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Class II HLA-DC β-chain DNA restriction fragments differentiate among *HLA-DR2* individuals in insulin-dependent diabetes and multiple sclerosis

(histocompatibility antigens/restriction fragment length polymorphism/multigene family/gene conversion)

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ABSTRACT ***HLA-DR2* allele is negatively associated with insulin-dependent diabetes and positively associated with multiple sclerosis (MS). A 2.2-kilobase-pair *Eco*RI DNA restriction fragment detected with a β-chain HLA-DC cDNA probe was found to be strongly correlated with *HLA-DR2* in the normal population, but was absent in *HLA-DR2* insulin-dependent diabetic patients. This fragment was found in *HLA-DR2* multiple sclerosis patients with the same frequency as in controls. A β-chain HLA-DC 12-kilobase-pair *Bam*HI fragment might differentiate multiple sclerosis patients from healthy individuals.**

The major histocompatibility complex codes for at least three classes of products involved in the immune response (class I, class II, and class III) (1). Class II products are membrane glycoproteins composed of two chains, α and β. The class II products can be divided into three subgroups according to their primary structure and the mapping of their genes: HLA-DR, HLA-DC, and HLA-SB. The genes of these proteins, *HLA-DR*, *HLA-DC*, and *HLA-SB*, are highly polymorphic. Most of the alleles at these loci have been immunologically defined by serological or cellular methods. This polymorphism also has been defined recently with restriction endonuclease fragments detected by probes specific for α- or β-chain genes (2–6).

The association between *HLA* alleles and disease susceptibility remains one of the most intriguing discoveries of modern genetics (7). Associations reported have been mainly partial ones. *HLA-DR2* raises a particular problem because this allele has been shown to be negatively associated with insulin-dependent diabetes (IDD) and positively associated with multiple sclerosis (MS) (8, 9). Susceptibility to MS and resistance to IDD could depend on the same genes being found more often, or more rarely, in *HLA-DR2* haplotypes. Alternatively, different genes can be responsible for the two characteristics. In order to favor one of these two possibilities, an analysis of class II β-chain restriction fragment-length polymorphism was performed on the DNA of *HLA*-typed IDD patients, MS patients, and healthy individuals.

MATERIAL AND METHODS

Techniques for extraction and restriction endonuclease digestion of human cellular DNA and for electrophoresis, transfer, and hybridization of restriction endonuclease fragments have been described (10). After hybridization, the membranes were washed four times (45 min each) at 60°C with 2 × NaCl/Cit (1× NaCl/Cit = 0.15 M NaCl/0.015 M sodium citrate, pH 7), once with 0.2× NaCl/Cit (45 min), and finally once with 0.1× NaCl/Cit (45 min). The restriction endonucleases *Eco*RI and *Bam*HI (obtained from Boehringer Mannheim and from Amersham) were used according to manufacturers' recommendations. The probe in these studies was the cDNA clone containing most of the coding sequence for an *HLA-DC* gene provided by Larhammar *et al.* (11).

RESULTS AND DISCUSSION

A 2.2-Kilobase-Pair (kb) *Eco*RI Fragment Differentiates Between *HLA-DR2* Healthy Individuals and *HLA-DR2* IDD Patients. The DNA of randomly selected and *HLA-DR2*-selected healthy individuals or IDD patients were digested by *Eco*RI. After electrophoresis and transfer, the restriction fragments were probed with a full-length β-chain HLA-DC (β DC) cDNA. A 2.2-kb fragment was noted among the 15–20 bands detected.

Thirty-four healthy individuals were tested: 22 of these were randomly selected, 5 were selected for their *HLA-DR2* homozygosity, and 7 were HLA-DR-matched with the IDD patients (Fig. 1). The 2.2-kb fragment was found in all *HLA-DR2* individuals except 1 (DR2,1). This fragment was also found in 2 non-*HLA-DR2* individuals (DR3,−; DR4,−). In contrast, however, it was absent in all *HLA-DR2* IDD patients but present in 1 DR4,− patient (Fig. 1). Thus, in the normal population, a strong correlation ($P = 0.001$) exists between this fragment and *HLA-DR2*, which is not found in *HLA-DR2* IDD patients ($P = 0.0003$). In non-*HLA-DR2* individuals, the frequency of the 2.2-kb fragment did not differ significantly in these small samples (2 out of 18 in the controls and 1 out of 20 in the IDD patients). Thus, the presence of the *Eco*RI 2.2-kb fragment noted in 1 (non-*HLA-DR2*) patient indicates that the polymorphic sequence (or sequences) that determines this fragment is not solely responsible for the protection.

