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Genetic Heterogeneity, Modes of Inheritance, and Risk Estimates for a Joint Study of Caucasians with Insulin-dependent Diabetes Mellitus

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Summary

From 11 studies, a total of 1,792 Caucasian probands with insulin-dependent diabetes mellitus (IDDM) are analyzed. Antigen genotype frequencies in patients, transmission from affected parents to affected children, and the relative frequencies of HLA-DR3 and -DR4 homozygous patients all indicate that DR3 predisposes in a "recessive"-like and DR4 in a "dominant"-like or "intermediate" fashion, after allowing for the DR3/DR4 synergistic effect. Removal of DR3 and DR4 reveals an overall protective effect of DR2, predisposing effects of DR1 and DRw8, and a slight protective effect of DR5 and a predisposing effect of DRw6. Analysis of affected-parent-to-affected-child data indicates that a subset of DR2 may predispose. The non-DR3, non-DR4 antigens are not independently associated with DR3 and DR4; the largest effect is a deficiency of DR2, followed by excesses of DR1, DRw8, and DRw6, in DR4 individuals, as compared with DR3 individuals. HLA-B locus distributions on patient haplotypes indicate that only subsets of both DR3 and DR4 are predisposing. The presence or absence of Asp at position 57 of the DQβ gene, recently implicated in IDDM predisposition, is not by itself sufficient to explain the inheritance of IDDM. At a minimum, the distinguishing features of the DR3-associated and DR4-associated predisposition remain to be identified at the molecular level. Risk estimates for sibs of probands are calculated based on an overall sibling risk of 6%; estimates for those sharing two, one, or zero haplotypes are 12.9%, 4.5%, and 1.8%, respectively. Risk estimates subdivided by the DR type of the proband are also calculated, the highest being 19.2% for sibs sharing two haplotypes with a DR3/DR4 proband.

Introduction

Because of the strong HLA-DR3 and -DR4 associations (see Svejgaard et al. 1980; Tiwari and Terasaki 1985) and relatively high prevalence and severity of the disease, a great deal of attention has focused on study of insulin-dependent diabetes mellitus (IDDM). Precisely because the associations with DR3 and DR4 are so strong, it is sometimes difficult in individual studies to determine the extent of the effects of non-DR3, non-DR4 antigens on disease predisposition. Also, evidence found in any one study for specific effects of the DR3- and DR4-associated predisposing components is

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strengthened if the effects are also observed in additional studies. For this reason, it is opportune that a number of large Caucasian IDDM data sets were made available for a joint study of various features of IDDM pathogenesis. The studies, of which there are 11, with a total of 1,792 probands, are from Europe, North America, and Australia. Each data set has been individually analyzed in a number of publications (see table 1).

The analysis of affected-sib-pair HLA haplotype-sharing data initially suggested a recessive mode of inheritance for IDDM. The first set of data on 15 affected sib pairs (Cudworth and Woodrow 1975; Thomson and Bodmer 1977*a*, 1977*b*), as well as accumulated data from 538 families with two or more affected sibs (Payami et al. 1985), are virtually identical with recessive expectations and reject a dominant mode of inheritance. The recessive model, however, predicts a very high frequency for the disease allele, namely, .367 (Payami et al. 1985). The discrepancy between such a high predicted allele frequency and known penetrance and incidence values for the disease could be alleviated by assuming that additional (non-HLA) loci are also involved in predisposition to disease (Thomson 1980). A number of associations of non-HLA loci with IDDM have been suggested, including the polymorphic region 5′ (5′ FP) to the insulin gene (Bell et al. 1984, 1985; Hitman et al. 1985; Thomson et al, in press), but no associations comparable in strength to that with HLA-DR have been found so far.

The possibility of heterogeneity of IDDM predisposition in the HLA region was raised with the demonstration of increased risk, initially for B8/B15(w62) heterozygotes (Svejgaard et al. 1975) and later for DR3/DR4 heterozygotes (Svejgaard et al. 1980; Svejgaard and Ryder 1981). Further, MacDonald (1980) provided evidence for a dominant mode of inheritance for IDDM, on the basis of the observation that the frequency of IDDM was compatible with the estimated admixture of Caucasian genes in American blacks—in contrast to the affected-sib-pair haplotype-sharing data which rejected a dominant model and favored a recessive (Thomson 1980) or intermediate (Spielman et al. 1980) model. The excess frequency of DR3/DR4 in IDDM over all single locus (recessive, dominant, intermediate, and general model) expectations (Rotter et al. 1983; Thomson 1983; Louis and Thomson 1986) proved the involvement of more than one predisposing allele. Extension of theoretical considerations to a three-allele synergistic model (Hodge et al. 1980) provided an explanation of these apparent anomalies. The question is then raised of the modes of inheritance of the DR3-associated and DR4-associated predisposing components. When there is a synergistic effect (DR3/DR4 in this case), the affected-sib-pair haplotype-sharing values move toward the recessive expectations (Rotter and Hodge 1980; Louis et al. 1984; Payami et al. 1985; Louis 1986). Thus, the results of MacDonald (1980) implicating a dominant component to IDDM predisposition and the sib-pair data implicating a recessive component are not incompatible if there is a dominant component to IDDM predisposition in the absence of the DR3/DR4 synergistic effect.

Two lines of evidence support a model in which the DR3-associated predisposing allele is recessive in its inheritance in the absence of DR4 and in which the DR4-associated predisposing allele is additive (dominant) in the absence of DR3. The first observation, by MacDonald et al. (1986*a*, 1986*b*), is of an excess transmission of DR4 alleles and deficiency of DR3 alleles from affected parents to affected children, compared with IDDM population frequencies. This trend is observed in other Caucasian IDDM data sets (Thomson et al. 1986, and in press). The other evidence comes from the pattern of deviations of the DR genotype frequencies from those expected under a single-locus recessive model (Louis and Thomson 1986).

The DR4-bearing haplotypes in IDDM patients show significant excess of B15(w62) and deficiency of B12(44) compared with control frequencies (Thomson et al. 1986, and in press; Risch, in press). For DR3 haplotypes the fit-to-population expectation is very close in some populations but significantly different in others (Thomson et al. 1986, and in press). The lack of fit of B-DR haplotypes to population expectations excludes the direct involvement of DR3 and DR4, as presently defined, in disease pathogenesis, as have studies with mixed lymphocyte culture (MLC) typing, further serological subdivision, and RFLP analysis (Cohen-Haguenauer et al. 1985; Sheehy et al. 1985*a*, 1985*b*; Festenstein et al. 1986; Hitman et al. 1986; Nepom et al. 1986; Tait and Boyle 1986; Tosi et al. 1986). Heterogeneity within DR3 haplotypes in IDDM patients (heterogeneity determined on the basis of their B allele distributions) has been observed for the genotypic classes DR3/DR3, DR3/DR4, and DR3/DRX (DRX denotes non-DR3, non-DR4 antigens) (Thomson et al. 1986, and in press) and for their frequency of occurrence in zero, only one, or two or more diabetics in multiplex families (Field, in press).

After the DR3 and DR4 associations with IDDM have been removed from the analysis, the remaining DR

antigens are not neutral with respect to disease predisposition. DR2 is protective, while DR1 and possibly DRw8 exhibit a higher frequency than expected in patients (Svejgaard et al. 1980; Thomson 1984; Clerget-Darpoux et al. 1986; Field et al. 1986; Thomson et al. 1986).

