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Relative Predispositional Effects (RPEs) of Marker Alleles with Disease: HLA-DR Alleles and Graves Disease

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Summary

A method is described to reveal the relative predispositional effects (RPEs) (predisposing, protective, or neutral) of the HLA alleles or of any other marker system that is associated with a disease. When the disease is associated with two or more alleles of a locus, the RPE method identifies the associations sequentially according to their strength; thus the problem that a strong association with one allele can create misleading deviations in the frequencies of other alleles is alleviated. Using this method, we have examined the relative effects of HLA-DR alleles in susceptibility to Graves disease in the Caucasian population. The well-established positive association with DR3 was confirmed as the strongest effect. In addition, a negative association was found between DR5 and Graves disease. The reduced frequency of DR5 among patients is statistically significant and is not a result of the increase in DR3. Finally, when patients were divided according to the presence or absence of eye disease, the latter showed a significant increase in the frequency of DR4. With family data, linkage to HLA of Graves disease was established in both Caucasian and Chinese families by the sib-pair method.

Introduction

Graves disease is associated with HLA-DR3 (Bech et al. 1977; Farid et al. 1979; Allannic et al. 1983; Schleusener et al. 1983; Stenszky et al. 1983, 1986; Farid and Thompson 1986). Frecker et al. (1988) have shown that the frequency of DR4 is lower in Graves disease patients who have eye disease than in those who do not have eye disease and have proposed a negative association between DR4 and eye disease. They also have suggested that DR7 in combination with B8 increases the risk for eye disease. Stenszky et al. (1983) have made a similar observation for the DR7-B8 combination among eastern Hungarian patients. There have been a number of studies that have examined DR5, but the results have not been consistent: Schleusener et al. (1983) reported an increased frequency of DR5 among patients without eye disease, while in the data presented by Stenszky et al. (1983) and Allannic et al. (1980) patients with Graves disease had a slightly lower frequency of DR5 than did controls; the difference, however, was not statistically significant.

When one or more HLA alleles show strong associations with a disease, the relative predispositional effects (RPEs) of the "other" alleles cannot readily be determined using odds-ratio (OR) estimates. For example, when the disease has a strong positive association with one allele (in this case Graves disease and DR3), it is difficult to assess whether a decrease in the frequency of another allele (possibly DR5) is just the expected consequence given the increased frequency of the first allele or is a true negative association. Similarly, a second positive association may be masked because of the expected decreased frequency imposed by the stronger primary association.

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In the present report we describe a method that reveals the RPEs of the "other" HLA alleles by using allele frequency data. (Also see Thomson et al. [1986, 1988, 1989] for application of the method to insulin-dependent diabetes mellitus, and see Thomson [1984] for a method to reveal RPEs by using phenotype data.) Data on Graves disease will be analyzed using this method. The aim is to determine the relative effects of the DR alleles in predisposing to or protecting against Graves disease. The analysis will also be applied to Graves patients who have eye disease and to Graves patients who do not have eye disease. HLA haplotype sharing distributions in sib pairs, Caucasian and Chinese, will be used for linkage and mode-of-inheritance studies.

Material and Methods

The RPE Method

The RPE method sequentially compares allele frequencies in patients and in controls to determine their predispositional, protective, or neutral effects relative to each other. The procedure to determine RPEs is as follows:

The overall frequency distribution of all alleles of a given HLA locus in patients is compared with the distribution in controls by using a χ^2 test to detect significant deviations. To identify the allele with the greatest predispositional effect, the individual alleles are reviewed for their contribution to the overall χ^2 value. For the allele with the largest deviation, the frequencies in patients and controls are compared using the normal distribution (Z statistic)—or the Poisson distribution if the allele is rare. When individuals cannot be uniquely classified as homozygotes or heterozygote/blanks, the above procedure should be performed twice for the two extremes, once under the assumption that all the ambiguous cases are homozygotes and then under the assumption that they are all heterozygote/blanks.

The above procedure is repeated to find the next largest RPE, except that (1) the allele which was detected in the previous round is excluded from both patients and controls and (2) the expected frequency distribution of the remaining alleles in the controls is normalized accordingly. This sequential process of identifying associated alleles and removing them is continued until no significant overall deviation is observed.

Compared with the OR calculation (Woolf 1955), which is the usual means for detecting associations, the RPE method has two main advantages: (1) the associated alleles, predisposing or protective, are detected sequentially according to the strength of their association with the disease, and (2) the problem that deviations in the frequency of an associated allele can create misleading deviations in the frequencies of other alleles (or can mask their effect) is alleviated by the RPE method.

The RPE analysis requires slightly different data than does the OR method. To calculate the OR, one needs to know how many of the subjects have the antigen and how many do not. The genotypes need not be known because no distinction is made between those who have one copy of the antigen (heterozygotes) and those who have two copies of it (homozygotes). The RPE method, on the other hand, is based on genotypes and uses allele frequencies.

