *C4 typing*

The C4 variants were revealed through high-voltage agarose electrophoresis with subsequent immunofixation and hemolytic overlay [2,4,9]. Heterozygosity for C4A*Q0 was determined by comparing the intensities of stained C4A and C4B bands in all the family members.

2.3. *Preparation of genomic DNA and Southern blot analysis*

High-M_r genomic DNA was prepared from peripheral blood collected on K_3-EDTA using standard phenol/chloroform/isoamyl alcohol extraction [10]. 10–12 μg DNA samples were digested in a final volume of 300 μl with 30 U restriction endonuclease *Hin*dIII according to manufacturer's instructions (Appligene, Strasbourg). The digested DNA were analysed by electrophoresis in 0.6% agarose gels, transferred onto nitrocellulose, prehybridized in 40% formamide solution and hybridized with nick-translated heat-denatured DNA probes for 48 h at 42°C.

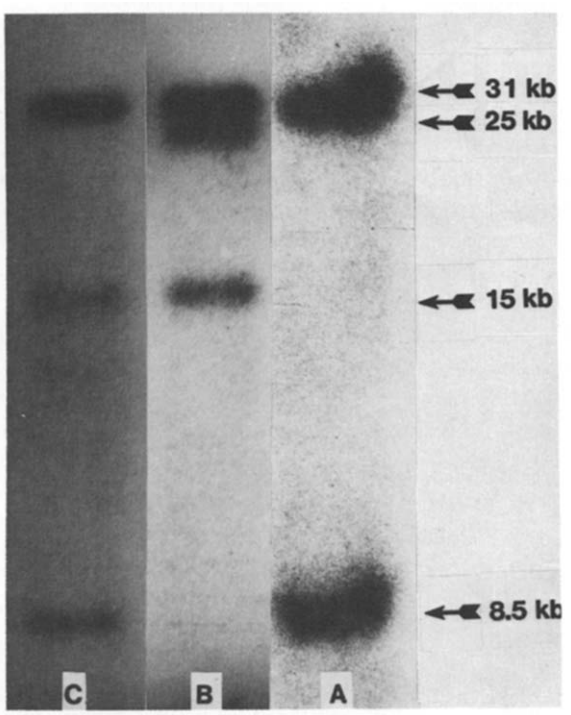

Fig.1. Patterns A, B and C observed after *Hin*dIII digestion and hybridization with the C4 pAT-A probe.

2.4. *Probes*

The C4 cDNA probe is clone A, an almost full-length C4B gene, 4.8 kb long [3]. The 21-OH probe is pC21/3c, a cDNA probe of approx. 2 kb which codes for all but a few hundred nucleotides at the 5′-end of the human 21-OH gene [11].

3. RESULTS

26 families selected for the segregation of a C4A*Q0 allele were studied. Among the in-

Table 1

Extended HLA haplotypes (HLA-A, C, B, C2, Bf, C4A, C4B, DR) with a C4A null (C4A*Q0) allele examined by Southern blot with the C4 and 21-OH probes

Extended haplotypes with deleted C4A + 21-OHA genes						
A1	Cw7	B8	n.d.	BfS	C4AQ0	B1 DR3
A2	Cw7	B8	C2C	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	C2C	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	C2C	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	C2C	BfS	C4AQ0	B1 DR3
A2	Cw7	B8	n.d.	BfS	C4AQ0	B1 DR3
Aw32	Cw7	B8	C2C	BfS	C4AQ0	B1 DR7
A1	Cw7	B8	n.d.	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	C2C	BfS	C4AQ0	B1 DR3
A2	Cw7	B8	C2C	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	C2C	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	n.d.	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	n.d.	BfS	C4AQ0	B1 DR3
A1	Cw7	B8	n.d.	BfS	C4AQ0	B1 DR3
Extended haplotypes with non-deleted C4A + 21-OH genes						
Aw30	Cw6	B14	C2C	BfS	C4AQ0	B1 DR7
A2	Cw3	Bw60	C2C	BfS	C4AQ0	B2 DRw6
Aw30	Cw3	Bw60	C2C	BfS	C4AQ0	B2 DR3
A2	n.i.	B14	C2C	BfS	C4AQ0	B1 DR7
A3	Cw7	B7	C2C	BfS	C4AQ0	B1 DR2
A3	Cw3	Bw62	C2C	BfS	C4AQ0	B1 DR5
Aw30	Cw6	B13	C2C	BfS	C4AQ0	B1 DR7
A2	Cw5	Bw44	C2C	BfS	C4AQ0	B1 DR4
A29	Cw1	Bw22	C2C	BfS	C4AQ0	B1 DR5
Aw33	Cw7	Bw44	C2C	BfS	C4AQ0	B2 n.i.
Aw30	Cw3	Bw60	C2C	BfS	C4AQ0	B2 DR4
A3	Cw4	B17	C2C	BfF	C4AQ0	B1 DRw6

n.d., not done; n.i., not informative

dividuals having a C4A*Q0 allele, 52 were heterozygous and 3 were homozygous. After *Hin*dIII digestion and Southern blot analysis using the C4 cDNA probe, three different patterns were identified:

Pattern A was composed of two bands of 31 and 8.5 kb, respectively.

Pattern B associated one band of 31 kb, sometimes duplicated with an additional band of 25 kb, and a band of 15 kb.

Pattern C corresponded to a heterozygous state with three fragments of 31 kb (also sometimes duplicated), 15 and 8.5 kb (fig.1).

