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Investigation of the Mode of Inheritance of Insulin-dependent Diabetes Mellitus in Japanese Subjects

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Summary

Previous studies have shown that insulin-dependent diabetes mellitus is positively associated with HLA-DR4 and HLA-DR9 in Japanese populations. It was proposed that susceptibility to the disease is determined by a single HLA allele associated with both DR4 and DR9. DR genotypes in a Japanese population with insulin-dependent diabetes mellitus were determined by DRB/DQB RFLP analysis. A single disease-susceptibility-allele model was tested by the antigen-genotype-frequency-among-patients method. Recessive and additive inheritance of a single susceptibility allele were rejected. The DR9-associated disease-susceptibility allele in Japanese subjects is distinct from both the DR3- and DR4-associated susceptibility alleles in white Caucasians. The data suggest further complexity in the inheritance of HLA-associated susceptibility to insulin-dependent diabetes mellitus.

Introduction

Insulin-dependent diabetes mellitus (IDDM) is determined by both genetic and environmental factors (Todd 1990). Inherited predisposition to the disease is positively associated with alleles of HLA genes. The mode of inheritance of these alleles is obscured by their incomplete penetrance and by uncertainty concerning the number of distinct alleles which contribute to disease susceptibility (Wassmuth and Lernmark 1989). In white Caucasian subjects, family and population studies have excluded models of disease susceptibility in which a single HLA-associated allele predisposes either additively or recessively to IDDM (Svejgaard et al. 1980; Rotter et al. 1983; Thomson 1983). Louis and Thomson (1986) have tested various models in which two distinct alleles (one associated with HLA-DR3, the other with HLA-DR4) predispose to IDDM. Data from white Caucasian populations fit most closely to a model in which the DR4-associated allele is inherited additively in the absence of DR3 and in which the DR3-associated allele is inherited recessively in the absence of DR4. In DR3/4 heterozygotes the two alleles predispose to IDDM synergistically. These data indicate that the DR3- and DR4-associated susceptibility factors are distinct.

One approach to resolving the modes of inheritance of these distinct susceptibility alleles is to study populations in which one or the other allele is uncommon. This approach in north Indian Asians supported recessive inheritance of DR3-associated susceptibility to IDDM (Jenkins et al. 1991*a*). In Japanese populations, IDDM is positively associated with both HLA-DR4 and HLA-DR9, although there is no clear synergism between these alleles in DR4/9 heterozygotes (Ito et al. 1988; Kida et al. 1989). DR3 is rare in this ethnic group and is not associated with the disease (Aparicio et al. 1988). Disease-predisposing DR4 haplotypes in Japanese differ from those in white Caucasians, but the consistent association between DR4 and IDDM suggests that all DR4 susceptibility haplotypes carry a common IDDM-predisposing allele (Jenkins et

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al. 1990). In white Caucasians, DR9 is uncommon and is not associated with IDDM. Inheritance of IDDM in Japanese may, therefore, differ from that in white Caucasians. We proposed that susceptibility to IDDM in Japanese subjects is encoded by a single allele which is associated with both DR4 and DR9. This hypothesis was tested by measuring the frequencies of DR4 and DR9 in a Japanese population with IDDM, by using RFLP analysis. Additive and recessive inheritance of a single susceptibility allele linked to DR4 and DR9 were analyzed using the antigen-genotype-frequency-among-patients (AGFAP) method (Thomson 1983).

Subjects and Methods

Subjects

Fifty-three Japanese subjects with IDDM and 69 racially matched control subjects were selected for study. Subjects with IDDM were recruited from the diabetic clinic of the Tokyo Women's Medical College. They were diagnosed at less than 30 years of age, ketosis prone, and, from the time of diagnosis, continuously dependent on insulin. Fasting C-peptide levels were less than 0.6 ng/ml. Control subjects were resident in Tokyo and had neither personal nor family history of diabetes.

