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DRB Genotyping Supports Recessive Inheritance of DR3-associated Susceptibility to Insulin-dependent Diabetes Mellitus

David Jenkins,* Jeremy Fletcher,* Michelle A. Penny,* Catherine H. Mijovic,* Karen H. Jacobs,* Arthur R. Bradwell,† and Anthony H. Barnett*

Departments of *Medicine and †Immunology, University of Birmingham and East Birmingham Hospital, Birmingham

Summary

The mode of inheritance of HLA-associated susceptibility to insulin-dependent diabetes mellitus was investigated by the antigen genotype frequency among patients method in a white Caucasian population and a North Indian Asian population. DR genotypes were determined by DRB/DQB RFLP analysis. In white Caucasians, simple recessive and simple additive inheritance of a single HLA-associated disease susceptibility allele were rejected ($P < .025$ and $P < 10^{-6}$, respectively). The data were compatible with a three-allele model of disease susceptibility. In North Indian Asians, simple additive inheritance was rejected ($P < 10^{-6}$). The observed genotype frequencies were compatible with a single DR3-associated disease susceptibility allele which is inherited recessively. These data show that study of DR genotypes in populations of different ethnic origins may further the understanding of inherited susceptibility to insulin-dependent diabetes mellitus.

Introduction

Insulin-dependent diabetes mellitus (IDDM) in white Caucasian subjects is strongly associated with HLA-DR3 and HLA-DR4. The DRB1 alleles which define DR3 and DR4 are in linkage disequilibrium with one or more alleles which determine susceptibility to IDDM directly (Jenkins et al. 1990). The mode of inheritance (dominant, intermediate, or recessive) has proved difficult to determine, partly owing to environmental determinants (the susceptibility genes are incompletely penetrant), and also owing to uncertainty concerning the number of disease susceptibility loci (Wassmuth and Lernmark 1989).

Thomson (1983) has devised a method of analyzing inheritance of HLA-linked susceptibility to IDDM from the frequencies of HLA markers in diabetic populations. The antigen genotype frequency among patients (AGFAP) method has excluded both simple additive and simple recessive models of inheritance of a single HLA gene which determines predisposition to IDDM. The data were compatible with a DR4-associated allele inherited additively in the absence of DR3 and with a distinct DR3-associated allele inherited recessively in the absence of DR4 (Thomson et al. 1988).

Louis and Thomson (1986) have indicated that the accuracy of DR genotyping may affect the results obtained by this method. Most studies have used serological DR-typing, which is limited by the reagents available. Subjects who type as DR4 only may be heterozygous for DR4 and a "blank" specificity which is not recognized by available antisera. The subject is designated a DR4 homozygote unless family members are typed. The number of homozygotes ascertained by serology, therefore, may be erroneously high. The AGFAP method can be modified to allow for blanks, but complex calculations are required (Louis and Thomson 1986).

RFLP analysis of the DRB1 gene is an alternative method of determining DR genotypes (Cox et al. 1988). RFLP analysis allows unambiguous genotyping without the need for family studies.

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Address for correspondence and reprints: Dr. David Jenkins, Department of Medicine, Queen Elizabeth Hospital, Edgbaston, Birmingham B15 2TH, England.

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IDDM occurs in ethnically distinct populations and is assumed to be identical to the disease in white Caucasians (Jenkins et al. 1990). Analysis of susceptibility to IDDM may be simplified by investigating populations in which one disease marker is infrequent. In North Indian Asians (of Aryan descent), DR4, although positively associated with IDDM, occurs less commonly than in white Caucasian populations, and is less strongly associated with IDDM than DR3 (Bhatia et al. 1985; Odugbesan et al. 1987; Fletcher et al. 1988*a*). This is in contrast with genetically distinct South Indians (of Dravidian descent), in whom DR3 and DR4 occur equally commonly among patients with IDDM (Serjeantson et al. 1987). This group, therefore, allows examination of DR3-associated susceptibility to IDDM with little confounding influence of DR4.

In this study DR genotypes were determined by RFLP analysis in two populations. The AGFAP method was used to test the hypothesis that susceptibility to IDDM that is associated with the major histocompatibility complex (MHC) is transmitted by a single gene. A white Caucasian population was studied to compare the data obtained with those produced by serological methods. A North Indian Asian population was studied to examine inheritance of DR3-associated susceptibility, with less influence from DR4 than in white Caucasians.