The correlation between the patterns and the extended haplotypes showed that the pattern A was characteristic of the three individuals homozygous for the C4A*Q0 allele and also for the HLA-A1 Cw7 B8 C2C BfS C4AQ0B1 DR3 haplotype.

Pattern C was observed in eight families where the HLA-A1 Cw7 B8 BfS C4AQ0B1 DR3 haplotype was segregating. It was also correlated with the HLA-A2 Cw7 B8 BfS C2C C4AQ0B1 DR3 haplotype in three families. In one family, the extended haplotype associated with the pattern C was Aw32 Cw7 B8 C2C BfS C4AQ0B1 DR7.

Pattern B was observed in the family members not bearing a C4A*Q0 allele and who could be considered as controls. It was also present in the individuals carrying a C4A*Q0 allele occurring within various extended haplotypes as shown in table 1.

RFLP analysis with the 21-OH probe was done on *Hin*dIII digests of the DNA extracted from individuals showing pattern A or C with the C4 cDNA probe. The pattern obtained showed only the fragment of 31 kb duplicated with a second band of 25 kb. The 8.5 kb fragment was not recognized with this probe. The 15 kb fragment corresponding to the 5'-end of the C4 gene was also not recognized.

4. DISCUSSION

Pattern B is common to people expressing four C4 genes and is also observed in some individuals having a C4A*Q0 allele. This pattern results from the presence of *Hin*dIII restriction sites inside and outside the C4 genes (fig.2). All the restriction fragments are recognized with the full-length C4 cDNA probe.

Pattern A shows a 8.5 kb fragment which corresponds to a deletion taking away the restriction site for *Hin*dIII normally present in the C4A gene. This deletion of approx. 30 kb also includes the 21-OHA gene as the 8.5 kb fragment is not recognized by the 21-OH probe [1,12].

Pattern C corresponds to the heterozygous state with one haplotype HLA-A1 Cw7 C2C B8 BfS C4AQ0B1 DR3 carrying the deletion and a normal haplotype with two expressed C4 genes.

A polymorphism of the C4B gene size is also detected on the same blots. It is responsible for the

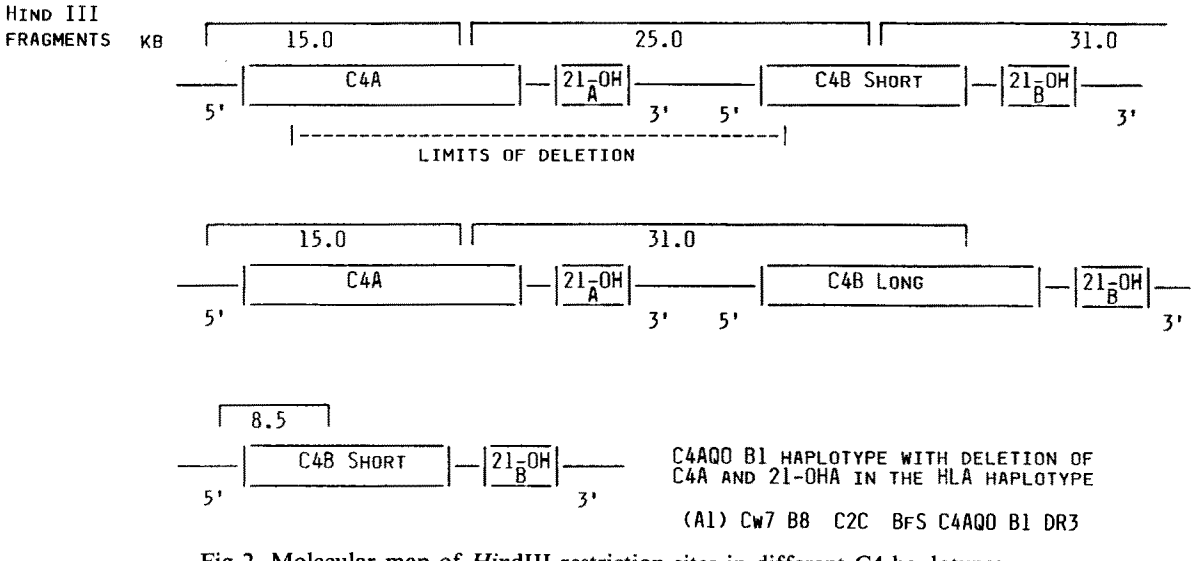

Fig.2. Molecular map of *Hin*dIII restriction sites in different C4 haplotypes.

duplication of the large fragment (31 kb) sometimes evident. The 3′-end of the C4B gene is located in a 31 kb fragment. The fragment containing the 3′-end of a C4A and the 5′-end of a C4B may have two possible sizes: 25 kb for a short C4B gene, 31 kb for a long C4B gene (fig.2). It was previously shown that the size difference in the C4B gene is due to an insertion of about 6 kb near the 5′-end [13].

The screening of a large number of haplotypes with a C4A null allele shows clearly that the molecular basis for this null allele can be different. In this large series, the deleted C4A and 21-OHA genes were only found in the extended HLA-Cw7 B8 DR3 C2C BfS C4AQ0 B1 DR3 haplotype. This haplotype is common in patients with autoimmune diseases but may be found, as in our study, in healthy individuals although it has been shown that these individuals have differences in their immune responses predisposing to the development of autoimmune disorders [14]. Whether the deletion of the C4A and 21-OHA genes plays a role in the occurrence of immune response abnormalities remains however to be demonstrated.

ACKNOWLEDGEMENT

This work was supported by a grant from the INSERM (CRE 843005) to G.H.

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