DR Genotyping

DR genotypes were deduced from *Taq*I and *Hin*dIII DRB RFLP analysis and from *Bam*HI DQB RFLP analysis. DNA samples (7.5 μg) were digested separately with *Taq*I, *Hin*dIII, and *Bam*HI 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 h. The fragments were blotted onto nylon filters (Hybond-N; Amersham International, UK). *Taq*I and *Hin*dIII 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-1 (full-length DQB1 gene). The probes were labeled to a specific activity of 10^9 counts/min/μg DNA, by the oligonucleotide-primer method. Prehybridization and hybridization were performed according to a method described elsewhere (Jenkins et al. 1991*a*).

Correlations previously observed between serological DR types and DRB/DQB RFLPs in white Caucasian, black, and Japanese subjects were used to define the DR haplotypes in each subject (Aparicio et al. 1988; Fletcher et al. 1988; Paulsen et al. 1989). Haplotypes were classified as DR4, DR9, or DRX, where "X" is any haplotype apart from 4 and 9. DR4 haplotypes were recognized by 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. DR7 and DR9 haplotypes were recognized by a 16-kb + 7-kb + 4-kb + 2.5-kb or a 16-kb + 6-kb + 4-kb + 2.5-kb *Taq*I RFLP (Fletcher et al. 1988). *Hin*dIII DRB RFLP analysis distinguished DR9 haplotypes from DR7 haplotypes. DR9 haplotypes lacked the 9.6-kb and 8.4-kb *Hin*dIII DRB fragments characteristic of DR7(w11) and DR7(w17) haplotypes (Paulsen et al. 1989). A 4-kb + 3.2-kb *Bam*HI DQB RFLP distinguished the DR7(Dw17) haplotype in DR4/7(w17) heterozygotes, allowing DR4/9 heterozygotes to be distinguished from DR4/7(w17) heterozygotes. Genotypes were then classified as 4/4, 4/9, 9/9, 4/X, 9/X, and X/X.

AGFAP Method

The AGFAP method uses a simple two-locus disease-association model. It was proposed that the DR4 and DR9 alleles of the DRB1 gene are in linkage disequilibrium with a single susceptibility allele of a diallelic gene which determines predisposition to IDDM. The other allele does not confer disease susceptibility. For the genotypes 4/4, 4/9, 9/9, 4/X, 9/X, and X/X in subjects with IDDM, the expected frequencies predicted by this model under recessive inheritance were calculated from the observed genotype frequencies as described by Thomson (1983). The frequencies expected under additive inheritance were calculated from the observed genotype frequencies and from the gene frequencies of DR4 and DR9 in the control population, by means of the AGFAPK computer package. (This uses a Newton-Raphson iteration procedure to solve for the maximum-likelihood estimates of the disease-association parameters of DR4 and DR9.) The expected frequencies were compared with those observed by using the χ^2 test with 3 df, a df representing each independent comparison made. The χ^2 test has been shown to be valid, even when the expected values of some classes are less than 5 (Roscoe and Byars 1971). *P* values smaller than .05 were considered to indicate statistical significance.

Table 1

Observed and Expected Frequencies of DR Genotypes (4/4, 4/9, 9/9, 4/X, 9/X, and X/X) in 53 Japanese Subjects with Insulin-dependent Diabetes Mellitus

Genotype[a]	No. Observed	No. Expected	
		Additive[b]	Recessive[c]
4/4	2	4.0	3.4
4/9	4	8.6	8.7
9/9	8	4.6	5.5
4/X	19	14.0	11.5
9/X	14	15.2	14.4
X/X	6	6.6	9.6

[a] X = any non-DR4, non-DR9 haplotype.
[b] Additive inheritance—χ^2 = 7.9, 3 df, $P < .05$.
[c] Recessive inheritance—χ^2 = 10.5, 3 df, $P < .025$.