Methods

Subjects

Two hundred and four unrelated white Caucasian subjects of European ancestry with IDDM and 106 unrelated, racially-matched, healthy control subjects were recruited. Forty-four North Indian (Punjabi) subjects with IDDM and 93 racially matched control subjects were also studied. All subjects were resident in the United Kingdom. Subjects with IDDM were diagnosed at less than 30 years of age, ketosis prone, and continuously dependent on insulin from the time of diagnosis. Control subjects had neither personal nor family history of diabetes.

Determination of DR Genotypes

DRB and DQB gene RFLP analysis of genomic DNA from peripheral blood lymphocytes was performed. DNA (7.5 μg) was digested with *Taq*I and *Bam*HI restriction enzymes under conditions recommended by the manufacturer (BRL, Glasgow). Digested DNA fragments were separated by electrophoresis in 0.7% agarose at 50 V, 25 mA for 18–22 h. The fragments were blotted onto nylon filters (Hybond-N, Amersham). *Taq*I fragments were hybridized with radiolabeled cDNA consisting of the 500-bp *Pst*I fragment of pII-β-4 (corresponding to the second domain, transmembrane, cytoplasmic and 3′ untranslated portion of the DRB1 gene). *Bam*HI fragments were hybridized with radiolabeled cDNA consisting of the *Hin*dIII/*Pst*I insert of pII-β-I (full-length DQB1 gene). Both probes were provided by P. A. Peterson of Uppsala. Probes were labeled to a specific activity of 10^9 counts/min/μg DNA by the oligonucleotide primer method. Prehybridization and hybridization were performed at 65°C in 6 × standard sodium citrate solution (SSC; 1 × SSC = 0.15M NaCl, 0.015M Na citrate), 5 × Denhardt's solution, and 0.5% SDS with 250 μg/ml denatured salmon sperm DNA. Ten percent dextran sulphate was used in the hybridization solution. After hybridization, filters were first washed in 2 × SSC, 0.1 SDS, then washed in the same solution at 65°C for 30 min. The filters were then washed in 0.5 × SSC, 0.1% SDS for 60 min, followed by four 15-min washes in 0.1 × SSC, 0.1% SDS, all at 65°C. The filters were then dried in air and autoradiographed for 72 h at −70°C.

DRB genotypes were determined from the DRB RFLPs from associations described in Fletcher et al. (1988*b*). Haplotypes were classified as DR3, DR4, or DRX, where X is any haplotype apart from 3 and 4. DR3 haplotypes were recognized by a 12-kb + 7-kb + 4-kb or a 10-kb + 7-kb + 4-kb *Taq*I DRB RFLP in combination with a 7-kb + 4-kb *Bam*HI DQB RFLP (Fletcher et al. 1988*a*). DR4 haplotypes were recognized as a 16-kb + 7-kb + 5.5-kb + 2.5-kb or a 16-kb + 6-kb + 5.5-kb + 2.5-kb *Taq*I RFLP. Any other RFLP was classified as X. Genotypes were then classified as 3/3, 3/4, 4/4, 3/X, 4/X, and X/X.

In the North Indian population, DR haplotypes were also classified as DR3 and DRY, where Y is any haplotype other than DR3. Genotypes were then classified as 3/3, 3/Y, and Y/Y.

Analysis of Mode of Inheritance

The AGFAP method (Thomson 1983) uses a simple two-locus disease association model where the DR3 allele and the DR4 allele of the DRB1 gene are linked to the same susceptibility locus for IDDM. The expected frequencies of the genotype classes produced by such a model under recessive and additive inheritance were calculated from the observed genotype frequencies as described in Thomson (1983). The frequencies

expected under dominant inheritance (where homozygotes and heterozygotes are equally susceptible) are very similar to the frequencies expected under additive inheritance (where the heterozygote has intermediate susceptibility) when the frequency of the disease allele is small. The frequencies expected under additive inheritance were calculated with the AGFAPK computer package. The observed frequencies were compared with those expected using the χ^2 test.

For the North Indian population, the AGFAP method was also used to test a two-locus association model where inherited susceptibility to IDDM is determined by a single allele linked to DR3. The expected genotype frequencies produced by this model under recessive and additive inheritance were compared with those observed using the χ^2 test.