A β DC cDNA Probe Detects a Fragment that Correlates with an HLA-DR Specificity. Such correlations have already been reported (4, 6). They raise several problems.

(*i*) The structural homology between *HLA-DR*, *HLA-DC* (12), and even *HLA-SB* (13) β-chain genes probably allows a cross-hybridization under relaxed conditions of hybridization and washing. We already have noted that this quasi-full-length β DC cDNA probe detects an increasing number of

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Abbreviations: kb, kilobase pair(s); IDD, insulin-dependent diabetes; MS, multiple sclerosis; β DC, HLA-DC β-chain (fragment or cDNA probe); *DC 2.2*, gene that carries the β-chain HLA-DC *Eco*RI 2.2-kb fragment; *DC 12*, gene that carries *Bam*HI 12-kb fragment.

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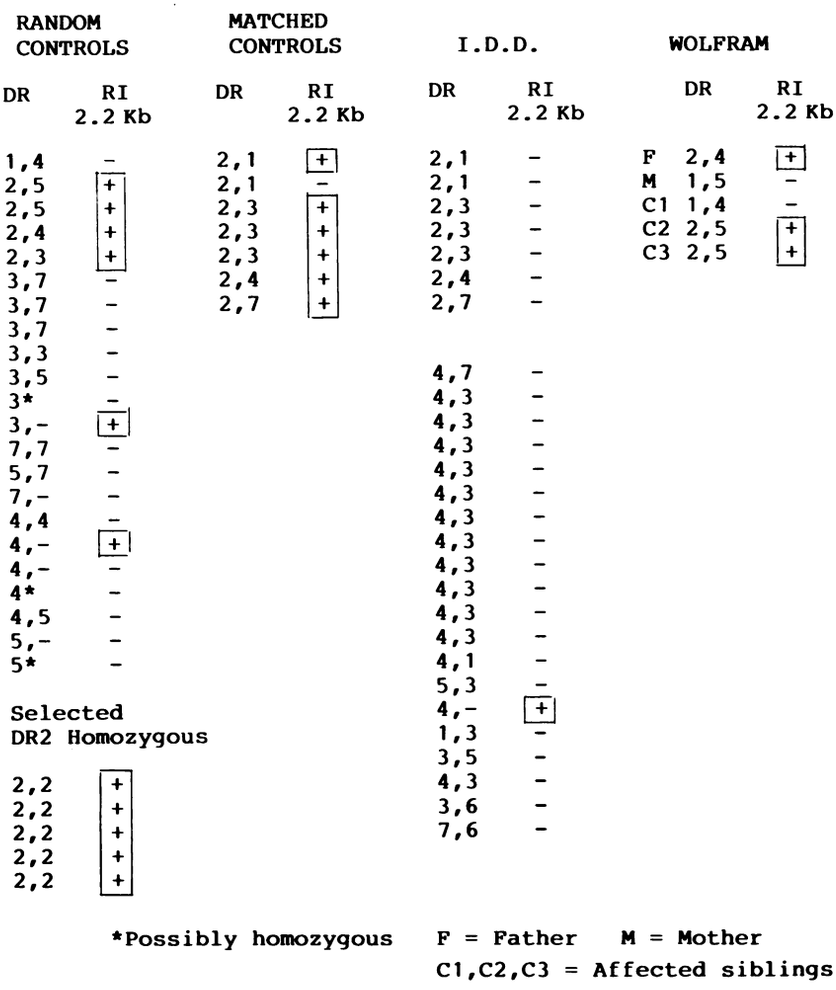

FIG. 1. The presence (+) or absence (−) of the *Eco*RI 2.2-kb β DC restriction fragment in randomly selected controls, in *HLA-DR2* homozygous healthy individuals, in *HLA-DR2* IDD patients and *HLA-DR*-matched controls, in randomly selected non-*HLA-DR2* patients, and in one family of Wolfram syndrome.

bands (from 7 or 8 to about 15 per individual) when the salt concentration in the washing solution is varied from 0.1× to 0.2× NaCl/Cit. Thus, it is quite possible that some of the bands correspond to *HLA-DR* genes.