Our aim in the current study is to investigate the aspects of HLA-DR associations with IDDM mentioned above. In addition, relations between age at onset, sex, and DR type will be considered. The relative frequencies of the DR3 and DR4 homozygous classes in patients will be analyzed, including implications for modes of inheritance. An appropriate method to detect synergistic effects apart from the DR3/DR4 effect will be presented. DR3 and DR4 haplotypes transmitted from an affected parent to an affected child will be compared with these haplotypes in the IDDM population. Risk estimates for sibs of probands, based on DR type and haplotype sharing, will be determined.

HLA-DR Associations with IDDM

In table 1 the country, investigators, sample size of IDDM patients, and previous publications (which give details of the ascertainment of patients) for each study are indicated. For family data, only one affected individual per family (the proband if one was indicated, otherwise the eldest) was counted. Several families appeared in more than one study; these were counted as part of the most recent study only. The antigen frequencies among patients for each study are listed in table 2. The USA2 study (data set 9) is divided into two sets, randomly and multiplex ascertained; the GAW5 data were all multiplex ascertained. The antigen frequencies for the totals of the simplex and multiplex data are not significantly different, although the frequencies of the classes DR3, DR4, DR3/DR4, and DR3 and/or DR4 are greater in the multiplex data than in the randomly ascertained data.

Throughout the present paper, the term "DRX" is used to refer to all DR alleles other than DR3 and DR4.

Sex, Age at Onset, and DR Type

The distribution of affected males and females based on DR type was analyzed in data sets 1 and 3–9. Males represented 53% of the sample, females 47%. No significant differences were found for sex versus DR type in the combined data. Previous studies analyzed by Ludvigsson et al. (1986) have indicated an excess of females among DR4/non-DR3 patients, especially in the USA study from Pittsburgh, where the excess is significant (observed 45, expected 35.7, overall $\chi^2 = 11.34$ (df = 3), $P < .01$). A nonsignificant excess is observed in the present study (observed 210, expected 200). When the nine studies are considered separately (for study 9, random and multiplex are considered separately), an overrepresentation of DR4/non-DR3 females over ex-

Table 1

Data Sets Used in Study

Country (Sample Size)	Investigators (Reference[s])
1. Sweden (126)	J. Ludvigsson and B. Lindblom (Ludvigsson and Lindblom 1984; Ludvigsson et al. 1986)
2. Finland (233)	J. Partanen (Partanen et al. 1986)
3. Canada (77)	N. Farid (Farid et al. 1979; Skanes et al. 1986; Farid and Thompson 1986)
4. Austria (159)	E. Schober and W. M. Mayr (Schober et al. 1981; Ludvigsson et al. 1986)
5. Australia (231)	B. Tait (Tait and Boyle 1986)
6. USA1 (105)	M. MacDonald and J. Gottschall (MacDonald et al. 1986*a*; 1986*b*)
7. Germany[a] (154)	J. Bertrams and M. Baur (Bertrams and Baur 1984; Baur 1986)
8. France[a] (45)	I. Deschamps (Deschamps et al. 1980; Contu et al. 1982; Ludvigsson et al. 1986)
9. USA2 random (131) and multiplex (120)	J. Barbosa and S. Rich (Barbosa et al. 1982; Dunsworth et al. 1982; Morton et al. 1983; Rich et al. 1984; Rich 1986)
10. Denmark (317)	A. Svejgaard (Svejgaard et al. 1986)
11. GAW5[b] (94)	R. Spielman, F. Clerget-Darpoux and M. Baur (organizers) (Spielman et al., in press)

[a] Analyzed in Genetic Analysis Workshop 4.
[b] Data from Europe, North America, and Australia analyzed in Genetic Analysis Workshop 5.

Table 2

DR Antigen Frequencies (in % for Each Study)

A. Randomly Ascertained Probands

Antigen	Study 1	2	3	4	5	6	7	8	9	10	Total
DR1	17.5	17.2	9.1	18.9	15.1	16.2	23.4	24.4	16.8	14.2	16.8
DR2	1.6	2.1	6.5	8.8	5.2	4.8	5.2	8.9	3.0	2.5	4.2
DR3	56.3	39.5	53.2	57.9	58.4	54.3	52.6	64.4	45.0	50.5	51.8
DR4	77.0	79.8	66.2	67.9	69.3	80.9	76.6	64.4	74.8	71.9	73.5
DR5	.8	1.7	3.9	4.4	5.2	9.5	6.5	13.3	9.2	4.4	5.0
DRw6	.8	9.0	10.4	.6	8.7	12.4	9.1	4.4	6.9	7.3	7.1
DR7	4.8	4.3	15.6	10.7	11.3	2.9	6.5	11.1	7.6	4.7	7.2
DRw8	3.2	15.0	5.2	.6	3.9	5.7	...	...	7.6	9.1	6.2
DRw9	1.6	6.4	.0	...	...	2.9	...	...	...	...	1.3
DR3/4	35.7	28.3	27.3	34.6	38.1	37.1	34.4	40.0	26.7	30.0	32.6
DR3 or 4	97.6	91.0	92.1	91.2	89.6	98.1	94.8	88.8	93.1	92.4	92.7
N	126	233	77	159	231	105	154	45	131	317	1578

B. Multiplex Ascertained Probands

Antigen	Study 9	11	Total	Control[a]
DR1	15.8	8.5	12.6	18.1
DR2	5.8	2.1	4.2	29.1
DR3	55.8	57.5	56.6	22.6
DR4	80.8	80.9	80.8	23.8
DR5	5.0	4.3	4.7	26.6
DRw6	13.3	10.6	12.1	21.2
DR7	3.3	8.5	5.6	12.0
DRw8	4.2	5.3	4.7	3.0
DRw9	...	2.1	2.1	0.8
DR3/4	39.2	40.4	39.7	3.05
DR3 or 4	97.4	98.0	97.7	43.3
N	120	94		

[a] Ninth HLA Workshop, healthy Caucasians (Baur et al. 1984).

pected is observed in eight of the nine studies, and this asymmetry is significantly different from random expectations ($P = .02$, one-sided test). Thus there is evidence of overrepresentation of females in the DR4/non-DR3 class, but the effect is very small.

In the joint data there is some evidence for incidence peaks in age at onset versus DR type, as reported by Ludvigsson et al. (1986), although males and females differ in the peaks and the trends are not seen consistently in each study considered separately. The mean ages at onset for males and females, ignoring DR type, are virtually identical: 9.89 and 9.87 years, respectively. A test of heterogeneity was performed considering three variables: age at onset (divided into four classes 0–3, 4–7, 8–11, and 12+), sex (male vs. female), and DR type (DR3/non-DR4, DR3/DR4, DR4/non-DR3, and others) for studies 1, 4, 6–9, and 11. There was no evidence of heterogeneity for the three possible pairwise interactions or for a three-way interaction. Seasonal variation in onset versus DR type (Ludvigsson and Lindblom 1984) was not considered in the present study.