Results

White Caucasians

The observed frequencies of the different genotype classes among the white Caucasian diabetic subjects are shown in table 1. The frequencies of the DR3 and DR4 RFLPs in the control population were .139 and .204, respectively. The genotype frequencies expected under additive and recessive inheritance of a single susceptibility allele linked to both DR3 and DR4 are shown in table 1. The frequencies observed were significantly different from those expected under additive inheritance ($P < 10^{-6}$). The largest discrepancies occurred in the 3/4 class, where more were observed than expected (76 vs. 33.6), and in the 3/X class, where fewer were observed than expected (32 vs. 80.5).

Table 1

Observed and Expected Frequencies of DRB Genotypes in 204 White Caucasian Subjects with IDDM

Genotype	Observed	Expected (Additive)	Expected (Recessive)
3/3.....	33	16.5	37.1
3/4.....	76	33.6	59.7
4/4.....	15	13.8	24.0
3/X.....	32	80.5	40.1
4/X.....	34	48.1	32.2
X/X.....	14	11.5	10.8

NOTE.—χ^2 (additive) = 104, 3 df, $P < 10^{-6}$; χ^2 (recessive) = 10.9, 3 df, $P < .025$. X denotes any haplotype other than DR3 or DR4.

Table 2

Observed and Expected Frequencies of DRB Genotypes in 44 North Indian Asian Subjects with IDDM

Genotype	Observed	Expected (Additive)	Expected (Recessive)
3/3.....	16	5.1	15.4
3/4.....	9	2.8	7.7
4/4.....	0	.5	1.0
3/X.....	11	25.7	13.5
4/X.....	4	6.6	3.4
X/X.....	4	3.5	2.9

NOTE.—χ^2 (additive) = 38.4, 1 df, $P < 10^{-6}$; χ^2 (recessive) = 0.8, 1 df, not significant. Expected classes were combined if less than 5. X denotes any haplotype other than DR3 or DR4.

The frequencies observed were significantly different from those expected under recessive inheritance ($P < .025$). The largest discrepancies occurred in the 3/4 class, where more were observed than expected (76 vs. 59.7), and in the 4/4 class, where fewer were observed than expected (15 vs. 24).

North Indian Asians

The observed frequencies of the different genotype classes among the group of North Indian diabetic subjects are shown in table 2. The frequencies of the DR3 and DR4 DRB RFLPs in the control population were .153 and .048, respectively. The expected frequencies of the different genotype classes occurring under recessive and additive inheritance are also shown in table 2. The frequencies predicted by additive inheritance were significantly different from those observed ($P < 10^{-6}$). The largest discrepancies occurred in the 3/4 class, where more were observed than expected (9 vs. 2.8), and in the 3/X class, where fewer were observed than expected (11 vs. 25.7). The frequencies predicted by recessive inheritance were not significantly different from those observed.

DR3-associated susceptibility to IDDM in the North Indian population was investigated further by classifying the DRB genotypes as 3/3, 3/Y, and Y/Y. The numbers of diabetic patients observed, and the numbers expected under simple additive and simple recessive inheritance, are shown in table 3. The frequencies predicted by additive inheritance were significantly different from those observed, ($P < 10^{-6}$). The frequencies predicted under recessive inheritance were not significantly different from those observed.

Table 3

Observed and Expected Frequencies of DRB Genotypes in 44 North Indian Subjects with IDDM

Genotype	Observed	Expected (Additive)	Expected (Recessive)
3/3.....	16	5.7	15.4
3/Y.....	20	30.9	21.3
Y/Y.....	8	7.5	7.4

NOTE.—χ^2 (additive) = 33.3, 1 df, $P < 10^{-6}$; χ^2 (recessive) = 0.16, 1 df, not significant. Y denotes any haplotype other than DR3.

Discussion

In this study, DR genotypes were defined by DRB and DQB RFLP analysis. All class II alleles produce a restriction pattern, and blanks do not occur. Homozygosity for DR types, therefore, can be determined without data from the family of the subject, thus allowing unambiguous genotyping without the need for family studies. This study has demonstrated how RFLP analysis can be used to investigate the inheritance of MHC-associated disorders.