(*ii*) The 2.2-kb *Eco*RI fragment remains even under highly stringent conditions of washing (Fig. 2) and gives a strong signal, indicating that it could be part of an *HLA-DC* gene; in this case, the *Eco*RI 2.2-kb fragment would not be detected with a full-length β DR cDNA probe. However, the divergence between β-chain genes at the *HLA-DR* and *-DC* loci might depend on the haplotype in question. We cannot eliminate the possibility that in *HLA-DR2* haplotypes there is more homology between *HLA-DC* and *-DR* β-chain genes than in other haplotypes. A gene conversion mechanism (14, 15) could lead to such variability in the divergence between genes at these two loci. In other words, the human class II β-chain genes could constitute a kind of "mosaic" in which strict locus specificity does not exist (*HLA-DR* or *-DC* series would contain *HLA-DC-* or *-DR*-like alleles). Nevertheless, we propose the name *β DC Eco*RI 2.2 kb (in short, *DC 2.2*) for the gene(s) that carries the *Eco*RI 2.2-kb fragment.

(*iii*) If *DC 2.2* belongs to the *HLA-DC* locus and not to the *HLA-DR* locus, the correlation with *HLA-DR2* remains to be explained by classic linkage disequilibrium.

What Role Does *DC 2.2* Play in the Protection Against IDD? Because *HLA-DR2* is found only rarely in IDD patients, it has been proposed that one or several resistance genes are located more often in *HLA-DR2* haplotypes. Alternatively, one or several susceptibility genes, relatively absent from *HLA-DR2* haplotypes, might also explain the decrease of *HLA-DR2* in IDD patients. Our findings indicate that resistance genes (or susceptibility genes) are more often present (or absent) in haplotypes carrying *both DC 2.2* and *HLA-DR2*.

Several hypotheses can be advanced if *HLA-DR2* and *DC 2.2* are assumed to be in linkage disequilibrium rather than belonging to the same locus: (*i*) a subtype of *HLA-DR2* or a subtype of *DC 2.2* confers resistance; (*ii*) haplotypes carrying both *DC 2.2* and *HLA-DR2* are in positive linkage disequilibrium with a resistance gene(s); and (*iii*) they are in negative linkage disequilibrium with a susceptibility gene(s). These hypotheses *i–iii* are not mutually exclusive, nor do they exclude the following one: (*iv*) *DC 2.2* or a gene in linkage disequilibrium and *HLA-DR2* or a gene in linkage disequilibrium act synergistically in the protection.

As we cannot eliminate the possibility that *DC 2.2* and *HLA-DR2* are located at the same locus, it is also possible that protection could be due either to a single gene carrying the *Eco*RI 2.2-kb fragment and coding for HLA-DR2 antigenic determinants or to the relative absence of a susceptibility gene(s) from haplotypes carrying such a gene.

Interestingly, Bach *et al.* (16) reported that none of the five *HLA-DR2* patients tested were found to be *Dw2* or *Dw12*, the most common *Dw* haplotypes associated with *HLA-DR2*. Thus *DC 2.2* should be a good marker of haplotypes coding for Dw2 or Dw12 specificities. Similarly, the HLA-DR2 antigen has been serologically divided into subtypes (17–20).