Antigen Genotype Frequencies among Patients (AGFAP)

Family data were available for the German, French, USA2 (randomly and multiplex ascertained), and GAW5 studies (data sets 7, 8, 9, and 11, respectively),

so that most individuals could be classified unambiguously as to whether they were homozygous or heterozygous for a blank. (AGFAP analyses were performed twice, once assuming that all remaining ambiguous cases were homozygous and once assuming they were heterozygous; the results were substantially the same.) Results presented below assume that ambiguous cases are homozygous.

The DR3 and DR4 genotype frequencies in the five data sets and in the combined data all differ from expectations under a single-locus recessive, additive, or intermediate model, when using the AGFAP method (Thomson 1983) (see Thomson et al. 1986 for analysis of the German and French data, Thomson et al., in press for the GAW5 data, and table 3 for the USA2 and combined data). The largest deviations from recessive expectations are an excess of DR3/DR4 genotypes, an excess of DR4/DRX heterozygotes, and a deficiency of DR4/DR4 homozygotes, DR3/DRX heterozygotes, and DR3/DR3 homozygotes. The pattern observed implies that, of the DR-associated predisposing components, the mode of inheritance of the DR4-associated component is more like "dominant" and that of the DR3-associated component is more like "recessive," after allowing for the synergistic DR3/DR4 effect (Louis and Thomson 1986).

Relative Predispositional Effects of the Non-DR3, Non-DR4 Antigens

The rank-order method outlined by Thomson (1984) was applied using the 10 simplex data sets (table 1) and, in addition, the nonoverlapping published frequency data of Wolf et al. (1983), Murphy et al. (1983), Suciu-Foca et al. (1979), and Solow et al. (1979) (data sets 12, 13, 14, and 15, respectively). The DR antigens other than DR3 and DR4 were ranked according to their relative frequencies in both the patient and control groups (control data were available for data sets 1, 2, 5, 10, and 11–15; for the other data sets 9th HLA Workshop Caucasian data were taken as controls [Baur et al. 1984], since ethnically matched control data were not available). The most frequent of these "other" antigens in the patient group was given a rank of 1, the next 2, etc., and the same was done for the control group. For each antigen the rank numbers in the patients and controls were compared, as was done by Thomson (1984). Under a model where DR3 and DR4 directly predispose to IDDM and in which all other DR antigens are equivalent in their effect on predisposition to the disease, the rank numbers in patients and controls for an antigen are expected to be equal.

Eight of the studies (2–6, 9, 10, and 12) allowed com-

Table 3

DR Antigen Genotype Frequencies Compared with Single-Locus Expectations

	3/3	3/4	4/4	3/X	4/X	X/X	
A. USA2 Simplex (9)							
Observed	10	35	16	14	47	9	Total: 131
Recessive	9.09	30.03	24.80	20.81	34.37	11.91	χ^2 = 11.59** (df = 3)
Additive	4.01	14.68	11.04	26.47	66.80	8.00	χ^2 = 46.14*** (df = 2)
B. USA2 Multiplex (9)							
Observed	6	47	8	14	42	3	Total: 120
Recessive	5.10	31.94	22.97	18.86	27.12	8.01	χ^2 = 31.75*** (df = 3)
Additive	4.09	14.22	10.46	26.09	62.48	2.66	χ^2 = 89.21*** (df = 2)
C. Combined Data (7–9 and 11)							
Observed	37	191	46	59	178	33	Total: 544
Recessive	48.21	137.47	97.80	90.13	128.24	42.05	χ^2 = 144.13*** (df = 3)
Additive	18.53	61.74	44.59	120.89	269.27	28.98	χ^2 352.17*** (df = 3)

NOTE.—For details of analysis see Thomson (1983). When expected values are less than 5, this class is combined with the class with the next smallest expected value. X = non-DR3, non-DR4 antigens.

** $P < .01$.

*** $P < .001$.

parison of the antigens DR1–8, excluding DR3 and DR4. The results from this analysis are presented in table 4. The rank order of DR2 showed the greatest deviation, and DR2 was found to be less frequent than expected for the control data in all eight studies (table 4A), in agreement with the known "protective" effect of DR2 (Svejgaard et al. 1980; Thomson 1984). DR1 was more frequent than expected in seven of the eight studies and was equal to expectations in one study. This asymmetry continued to exist after removal of DR2 from the analysis (table 4B), a result in agreement with the previously reported predispositional effect of DR1 (Thomson 1984; Clerget-Darpoux et al. 1986; Field et al. 1986; Thomson et al. 1986).

After removal of DR2 and DR1, a predispositional effect of DRw8 (table 4C) showed the next largest deviation (rank less than expected in seven of the eight studies and equal in one study). After removal of DR2, DR1, and DRw8, asymmetries in the relative predispositional effects of DR5, DRw6, and DR7 were seen (table 4D), with the bias being that DR5 is protective, DRw6 predisposing, and DR7 neutral. No statistical significance can be attached to these latter observations, and the DRw6 finding in particular should be interpreted in light of the known difficulty in distinguishing DRw6 from DR3 when using pre–9th Workshop sera. The same pattern is also observed in analysis of only the antigens DR1–7, for which data was available from 12 studies (2–10, 12, 14, and 15).

From the 14 studies, antigens DR1, DR2, DR5, and DR7 were examined, with the predisposing effect of DR1 having the largest deviation, followed by the protective effect of DR2. After removal of these two antigens, there is asymmetry in the effects of DR5 and DR7; relatively, DR5 is protective and DR7 is predisposing.

Note that these analyses do not allow us to draw

Table 4

Rank-Order Analysis (Studies 2–6, 9, 10, 12)

DR	Rank Difference[a]			Average Rank		Difference
	>	=	<	Patients	Controls	
A. Antigens DR1-8, Excluding DR3 and DR4						
1	0	1	7	1.25	3.87	−2.62
2	8	0	0	4.69	1.25	3.44
5	4	3	1	4.56	3.50	1.06
6	3	1	4	3.19	3.75	−.56
7	4	1	3	3.19	2.87	.32
8	0	1	7	4.12	5.75	−1.63
B. Antigens DR1-DRw8, Excluding DR2, DR3, and DR4						
1	0	1	7	1.25	3.00	−1.75
5	6	2	0	4.00	2.50	1.50
6	4	1	3	3.06	2.75	.31
7	5	2	1	3.06	2.00	1.06
8	0	1	7	3.62	4.75	−1.13
C. Antigens DR1-DRw8, Excluding DR1, DR2, DR3, and DR4						
5	5	3	0	3.00	2.00	1.00
6	4	1	3	2.19	2.37	−.18
7	4	2	2	2.19	1.87	.32
8	0	1	7	2.62	3.75	−1.13
D. Antigens DR1-DRw8, Excluding DR1, DR2, DR3, DR4, and DRw8						
5	4	4	0	2.50	1.87	.63
6	0	5	3	1.75	2.37	−.62
7	2	4	2	1.75	1.75	.00

[a] Number of studies in which rank in patients was greater than (>), or equal to (=), or less than (<) that in controls. Antigens were ranked in order of frequency, with rank 1 being most frequent.

conclusions as to whether, for example, the overall protective effect of DR2 is due to negative linkage disequilibrium with a predisposing allele, positive disequilibrium with a "protective" allele, or a combination of both; this is also true for the predisposing effects of, say, DR1.