The following data were analyzed by both the RPE method and OR calculations (Woolf 1955).

Data

The data consist of 133 randomly selected Newfoundland patients with Graves disease, 64 of whom also have eye disease. Patients who were considered to have eye disease had grade 1c or greater according to the American Thyroid Association classification. Because Graves eye disease appears before, during, or after the hyperthyroidism, patients were followed for a mean of 7.5 years. Patients with no symptoms or with signs of lid lag only were considered to have no eye disease, whereas those with soft-tissue changes, proptosis, and/or extraocular muscle dysfunction were considered to suffer from eye disease. Eye disease was scored without knowledge of the genetic marker data. No correlation was found between disease severity and the HLA type. (Data and diagnosis have been described in more detail by Frecker et al. [1988]).

With the available data we were able to perform the RPE test on the DR alleles and to calculate OR for the A, B, C, and DR antigens. Because of the presence of a total of 24 individuals with ambiguous genotypes, the RPE analysis was performed twice, once under the assumption that all ambiguous cases were homozygous and then under the assumption that they were all heterozygous/blank . Of the ambiguous cases, those with eye disease included one DR1/–, one DR2/–, seven DR3/–, and one DR7/–, and those without eye disease included one DR2/–, eight DR3/–, two DR4/–, one DR6/–, and two DR7/–. The results shown in tables 1–3 were obtained by assuming that all ambiguous cases were heterozygote/blank. Similar results were obtained when all ambiguous cases were assumed to be homozygotes, with one exception: DR7 among patients with eye disease. This particular case will be addressed below.

Table 1

RPEs of DR Alleles in Graves Disease

		Round 1 of Comparison			Round 2: DR3 Removed			Round 3: DR3 and DR5 Removed		
(1) DR	(2) Observed	(3) Expected	(4) χ^2	(5) *P*	(6) Expected	(7) χ^2	(8) *P*	(9) Expected	(10) χ^2	(11) *P*
1.....	25	25.27	.00		20.51	.98		23.20	.14	
2.....	32	42.03	2.39		34.11	.13		38.59	1.13	
3.....	76	31.92	60.87	<.00001	...	...		...	...	
4.....	38	33.78	.53		27.42	4.08		31.02	1.57	NS
5.....	10	38.04	20.67		30.87	14.11	<.0001	...	...	
6.....	21	29.79	2.59		24.18	.42		27.35	1.47	
7.....	31	31.92	.03		25.91	1.00		29.31	.10	
8.....	7	7.98	.12		6.48	.08		7.33	.01	
10.....	2	2.13[a]			1.73[a]			1.95[a]		
Other	24	23.14	.03		18.78	1.45		21.25	.36	
Total...	266	266	87.23	<.001	190	22.25	<.01	180	4.78	NS

Note.—Significant values are underlined; NS = not significant.

[a] Because of the small numbers in the DR10 class, it was combined with the DR8 class.

Results

Table 1 shows the number of DR alleles in Graves patients (col. 2), the number expected from controls (according to the Ninth Histocompatability Workshop data) on the basis of the assumption that there are no differential predispositional effects of the DR alleles (col. 3), and the contribution of each allele to the overall χ^2 (col. 4). The overall χ^2 is statistically significant at $P < .001$ (col. 5). The allele with the largest contribution to this χ^2 is DR3, as expected (observed 76, expected 31.92; $P < .00001$). (The significance of the deviation in the DR3 frequency was determined by *Z* statistics.) After DR3 is removed and the comparison is repeated (cols. 6–8), the overall χ^2 is still significant ($P < .01$). The largest deviation is now due to the decrease in the frequency of DR5 among patients (observed 10, expected 30.87; $P < .0001$). Finally, there is a nonsignificant increase in DR4 (observed 38, expected 31.02) once DR3 and DR5 are excluded (cols. 9–11).

ORs were calculated for the A, B, and DR antigens. The most significant results were those for DR3 (OR = 4.57, $P < .0005$) and DR5 (OR = .23, $P < .0005$). The only other significant results were for B8 (OR = 3.94) and A1 (OR = 2.63), both of which are in linkage disequilibrium with DR3.

Presence or Absence of Eye Disease

Among the 133 Graves patients, 64 had eye disease. We subdivided the patients on the basis of presence or absence of eye disease and examined the DR allele frequency distribution in each group. As shown in table 2, the most significant deviation in the patients with eye disease is the increased frequency of DR3 (observed 40, expected 15.36; $P < .00001$), followed by the decreased frequency of DR5 (observed 4, expected 14.30; $P < .005$). When both DR3 and DR5 are removed, an increase in the frequency of DR7 is noted (observed 19, expected 13.68), but the significance of it cannot be assessed, because of the ambiguous genotypes of 10 individuals. Seven of these patients were DR3/–, others were DR1/–, DR2/–, and DR7/–. If they are all taken as heterozygote/blank, as shown in table 2, the deviation is not significant. But if we assume homozygosity, the increase in DR7 becomes statistically significant ($P < .02$). The OR for DR7 is slightly greater than one (1.45), but it is not statistically significant.