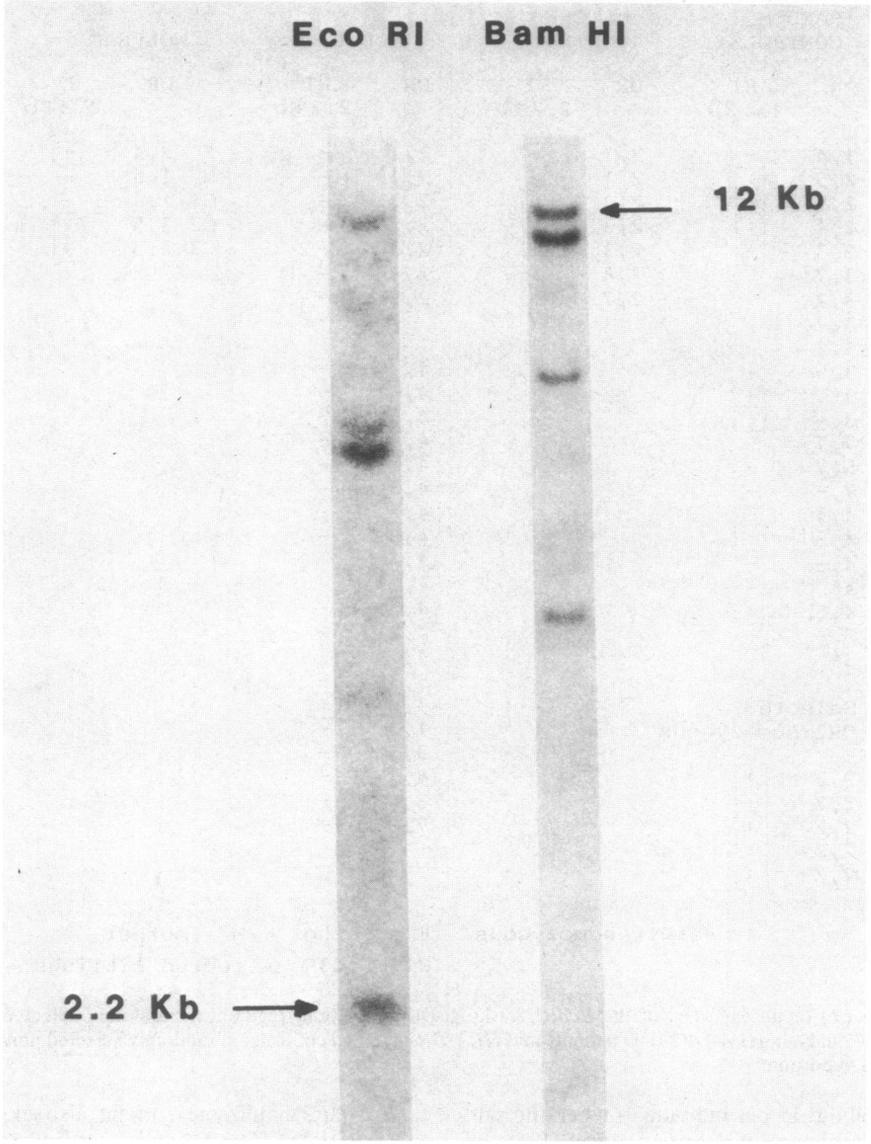

FIG. 2. *Eco*RI and *Bam*HI restriction fragment patterns detected under stringent conditions of washing (0.1 × NaCl/Cit at 60°C for 45 min).

Obviously, a correlation will have to be worked out between these two observations and our own. It is quite possible that all three methods could define the same haplotypes.

***DC 2.2* Was Tested in the Wolfram Syndrome and in MS.** The Wolfram syndrome is characterized by the occurrence of IDD and optic atrophy; a positive association was noted with *HLA-DR2* but in a rather small sample (21, 22). A family with three affected children of *HLA* genotypes *DR2,5*, *DR2,5*, and *DR1,4* was tested for *DC 2.2* (Fig. 1). The *Eco*RI 2.2-kb fragment was found to segregate with the *HLA* haplotype carrying *DR2*. Although these results will require to be confirmed in a larger study, they suggest that this disease should be considered as a specific entity.

Twenty-four randomly selected MS patients were tested for *DC 2.2* (Fig. 3); in this sample *HLA-DR2* was found to be significantly increased, from 26% in controls to 62% in patients ($P = 0.0004$). *DC 2.2* was noted in 14 of the 15 *HLA-DR2* patients and in 2 non-*HLA-DR2* patients (DR5,− and DR7,−). Thus, *DC 2.2* defines two phenotypes in *HLA-DR2* individuals: DR2(+), DC 2.2(+) and DR2(+), DC 2.2(−). The relative proportions of these two phenotypes are the same in *HLA-DR*-matched, healthy individuals (7:1) and MS patients (7:1) (Fig. 3). However, this ratio differs greatly in *HLA-DR*-matched healthy individuals (6:1) and IDD patients (0:7) ($P = 0.002$). These comparisons suggest that susceptibility to MS and resistance to IDD might not depend on the same haplotypes in *HLA-DR2* individuals. This hypothesis will have to be confirmed by larger studies, to show that the phenotype DR2(+), DC 2.2(−) is significantly more frequent in MS but not different in IDD.

A 12-kb *Bam*HI Fragment Might Differentiate MS Patients from Healthy Individuals. The DNA of 24 randomly selected MS patients was digested with *Bam*HI in parallel with 24 healthy individuals matched for HLA-DR types and with another 45 healthy individuals randomly selected. A 12-kb *Bam*HI fragment was noted, which gave a strong signal even under stringent conditions. Thus, we propose the name *DC12* for the gene(s) that carries this 12-kb fragment, the presence of which correlates positively with *HLA-DR4* in healthy individuals ($P = 0.0002$).