The DR4 *Taq*I DRB RFLP allows unambiguous definition of the diabetes-associated DR4 haplotype in white Caucasians and North Indians (Fletcher et al. 1988*b*). The DR3-associated DRB RFLPs are also associated with DRw6 in these races (Cox et al. 1988; Fletcher et al. 1988*b*). DQB RFLP analysis, however, allows distinction of DR3 and DRw6 haplotypes (Bidwell et al. 1987; Fletcher et al. 1988*b*). The 7-kb + 4-kb *Bam*HI DQB RFLP is specific to the DR3 haplotype in both white Caucasian and North Indian subjects (Fletcher et al. 1988*a*), allowing it to be defined unambiguously.

Various approaches have been used to investigate the inheritance of IDDM. A family study found DR4 to be transmitted from diabetic parents to diabetic offspring more frequently than from nondiabetic parents, indicating that the DR4-associated factor is inherited dominantly. The converse was true of transmission of DR3, suggesting that the DR3-associated factor is inherited recessively (MacDonald et al. 1986). These data support the existence of at least two disease susceptibility alleles which have distinct modes of inheritance (Risch 1984; Spielman et al. 1989).

Parametric analysis of population data has excluded simple recessive inheritance of susceptibility to IDDM (Rotter et al. 1983). Estimates of gene admixture between white Caucasian and American black populations suggested dominant inheritance (MacDonald 1980). Rotter and Hodge (1980), however, have shown that the data were also compatible with a three-allele model of diabetes susceptibility.

In this study the data from white Caucasian subjects rule out the hypothesis that susceptibility to IDDM is inherited either additively or recessively by a single allele linked to both DR3 and DR4. The frequency of DR3/DR3 homozygotes was approximately 16%, greater than observed in other studies (approximately 7%; Thomson et al. 1988). This difference may be due to ethnic variation between the British white Caucasians analyzed in this study and the non-British subjects analyzed by other workers. An alternative possibility is that the DR3-related RFLPs of some apparent DR3/DR3 homozygotes may be shared by other DR genotypes. Comparison of DR genotypes obtained by serological typing and DRB/DQB RFLP analysis, however, has shown a close correlation between the two methods in both white Caucasian and North Indian subjects (Fletcher et al. 1988*a*; J. Fletcher, unpublished data).

The observed excess of DR3/DR4 heterozygotes is consistent with the possibility that the DR3- and DR4-associated susceptibility alleles are distinct and have a synergistic effect on disease predisposition. The observed frequencies of the other DRB genotypes also deviate from those predicted by simple recessive inheritance. Using the model of Louis and Thomson (1986), the observed deviations are compatible with a three-allele model in which a DR3-linked allele acts recessively in the absence of DR4, and a DR4-linked allele acts additively in the absence of DR3. These findings concur with data produced by serological DR typing (Thomson et al. 1988), indicating that the different methods of genotyping produce similar results.

In North Indian subjects, additive inheritance of a single susceptibility allele linked to DR3 and DR4 was also rejected. The data, however, were consistent with a single allele which is inherited recessively. This suggests either that the DR4-associated susceptibility allele in North Indians is distinct from that in white Caucasians and is identical to the DR3-associated susceptibility allele, or that the low frequency of the DR4-associated susceptibility allele has little influence on the inheritance of DR3-associated susceptibility to IDDM in the North Indian population. The mode of inheritance of the DR3-associated factor was therefore considered without DR4. Additive inheritance of the DR3-associated factor was excluded by the AGFAP

method, but the data fitted recessive expectations very closely. These data suggest, therefore, that DR3-associated susceptibility to IDDM is inherited recessively in the absence of DR4. This supports the conclusions of Louis and Thomson (1986) obtained from a three-allele model.

These data demonstrate that MHC class II RFLP analysis is a simple method of determining DR genotype status. The application of the AGFAP method to a population in which DR4 is uncommon allowed investigation of the inheritance of DR3-associated susceptibility to IDDM, without the need for a complex three-allele model. The application of this method to other populations might shed light on the inheritance of DR4-associated susceptibility. In Japanese subjects, DR3 is rare and is not associated with IDDM (Aparicio et al. 1988). Although Japanese DR4 haplotypes differ from white Caucasian DR4 haplotypes at the DRB1 locus, they are positively associated with IDDM. It would be of interest to study the inheritance of DR4-associated disease susceptibility without the influence of DR3 in this race.

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