The relative predispositional effects of the non-DR3, non-DR4 antigens have also been considered for each study by using the observed allele frequencies of the antigens and the expected values from controls, based on the hypothesis of no differential predispositional effects of the antigens. In table 5 the antigens that show a significant deviation from expected frequencies are indicated (note that the *P* values indicated are not corrected for the number of comparisons). Heterogeneity as to which antigens show significant effects in each population, and the relative magnitude of these effects, is observed. Note, however, that, after removal of the significant effects given in table 5, the rule that DR1 and DRw8 were increased over expectations and that DR2 decreased held in all studies except the Austrian data set, where DR2 and DRw8 frequencies were the reverse of those expected.

Frequencies of DR3/DR3 and DR4/DR4 IDDM patients

The frequencies of DR3/DR3 and DR4/DR4 individuals among IDDM patients are of interest with respect to the predispositional effects of DR3 and DR4 in the absence of each other and to the modes of inheritance of the DR3- and DR4-linked predisposing components. Family data are needed for this analysis, and the German, randomly and multiplex ascertained USA2, and multiplex GAW5 studies (data sets 7, 9, and 11, respectively) are appropriate. Only a single proband (first born if more than one proband was indicated) was used per family in analysis of multiplex data.

The method of analysis is to consider the observed frequencies of the DR3/non-DR4 genotypes relative to the respective control-population allele frequencies of the non-DR4 antigens, and similarly for the DR4/non-DR3 genotypes. For example, see table 6 for the DR3/non-DR4 observed and expected distributions for the German data. A significant (overall $\chi^2 = 16.31$, df $= 3$, $P < .001$) excess of DR3/DR3 individuals is observed compared with the other DR3/non-DR4 individuals in the German data set (table 6A). For the USA2 randomly ascertained data set a significant excess of DR3/DR3 individuals is also seen (observed 10, expected 3.08, overall $\chi^2 = 9.32$, df $= 2$, $P < .05$), and nonsignificant excesses are seen in the USA2 multiplex data and the GAW5 data (data not shown). In each case, when DR3/DR3 is removed from the analysis, the remaining DR3/nonDR4 genotypes do not differ significantly from frequencies expected based on the control

Table 5

Non-DR3, Non-DR4 Antigens, by Study

Study	Antigen(s)
1. Sweden	DR1 ↑ (22 vs. 6.64)***
2. Finland	DR2 ↓ (5 vs. 26.37),*** DRw8 ↑ (35 vs. 22.68),** DRw9 ↑ (15 vs. 6.73)***
3. Canada	DR5 ↓ (3 vs. 8.69),* DR2 ↓ (5 vs 11.35)*
4. Austria	DR1 ↑ (31 vs. 10.40),*** DR7 ↑ (18 vs. 8.74)***
5. Australia	DR2 ↓ (12 vs. 30.22),*** DR1 ↑ (35 vs. 24.36)*
6. USA1	DR1 ↑ (17 vs. 7.80,*** DRw8 ↑ (6 vs. 1.97),** DRw6 ↑ (13 vs. 6.51)**
7. Germany	DR1 ↑ (36 vs. 12.10)***
8. France	. . .
9. USA2:	
Random	DR1 ↑ (23 vs 10.11),*** DRw8 ↑ (10 vs. 2.5),*** DR2 ↓ (4 vs. 10.97)*
Multiplex	DR1 ↑ (19 vs. 8.23),*** DRw6 ↑ (16 vs. 7.56)***
10. Denmark	DRw8 ↑ (29 vs. 8.02),*** DR1 ↑ (45 vs. 19.26),*** DR2 ↓ (8 vs. 21.56)***
11. GAW5	DR2 ↓ (2 vs. 9.49),** DR5 ↓ (4 vs. 10.70)*

NOTE.—The symbol ↑ indicates an excess over control value, while ↓ indicates a deficiency. Antigens found to differ significantly from expectations were removed one at a time in order of significance until no further significant effects were seen.

* $P < .05$.

** $P < .01$.

*** $P < .001$.

Table 6

DR3/Non-DR4 Genotypes in the German (7) Data Set

Genotype	No. Observed	No. Expected
A. DR1-7, Omitting DR4		
3/1	3	3.43
3/2	3	5.70
3/3	14	4.33
3/5	1	5.16
3/6	5	4.04
3/7	1	4.33
Overall	27	χ^2 = 16.31*** (df = 3)
B. DR1-7, Omitting DR3 and DR4		
3/1	3	1.97
3/2	3	3.27
3/5	1	2.96
3/6	5	2.32
3/7	1	2.48
Overall	13	χ^2 = .18, NS (df = 1)

NOTE.—NS = not significant.
*** $P < .001$.

population allele frequencies (table 6B for the German data).

In contrast, when the DR4/non-DR3 genotypes are considered the greatest deviations (based on contribution to the overall χ^2) are an excess of DR4/DR1 individuals for the USA2 random (table 7A), USA2 multiplex (observed 12, expected 5.69, overall χ^2 = 18.93, df = 5, $P < .01$), and German (observed 29, expected 8.05, overall χ^2 = 65.80, df = 5, $P < .001$) data sets and a deficiency of DR4/DR2 individuals for the GAW5 data set (observed 1, expected 7.37, overall χ^2 = 12.83, df = 5, $P < .05$) (data not shown). These observations do not in themselves imply synergistic DR4/DR1 or DR4/DR2 effects but could merely reflect the relative magnitudes of the predisposing effect of DR1 and protective effect of DR2 in these data sets, respectively (see table 5).

Of specific interest to us in this section are the relative predispositional effects of DR3/DR3 versus other DR3/non-DR4 genotypes and of DR4/DR4 versus other DR4/non-DR3 genotypes. The question of synergistic effects with DR3 and DR4 will be raised in the next section. In the GAW5 data, after removal of DR2 from the DR4/non-DR3 analysis, no further effects are detectable. In the USA2 (random) data significant deviations are still observed after removal of DR1 (table 7B), the largest effect being an excess of DR4/DR4; further removal of DR4 from the analysis still gives significant deviations (table 7C), with a deficiency of DR4/DR2 and excess of DR4/DRw8. Significant deviations are observed in the USA2 (multiplex) data after removal of DR1; in this case the largest effect is an excess of DR4/DRw6 (observed 12, expected 5.68), reflecting the predisposing effect of DRw6 in this data set (table 5).

The main observation from this analysis is that the excess of DR3/DR3 genotypes observed in the DR3/non-DR4 individuals overrides both the DR1 predisposing effect in the USA2 and German data sets and the protective effect of DR2 in the GAW5 data set, whereas, while there is evidence of a significant excess of DR4/DR4 in one of the four data sets (table 7B), the

Table 7

DR4/Non-DR3 Genotypes in the USA2 (9) Randomly Ascertained Data Set

Genotype	No. Observed	No. Expected
A. DR1-DRw8, Omitting DR3		
4/1	18	7.14
4/2	1	11.87
4/4	16	9.55
4/5	6	10.75
4/6	7	8.42
4/7	5	9.02
4/8	6	2.25
Overall	59	χ^2 = 35.23*** (df = 5)
B. DR1-8, Omitting DR3 and DR1		
4/2	1	9.39
4/4	16	7.55
4/5	6	8.50
4/6	7	6.66
4/7	5	9.13
4/8	6	1.78
Overall	41	χ^2 = 20.79*** (df = 4)
C. DR1-8, Omitting DR1, DR3, and DR4		
4/2	1	7.02
4/5	6	6.35
4/6	7	4.97
4/7	5	5.33
4/8	6	1.33
Overall	25	χ^2 = 12.33** (df = 3)

NOTE.—Classes were combined when necessary to give expected values greater than 5.
** $P < .01$.
*** $P < .001$.

predominant feature of the DR4/non-DR3 genotypes is reflection of the predisposing effects of DR1 and protective effect of DR2 in the respective data sets. These observations are compatible with a "recessive" mode of inheritance of the DR3-associated predisposing component in the absence of DR4 and of a mode of inheritance closer to "dominant"—or possibly "intermediate," given evidence of excess of DR4/DR4—for the DR4-associated predisposing component in the absence of DR3.