Results

The observed and expected frequencies of the different genotype classes among the Japanese diabetic subjects are shown in table 1. The gene frequencies of DR4 and DR9 among the control subjects were .2 and .21, respectively. The genotype frequencies observed in the diabetic subjects were significantly different from those predicted under additive inheritance (χ^2 = 7.9, 3 df, $P < .05$). The largest difference occurred in the 4/X class (19 observed vs. 14 expected).

The frequencies observed in diabetic subjects were significantly different from those expected under recessive inheritance (χ^2 = 10.5, 3 df, $P < .025$). The largest difference occurred in the 4/X class (19 observed vs. 11.5 expected).

Discussion

The AGFAP method rejected the hypothesis that DR4 and DR9 are associated with a single allele which directly predisposes to IDDM, either additively or recessively. It is of interest, however, that in Japanese subjects the fit of the observed frequencies to the expected frequencies was closer to additive inheritance than to recessive inheritance. This contrasts with other ethnic groups in which disease susceptibility more closely approximates recessive inheritance (Jenkins et al. 1991*a*). The significance of this finding is unclear, but inheritance of IDDM in Japanese subjects appears distinct from that in other races studied to date.

The rejection of the single-allele models is of considerable interest, since the data suggest that the susceptibility allele on DR4 haplotypes may be distinct from the disease-predisposing allele on DR9 haplotypes. Japanese DR4 and DR9 haplotypes carry the same DQA1 allele, DQA1*0301 (previously termed A3). This allele was significantly positively associated with IDDM in a Japanese population (Todd et al. 1990) and also in populations of white Caucasian (Khalil et al. 1990), north Indian (Jenkins et al. 1991*b*), and black origin (Todd et al. 1989). Preliminary data from the subjects in the present study indicate that DQA1*0301 was also significantly increased among the diabetic group (Jacobs et al. 1990). These data indicate that DQA1*0301 alone cannot account for both DR4- and DR9-associated predisposition to IDDM in the Japanese. This suggests either that DQA1*0301 is in linkage disequilibrium with distinct susceptibility alleles at other loci on DR4 and DR9 haplotypes or that any predisposing effect of DQA1*0301 is modified by another susceptibility allele on DR4 and/or DR9 haplotypes.

It is clear that the DR4- and DR9-associated factors do not interact in a manner similar to that shown by the DR3- and DR4-associated alleles in white Caucasians with IDDM. Both under additive inheritance and under recessive inheritance, the number of DR4/9 heterozygotes expected were greater than the number observed. This contrasts with the predictions made concerning DR3/4 heterozygotes in white Caucasian and north Indian subjects, in whom the expected numbers are less than those observed (Thomson 1983; Jenkins et al. 1991*a*). The DR4- and DR9-associated factors, therefore, do not predispose to IDDM synergistically. The DR9-associated factor is, therefore, distinct from the DR3-associated factor present in white Caucasians. The rarity of a DR3-associated disease-predisposing allele in Japanese populations may contribute to the low incidence of IDDM among Japanese subjects.

The disease-susceptibility alleles have not yet been identified. Candidate loci include the DQ (DQA1 and DQB1) and DRB1 genes. It is likely that analysis of associations between DR/DQ alleles and IDDM in various populations will contribute to identification of disease-susceptibility factors (Jenkins et al. 1990). The low frequency of DR3 in Japanese populations allowed investigation of the inheritance of DR4- and DR9-associated susceptibility to IDDM in the absence of DR3. Inheritance of IDDM in Japanese, as in white Caucasians, is complex and cannot be explained by a single-allele model. DR9-associated susceptibility to IDDM, therefore, is distinct from DR4-associated sus-

ceptibility. DR9-associated susceptibility is also distinct from DR3-associated susceptibility observed in white Caucasians. These findings indicate further complexity in the genetics of IDDM and support the examination of ethnically distinct populations as a method of investigating the inheritance of susceptibility to this disease.

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