DC12 was found more frequently in MS patients (33%) than in randomly selected controls (24%) or *HLA-DR*-matched controls (16%); but in these small samples, the differences are not statistically significant. However, *DC12* defines various phenotypes when combined with various HLA-DR specificities. The phenotype DR2(+), DC12(+) is significantly more frequent in MS patients (17%) than in randomly selected controls (2%) ($P = 0.03$). Thus, the disease

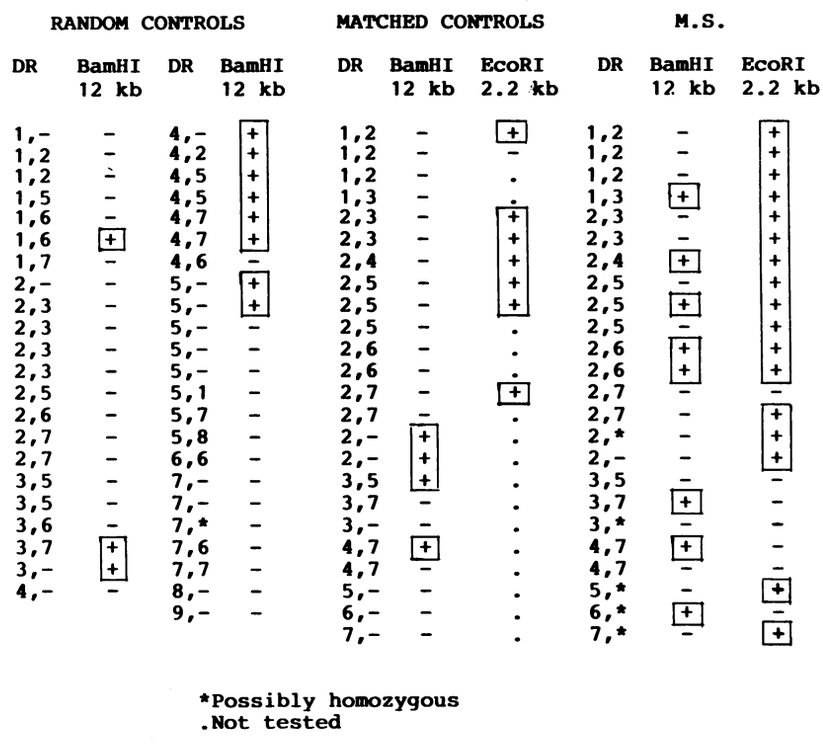

FIG. 3. The presence (+) or absence (−) of the *Bam*HI 12-kb β DC restriction fragment in randomly selected controls, in HLA-DR-matched healthy individuals, and in MS patients. The presence (+) or absence (−) of the *Eco*RI 2.2-kb β DC fragment is also indicated in MS patients and some HLA-DR-matched controls.

risk for individuals possessing the phenotype DR2(+), DC12(+) is 8.8, whereas a smaller relative risk (4.5) is found in this sample for DR2 alone (ignoring DC12). The same finding is noted for a phenotype combining DC12 and a DC1-like group of HLA-DR specificities: DR1, DR2, and DRw6. (The DC1 antigenic determinant(s) are most often found in haplotypes carrying *DR1* or *DR2* or *DRw6*). The phenotype DC1-like(+), DC12(+) is significantly more frequent in MS patients (25%) than in randomly selected controls (4%) (P = 0.01). The disease risk in this case is 7.1, significantly greater than for DC1-like alone when DC12 is ignored (2.5), not significantly different from 1.0.

The DC1-like risk was further examined by dividing cases and controls into two groups according to the presence or absence of *DC12*. For the group in which the fragment is present, the relative risk for DC1-like individuals is 12.5 (P = 0.02 : Fisher's exact test), whereas in the other group, the DC1-like risk is 1.3 (not significantly different from 1.0). Hence, the DC1-like phenotypes are at greater risk only when *DC12* is present. This increased risk may be due to linkage disequilibrium in the haplotypes carrying a *DC1*-like allele, *DC12*, and a susceptibility gene. However, because the haplotypes are unknown in the patients, we cannot rule out the alternative that the *DC1*-like allele and *DC12* increase the risk also when they are on different haplotypes.

It is noteworthy that *DC12* is present in all three *HLA-DRw6* patients, while it is found in only one of the nine *HLA-DRw6* controls (randomly selected or matched) (P = 0.02 : Fisher's exact test). This last finding alone might explain the increased risk of the phenotype DC1-like(+), DC12(+). It could also explain the increased risk of the phenotype DR2(+), DC12(+) because two of the three *HLA-DRw6* patients in this small sample are also *HLA-DR2* (Fig. 3).

These results, however, must be interpreted cautiously in view of the small size of the sample and the problem of *HLA-DRw6* typing, which remains uncertain. Gene typing using restriction fragment length polymorphism will provide new markers that will lead to a better definition of the polymorphism of the human major histocompatibility complex.

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