Are There Synergistic Effects Apart from the DR3/DR4 Effect?

A high frequency of DR1/DR4 individuals has been noted in a number of studies (Winearls et al. 1984; for the GAW5 data, see Clerget-Darpoux et al. 1986; Field et al. 1986; Thomson et al. 1986), which has been taken to imply a synergistic DR1/DR4 effect. Care, however, must be taken in choosing an appropriate analysis to determine synergistic effects. The differential behavior of DR3/DR3 relative to the other DR3/non-DR4 genotypes—versus the behavior of DR4/DR4 relative to that of the other DR4/non-DR3 genotypes—implies that the analysis to determine synergistic effects must be restricted to the comparison of DR3/DRX genotypes to DR4/DRX genotypes. Comparison of the observed values versus those expected under a random distribution of the DRX antigens with DR3 and DR4 will allow detection of synergistic effects.

Analysis of the combined randomly ascertained data (data sets 1–10) indicates heterogeneity in the association of the DRX antigens with DR3 and DR4 (table 8). The largest deviation (based on contribution to the overall χ^2) is a deficiency of DR2 in DR4 individuals compared with DR3 individuals, and the next three largest deviations are an excess of DR1, DRw8, and DRw6 in DR4 individuals compared with DR3 individuals. We stress that these effects are not due to the overall relative predispositional effects of these antigens but that they represent heterogeneity in their associations with DR3 and DR4 in IDDM patients. Note that these heterogeneity effects are not in general large and hence may not be found in individual studies (in this case, only the German data set [7] showed statistical significance for heterogeneity when considered alone). In addition, the effects reported here may represent serological problems of cross-reactivity; for example, an excess of DR1/DR4 individuals is observed in analysis of general population Provinces Françaises data (N. Borot, personal communication).

Table 8

DR3 and DR4 with the Non-DR3, Non-DR4 Antigens

	No. Observed (No. Expected[a])	
Combined Data	DR3	DR4
DR1	55 (62.01)	169 (161.99)
DR2	23 (13.01)	24 (33.99)
DR5	17 (14.95)	37 (39.05)
DRw6	18 (24.08)	69 (62.92)
DR7	24 (20.76)	51 (54.24)
DRw8	15 (21.31)	62 (55.69)
DRw9	8 (3.88)	6 (10.12)
Overall	160	418
		$\chi^2 = 23.55^{***}$ (df = 6)

[a] Based on a random assortment of the non-DR3, non-DR4 antigens with DR3 and DR4.

*** $P < .001$.

DR Transmission from Affected Parents to Affected Children

The distribution of DR alleles transmitted by affected parents to affected children is a source of information concerning the mode of inheritance of IDDM (MacDonald et al. 1986*a*, 1986*b*). Five data sets provided information on DR transmission from affected parents to affected children (data sets 6–9 and 11, excluding overlaps). If there is more than one affected child per family, the alleles transmitted per child are weighted to give a total weight of one per family. The number of DR alleles transmitted, their relative frequencies, and the allele frequencies in the IDDM proband population for the combined data are listed in table 9A. For the five data sets, an excess transmission of DR4 is observed, greater than the IDDM population frequency of this allele (64.9% vs. 42.7%), while DR3 is transmitted with a frequency less than its frequency in the IDDM patients (19.5% vs. 30.1%) (overall $\chi^2 = 15.63$, df = 3, $P < .005$). (When all affected children are included in the calculations, rather than an average of one per family, the frequencies of DR3 [23.4%] and DR4 [63.1%] transmitted do not differ greatly from those in table 9A.)

Under single-locus models the expectation for a recessive model is that a particular allele will be transmitted from an affected parent to an affected child with the same frequency as the allele occurs in the IDDM population, whereas for an additive or dominant model an allele that is positively associated with the disease will be transmitted with a frequency greater than that of

Table 9

DR Transmission from Affected Parent to Affected Child (Data Sets 6–9 and 11), Compared with Allele Frequency in IDDM Probands

A. DR1, DR3, DR4, and Other Haplotypes Transmitted

	DR1	DR3	DR4	Other	Total
Observed	3	15	50	9	77
Frequency	.039	.195	.649	.117	
Proband frequency	.074	.301	.427	.198	

B. Other Haplotypes Transmitted

	DR2	DR5	DRw6	DR7	DRw8	DRw9	Other	Total
Combined (6–9 and 11)	3	1	3	1	0	1	0	9
Frequency	.333	.111	.333	.111	.000	.111	.000	
Proband frequency	.092	.194	.189	.178	.134	.063	.153	

this allele in the IDDM population (G. Thomson, unpublished results). The observed results thus again favor a more "recessive" mode of inheritance of the DR3-linked IDDM-predisposing allele and a more "dominant" mode of inheritance of the DR4-linked IDDM-predisposing allele, after allowing for the DR3/DR4 synergistic effect, as originally observed by MacDonald et al. (1986*a*, 1986*b*).

Of further interest is the distribution of non-DR1, non-DR3, non-DR4 alleles transmitted from an affected parent to an affected child (table 9B). DR2 is transmitted in three (33.3%) of nine cases, a very high transmission rate given its frequency (9.2%) among the non-DR1, non-DR3, non-DR4 alleles in the IDDM population. For DR2 the difference between observed and expected is significant ($P < .05$). Molecular data subdivides DR2 between patients and controls (Bach et al. 1982; Cohen et al. 1984, 1986; Cohen-Haguenauer et al. 1985; Bohme et al. 1986; Cohen et al. 1986; Segall et al. 1986). The present analysis indicates that the subset of DR2 that is not protective may in fact be predisposing, rather than neutral, with respect to IDDM, a conclusion also supported by molecular data (Todd et al. 1987; Horn et al. 1988; Morel et al., in press).

Haplotype Patterns in the IDDM Population

The studies from Germany, France, USA2, and GAW5 (data sets 7, 8, 9, and 11, respectively) contained family information, and haplotypes could thus be assigned. For given DR-bearing haplotypes from the IDDM population, investigation of expected versus observed allele frequencies at the HLA-B locus can aid us in localization of the IDDM-predisposing genes and in possible subdivision of the DR-bearing haplotypes with respect to IDDM predisposition.

Under the hypothesis that, say, DR3 itself—rather than a disease-predisposing gene in linkage disequilibrium with DR3—is directly involved in predisposition to IDDM, the expected allele frequencies of DR3-bearing haplotypes at the non-DR HLA loci can be directly predicted from the control-population data on the association of DR3 with these alleles. The observed and expected allele frequencies at the HLA-B locus, under the separate hypotheses that each of the antigens DR1 through DRw8 is directly involved in disease predisposition, have been considered.

For DR1, DR2, DR7, and DRw8 the observed B allele distributions are not significantly different from population expectations, although for all but DR1 the sample sizes are quite small.

For DR3 haplotypes the overall fit of observed to expected values shows a significant difference ($P < .01$), the largest effects being an excess of B18 (observed 51, expected 32.82), a slight excess of B7, and slight deficiencies of B8 and B35. In analysis of the individual data sets, the French data (8) shows a large excess of B18 (observed 12, expected 2.98) and deficiency of B8 (observed 9, expected 17.45), indicating a difference in IDDM susceptibility associated with these two haplotypes. If the French data are removed from the DR3 analysis, there are no longer significant differences between the observed and expected values, although B18 is still increased in value over expected (observed 39,

expected 29.8). B18 is not substantially more frequent in French control data than in other populations, with a frequency of .061 (from the Provinces Françaises study; Cambon-Thomsen et al., in press), compared with .055 from 9th HLA Workshop data. Previous analysis of the German (7) IDDM data for GAW4 (Thomson et al. 1986) found no difference between the IDDM DR3 haplotypes and those found in the general population; in fact, the observed and expected values were remarkably similar, while analysis of the GAW5 data (11) (Thomson et al., in press) showed significant differences ($P < .05$). The present evidence for subdivision of the DR3 haplotypes must be viewed in light of the fact that, for most of the B alleles, the fit of observed to expected values is very close, particularly for B8, the most common DR3-associated B allele.

The DR4 haplotypes show highly significant differences ($P < .001$) from control population expectations, with a large excess of B15(w62) (observed 125, expected 56.29) and deficiency of B12(44) (observed 61, expected 99.68).

The DR5 haplotypes are significantly different from population expectations ($P < .001$), with the largest deviation being an excess of B8 (observed 6, expected .35) and smaller excesses of B21 (observed 6, expected 1.52) and B15(w62) (observed 8, expected 3.18). The DRw6 haplotypes are also significantly different ($P < .01$), the main effect being an excess of B15(w62) (observed 13, expected 5.6, $P < .005$).

From the data sets from Germany, France, USA2, and GAW5 (data sets 7, 8, 9, and 11, respectively; excluding overlaps), data from families with two or more affected sibs were considered to determine whether, when a parent is homozygous for DR3, both the DR3 haplotypes (distinguishable by their A and B locus alleles) are equally predisposing to disease, so that sib pairs would be equally likely to share either allele; and the same was done for DR4. From 23 DR3/DR3 parents 65% of affected sibs share the same DR3 allele, but this excess is not significantly different from 50%. The DR4 transmission from 45 DR4/DR4 parents does differ from 50% ($P < .008$), with 71% sharing the same DR4 haplotype, thus independently confirming that only a subset of DR4 is predisposing. For DRX parents the distribution of the parental alleles to the affected sibs is also nonrandom ($P < .025$), indicating that these alleles are not neutral with respect to disease predisposition.

On the basis of the genotypic class—DR3/DR4, DR3/DR3, or DR3/DRX—in which the DR3 haplotype occurred, heterogeneity ($P < .05$) was detected in the distribution of the B8, B18, and BX (non-B8, non-B18) alleles on DR3 haplotypes in previous analysis of the GAW5 data (11) (Thomson et al., in press). This observation was confirmed with analysis of data sets 6, 7, and 9 (table 10). On the basis of DR genotypic class, the largest deviation from a random association of the B locus alleles was a deficiency of B18 in DR3/DR3 individuals, followed by a deficiency of B8 in DR3/DRX individuals. This result provides evidence for the subdivision of the IDDM predisposition associated with DR3.

No significant effect was observed in the distribution of B locus alleles on DR4 haplotypes, on the basis of the occurrence of DR4 haplotypes in the genotypic classes DR3/DR4, DR4/DR4, and DR4/DRX from data sets 6, 7, and 9, although heterogeneity was observed in analysis of the GAW5 data (11) (Thomson et al., in press), with an excess of B44 in the DR4/DR4 IDDM class as the main effect.

The distribution of B allele frequencies on DR3 and DR4 haplotypes transmitted from an affected parent to an affected child were compared with these distributions in the IDDM population. A total of 15 DR3 haplotypes and 48 DR4 haplotypes (two relevant DR4 haplotypes from the GAW5 [11] data were not B locus typed) were available for study. The DR3 and DR4 haplotypes transmitted from an affected parent to affected child do not differ significantly from the IDDM population distributions, although for DR4 there is a trend for both an increased frequency of B15(w62) (observed 18.7, expected 13.34) and a decreased frequency of B12(44) (observed 2.5, expected 6.51). Under the hypothesis of a close to additive (dominant) mode of inheritance for

Table 10

HLA-B Locus Distribution on DR3 IDDM Haplotypes Subdivided by Genotype

	No. of B Allele Observed (No. Expected)			
Genotype	B8	B18	Non-B8, non-B18	Total
DR3/DR4	92 (87.5)	29 (24.8)	32 (40.6)	153
DR3/DR3	45 (38.9)	3 (11.0)	20 (18.1)	68
DR3/DRX	18 (28.6)	12 (8.1)	20 (13.3)	50
Total	155	44	72	271
	$\chi^2 = 18.94^{***}$ (df = 4)			

Note.—DRX denotes non-DR3, non-DR4 antigens.
*** $P > .001$.

the DR4-associated predisposing component in the absence of DR3, it is expected that the two distributions would differ, since the IDDM population would include some non-diabetogenic DR4 haplotypes. How big the difference would be is not known, although it would probably not be large. The trend observed is in the expected direction.

Risk

Given the overwhelming evidence for heterogeneity of IDDM predisposition according to HLA type, it is appropriate that consideration be given to more individualized risk estimates for family members. The risk estimate should be based on the family's particular configuration of susceptibility. Until the specific molecular alterations at the IDDM-predisposing loci are well characterized, an appropriate procedure is to give risk estimates based on DR type and haplotype sharing within each family.

Estimates of risks to siblings of IDDM probands vary: 5.9% (to age 30, with a standard error of 1.2%) (Degnbol and Green 1978), 5.6% (to age 16) (Gamble 1980), 8.5% and 4.6% (to age 40, if proband is diagnosed before or after age 10, respectively) (Chern et al. 1982), and 6.6% (lifetime) (Tillil and Kobberling 1987). We will take 6% as a representative value of the sibling risk to about age 30, as did Spielman et al. (1980). From 538 families with affected sib pairs (not all of which were DR typed), the observed frequencies sharing two, one, and zero haplotypes are 53.6%, 39.1%, and 7.3%, respectively (Payami et al. 1985). If we assume that all siblings of a proband (whether affected or not) share two, one, and zero haplotypes with random probabilities of 25%, 50%, and 25%, respectively (as is seen in the present study [data sets 7, 8, 9 and 11], but is not observed by Gorsuch et al. [1982]), we can estimate the risk to siblings who share two HLA haplotypes with a proband as being $R_2 = (.536 \times .06)/.25 = 12.9\%$. Similarly, if one haplotype is shared $R_1 = 4.7\%$, and if there is no common haplotype $R_0 = 1.8\%$. These estimates are very close to those found previously by Platz et al. (1981) who used this same approach, and by Gorsuch et al. (1982) and Tarn et al. (1988), who used empirical data.

Combining sib-pair data, from data sets 7–9 and 11 and nonoverlapping sib-pair data from the literature with DR typing (Payami et al. 1985), we have calculated risk estimates to sibs on the basis of the DR type of the proband as well as on the basis of the haplotype sharing of the sib and proband. The values, based on a total of 341 sib pairs and on DR genotype frequencies for the randomly ascertained patients in our total data set, are given in table 11. Risk values are calculated using all affected sibs from a family. The estimates are very similar if a weighted average of one sib pair per family is taken. The values of R_2, R_1, and R_0 in this case (with 95% confidence interval, considering only the variance from the affected sib-pair distribution, shown in parentheses) are 13.1% (11.8%–14.3%), 4.6% (3.9%–5.2%), and 1.8% (1.1%–2.4%), respectively—and, although based on a smaller sample size, are very similar to those calculated using the total affected sib-pair data given above. The highest risk, 19.2%, is for sibs sharing two haplotypes with a DR3/DR4 proband (95% confidence interval 15.7%–22.8%). When only one haplotype is shared, the risks have been been subdivided based on which haplotype is shared; that is, for probands who are DR3/DR4, DR4/DRX, or DR3/DRX risks are given based on whether DR3, DR4, or DRX is shared. As expected, the risks for sibs sharing zero haplotypes with the proband are not dependent on the DR genotype of the proband. The fact that the risk to siblings sharing zero haplotypes with a proband (1.8%) is greater than the population prevalence of the disease (0.4%) indicates that additional factors must be involved in disease predisposition.

Risk estimates for a child of an affected parent, on the basis of both the affected parent's DR type and the contribution of the unaffected parent to the child, can be calculated in a similar manner. Very high risks are found for some categories—for example, for DR3/DR4 offspring who inherit DR3 from an affected DR3/DRX

Table 11

Risk to Siblings on the Basis of DR Type of Proband and Haplotype Sharing

	Haplotype Sharing		
Proband	2	1	0
3/4	.192	.037 (.039, .035)	.013
4/4	.074	.035	.010
4/X	.111	.068 (.098, .037)	.016
3/3	.141	.039	.024
3/X	.112	.049 (.084, .014)	.028
X/X	.057	.033	.038
Overall	.131	.046	.018

Note.—The two numbers in parentheses for some of the haplotype-sharing-1 risk values are risks subdivided by whether the sib shares the first or second listed allele with the proband. Risk values are based on an overall risk to siblings of 6%. X = non-DR3, non-DR4 antigens.

father. We will not specify any risk values here, as we feel that additional data are needed to determine more accurate estimates; but preliminary observations indicate that this approach may provide interesting results.

Discussion

The DR3/DR4 synergistic effect in IDDM predisposition has been well established (Rotter et al. 1983; Thomson 1983; Louis and Thomson 1986) and is confirmed in our study. Two observations—(1) the excess transmission of DR4 and the deficiency of DR3, compared with IDDM population frequencies, transmitted from affected parents to affected children (MacDonald et al. 1986*a*, 1986*b*; Thomson et al. 1986, and in press) and (2) the excess frequency of DR4/DRX genotypes and the deficiency of DR3/DRX genotypes (DRX denotes non-DR3, non-DR4 antigens) in probands, compared with recessive expectations (Louis and Thomson 1986; Thomson et al. 1986, and in press)—independently indicate dominant- and recessive-like modes of inheritance, respectively, for the DR4- and DR3-associated disease-predisposing components in the absence of each other. The increased sample size from our joint study further confirms these two observations. Additionally, the differential frequencies of the DR3/DR3 class in the DR3/non-DR4 probands versus the DR4/DR4 class in the DR4/non-DR3 probands supports such a mixed model of inheritance. However, in one data set there is an excess of DR4/DR4 patients that is significantly above dominant expectations, indicating a more complicated model of inheritance. Additional family data are eagerly awaited to further investigate this observation.

Heterogeneity in predispositional effects is clearly established for the non-DR3, non-DR4 antigens, with the overall pattern (in order of magnitude of effects) being a protective effect of DR2, predisposing effects of DR1 and DRw8, and slight protective and predisposing effects of DR5 and DRw6, respectively, with DR7 being relatively neutral. Although DR2 haplotypes in IDDM patients have been subdivided at the molecular level, with 77% of IDDM DR2 chromosomes being DR2-AHZ compared with 6% of control DR2 haplotypes (Bach et al. 1982; Cohen et al. 1984; Cohen-Haguenauer et al. 1985; Bohme et al. 1986; Cohen et al. 1986; Segall et al. 1986; Todd et al. 1987), such a subdivision does not indicate whether the DR2-AZH haplotypes should be classified as neutral or predisposing with respect to IDDM. A high transmission rate of DR2 from affected parents to affected children is evidence of a predisposing component in a subset of DR2 haplotypes, in agreement with molecular data (see discussion below).

Further complexity in the analysis of IDDM is apparent from the observed heterogeneity, on the basis of genotypic class, of DR3 haplotypes in patients. The observed heterogeneity may be a consequence of the differential modes of inheritance of the different IDDM-predisposing components, so that the different DR3 genotypic classes cannot be directly compared.

The synergistic effects apart from DR3/DR4 are not as strong as originally expected. Previous evidence of a strong DR1/DR4 synergistic effect (Winearls et al. 1984; Clerget-Darpoux et al. 1986; Thomson et al. 1986) is shown not to hold in all studies. While there is significant heterogeneity for the combined data in the association of non-DR3, non-DR4 antigens with DR3 and DR4, with the largest deviation being a deficiency of DR2 in DR4 individuals compared with DR3 individuals, followed by an excess of DR1, DRw8, and DRw6, these heterogeneity effects are not large and hence in general may not be found in individual studies. Additionally, to some extent these effects may represent cross-reactivity of antigens.

We feel strongly that risk estimates for IDDM must take account of the HLA class II–associated heterogeneity of the disease. Our analysis has revealed that large differences in risk are found for sibs of probands on the basis of DR type and haplotype sharing; and preliminary analysis indicates very large risk differences to offspring based on the DR haplotypes transmitted from affected and unaffected parents. Our observations emphasize the need to collect relevant data to obtain more reliable estimates.

The presence of Asp at position 57 of the DQβ gene has been shown to have a strong negative association with IDDM; the DQβ alleles that are positively associated with IDDM have either Ala (DR3 and DR4.DQw3.2), Val (DR1), or Ser (DR2.AZH) at position 57 (Todd et al. 1987; Horn et al. 1988; Morel et al., in press). Furthermore, the non-Asp 57–associated predisposition appears to act in a recessive manner; that is, protection by Asp57 appears to be dominant (Todd et al. 1987; Morel et al., in press). The non-Asp association is implicated in both DR3- and DR4-associated predisposition—which is, at first sight, surprising, given our knowledge of the distinct features of DR3 versus DR4 predisposition. All DR3 haplotypes sequenced in both IDDM pedigrees and controls have Ala at position 57. For DR4 IDDM haplotypes 94% are non-Asp (Ala) at position 57 (Morel et al., in press), compared

with 75% of the control DR4 haplotypes (Todd et al. 1987).

The strongest case, however, for the involvement of non-Asp in IDDM predisposition comes from haplotypes other than DR3 and DR4, particularly DR2. DR2 control haplotypes can be divided into two common types, MLC subtypes Dw2 and Dw12, which are both DQβ Asp 57. However, most IDDM DR2 haplotypes are of the rare "AZH" type, and these carry Ser at position 57 (Todd et al. 1987; Horn et al. 1988). Overall, 87.5% of the non-DR3, non-DR4 haplotypes found in diabetics were non-Asp, compared with 32.4% of those in controls (Morel et al., in press).

Why does the non-Asp 57 genotypic distribution in IDDM patients imply recessive inheritance for the associated predisposition (Todd et al. 1987; Morel et al., in press) when we know that the mode of inheritance of IDDM is not recessive? If we consider the DR genotype frequencies and expectations under a recessive model (see table 3C), the deviations from recessive expectations are observed excesses of DR3/DR4 and DR4/DRX and deficiencies of DR3/DR3, DR4/DR4, DR3/DRX, and DRX/DRX; the overall deviations are highly significant. One can see intuitively from these values that, if DR3 and DR4 are considered together as one allele (called C for this discussion), the deviations from recessive expectations will be much less, as the excesses and deficiencies will tend to cancel each other out. When we do this analysis, the observed numbers for the genotype frequencies of the combined allele C do differ from recessive expectations—but to a much lesser degree than when we consider DR3 and DR4 separately (the χ^2 value is just significant—observed values for the genotypic classes CC, Cc, and cc are 274, 237, and 33, respectively, with recessive expectations of 283.2, 218.6, and 42.2, respectively; $\chi^2 = 3.85$, df = 1, $P < .05$). In other words, a genetic variant that is highly associated with the disease state—for example, DQβ non-Asp 57—but is associated with both DR3 and DR4 predisposition will tend toward recessive expectations in this test.

Is DQβ Asp 57 directly involved in protecting individuals from IDDM? The simultaneous occurrence of non-Asp 57 in a number of predisposing haplotypes—for example, DR3, DR4.DQw3.2, DR1, and DR2.AZH—is a powerful argument. This correlation also extends to the nonobese diabetic (NOD) mouse. The NOD Aβ gene, the mouse homologue of DQβ, has Ser at position 57, while nondiabetic mouse strains, including the nonobese normal (NON) strain, which is closely related to NOD, have Asp (Todd et al. 1988*b*).

A number of observations, however, demonstrate that additional factors are involved in IDDM predisposition (Klitz 1988). One specific exception to the position 57 pattern is given by the DR7 haplotype. DR7 is relatively neutral with respect to IDDM predisposition when compared with other DR antigens (see table 4). Yet, a large proportion of control DR7 haplotypes are non-Asp (six of 11 in the study of Morel et al., in press), and these haplotypes are identical to DR3 haplotypes in the region of DQβ position 57 (Horn et al. 1988). The DQα chain of DR7 is unique at three amino acid residues, and it is argued that interaction of variable DQα and β chains may be important in determining IDDM susceptibility (Nepom et al. 1987; Todd et al. 1988*a;* Morel et al., in press), although the explanation may not be this simple.

Similarly, although DR1 is positively associated with IDDM, its predispositional effect is much weaker than those of DR3 and DR4. Nevertheless, all control DR1 haplotypes analyzed have been non-Asp (Morel et al., in press). DR1 haplotypes have Val at DQβ position 57, in contrast to the Ala of DR3 and DR4.DQw3.2 and the Ser of DR2.AZH; this difference could possibly account for the differential predispositional effects. Also in this vein is the observation that in the BB rat class II, β-chains from both IDDM-sensitive and IDDM-resistant strains have identical first-domain sequences with Ser at position 57 (Todd et al. 1988*b*). These observations, in addition to the DR3- and DR4-associated heterogeneity in IDDM predisposition, indicate that position 57 of DQβ cannot by itself explain HLA-associated IDDM predisposition. Furthermore, although the evidence favors some direct role of Asp 57 in protecting against IDDM, the possibility that Asp 57 is acting as a marker for a closely linked protective element cannot be excluded.

The challenge now is to determine just what genetic elements in the HLA region, along with that marked by DQβ position 57, determine IDDM susceptibility. These elements and their interactions must ultimately be able to explain the HLA-based heterogeneity in predisposition described here. In addition, a full understanding of IDDM may well require the elucidation of non-HLA contributions. For HLA, Sheehy et al. (1988) have recently presented data demonstrating that in DR4 haplotypes certain alleles at both DRβ (Dw4 and Dw10) and DQβ (DQw3.2, which is non-Asp 57) are needed for maximum susceptibility to IDDM. In IDDM susceptibility, the involvement of more than one HLA-region locus would not be surprising, given the direct participation of many of the HLA-region genes

in the immune response, as well as their structural similarities. (HLA-DP antigens as well as DR2 have recently been implicated in susceptibility to multiple sclerosis, and again the two effects cannot be explained by linkage disequilibrium [Odum et al. 1988].)

We have demonstrated that nearly every DR antigen either increases or diminishes IDDM risk to varying degrees (table 4). The DR3- and DR4-associated risks can further be ranked based on their B allele distributions (Field et al., in press), and the risk associated with DR3-B18, for example, is shown to be significantly higher than that associated with DR3 (non-B8, non-B18) (W. Klitz, personal communication). It is most likely that eventually each DR antigen, including DR3 and DR4, will be subdivided into predisposing, protective, or possibly neutral effects. It may be that only a small subset of DR7 non-Asp 57 haplotypes carry the additional factors needed for IDDM predisposition, so that overall DR7 appears to be predispositionally neutral.

It is not yet clear whether some additional factors in the HLA region, beyond non-Asp 57 at DQβ, are always required for increased IDDM susceptibility—or whether these additional factors merely increase risk. Likewise, are the same factors involved in increasing risk in the presence of Asp 57, or is a different mechanism operating in these cases? When we know the HLA-region molecular variants involved in DR3- and DR4-associated predisposition, will we then understand their different modes of inheritance and their synergistic effect? Complex immune-system mechanisms, possibly involving tolerance induction, cross-reactivity, and molecular mimicry, can be expected to be involved. The same predisposing variants may be associated with more than one DR antigen, or a number of different variants may be involved. If the latter is the case (and there is some evidence to this effect from our analyses and those of others [see, e.g., Tait et al. 1988; Field, in press]), then different predispositional and synergistic effects and modes of inheritance may apply to each possible combination of predisposing variants; and the rarer of these will be difficult to identify.

Much work lies ahead before the mechanisms of genetic predisposition to IDDM will be fully understood. The extensive task of analyzing DNA sequences from the HLA region in IDDM patients and in controls to identify amino acid differences associated with the disease state, while allowing for the fact that the disease is heterogeneous and that we cannot uniquely identify whether both or only one of a particular individual's HLA haplotypes are involved in disease predisposition, is a daunting task. It is thus satisfying that the molecular story is emerging and that the stage is set for a complete understanding of the HLA-linked genetic predisposition to IDDM.

The final story may well be complex, and we should be prepared to assimilate a wide array of possibilities, many of them novel and unexpected. A close cooperation between the methods of molecular and population analysis will be necessary to fully elucidate the inheritance of IDDM.

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