When patients who did not have eye disease were considered (table 3), a different pattern of RPEs was observed. The increase in DR3 (observed 36, expected 16.56; $P < .00001$) was again the most significant deviation. However, the next significant deviation was now an increase in DR4 (observed 27, expected 14.72; $P < .001$), implicating a predispositional effect of DR4 to Graves disease without eye disease. After removal of DR3 and DR4 from the analysis, the overall χ^2 is not significant, although the decrease in DR5 by itself is significant (observed 6, expected 14.24; $P < .01$).

The most striking OR values in both groups of patients, with or without eye disease, were due to the in-

Table 2

RPEs of DR Alleles in Graves Disease Patients with Eye Disease

		Round 1 of Comparison			Round 2: DR3 Removed			Round 3: DR3 and DR5 Removed		
(1) DR	(2) Observed	(3) Expected	(4) χ^2	(5) *P*	(6) Expected	(7) χ^2	(8) *P*	(9) Expected	(10) χ^2	(11) *P*
1.....	12	12.16	.00		9.50	.66		10.83	.13	
2.....	14	20.22	1.91		15.80	.20		18.01	.89	
3.....	40	15.36	39.53	<.00001	...	...		...	...	
4.....	11	16.26	1.70		12.70	.23		14.47	.83	
5.....	4	18.30	11.17		14.30	7.42	<.005	...	...	
6.....	13	14.34	.12		11.20	.29		12.76	.00	
7.....	19	15.36	.86		12.00	4.08		13.68	2.07	NS
8.....	3	3.84[a]			3.00[a]			3.42[a]		
10.....	2	1.02[a]			.80[a]			.91[a]		
Other	10	11.14	.06		8.70	.050		9.92	.04	
Total...	128	128	55.35	<.001	88	13.38	NS	84	3.96	NS

NOTE.—Significant values are underlined; NS = not significant.

[a] Because of the small numbers in DR8 and DR10 classes, they were combined with the "other" DR class.

crease in DR3, B8, and A1 and to the decrease in DR5. Furthermore, in the group that did not have eye disease, the OR for DR4 was 2.06 ($P < .005$), which also supports the existence of a DR4-associated predisposition to Graves disease without eye disease. There were no other significant results from the OR analysis.

Sib-Pair Studies

Sib-pair data were used to test linkage to HLA. We pooled the reported data on HLA haplotype sharing in affected sibs from Caucasian families (McMichael 1975; Farid 1979, 1980*a*, 1980*b*, 1981; Raffoux 1979; Torfs 1983; Bertrams 1984; Torfs et al. 1986) and from Chinese families (Chan et al. 1980; Torfs 1983; Stenszky et al. 1986; Tian et al. 1986). There are a total of 45 Caucasian families with 64 sib pairs, and 30 Chinese families with 43 sib pairs. Among the Caucasians, 28 sib pairs shared two haplotypes, 25 shared one, and 11 shared zero; among the Chinese, 19 shared two haplotypes, 22 shared one, and 2 shared none.

Table 3

RPEs of DR Antigens in Graves Disease Patients without Eye Disease

		Round 1 of Comparison			Round 2: DR3 Removed			Round 3: DR3 and DR4 Removed		
(1) DR	(2) Observed	(3) Expected	(4) χ^2	(5) *P*	(6) Expected	(7) χ^2	(8) *P*	(9) Expected	(10) χ^2	(11) *P*
1.....	13	13.11	.00		11.01	.36		9.46	1.32	
2.....	18	21.80	.66		18.31	.01		15.74	.32	
3.....	36	16.56	22.82	<.00001	...	...		...	...	
4.....	27	17.53	5.12		14.72	10.24	<.001	...	...	
5.....	6	19.73	9.55		16.57	6.74		14.24	4.77	<.01
6.....	8	15.46	3.60		12.98	1.91		11.16	.89	
7.....	12	16.56	1.26		13.91	.26		11.95	.00	
8.....	4	4.14[a]	.29		3.48[a]			2.99[a]		
10.....	0	1.10[a]			.93[a]	.85		.80[a]	2.46	
Other	14	12.01	.33		10.08[a]			8.67[a]		
Total...	138	138	43.63	<.001	102	20.37	<.001	75	9.76	NS

NOTE.—Significant values are underlined; NS = not significant.

[a] Because of the small numbers, classes DR10 and DR8 were combined in round 1 and DR8, DR10, and "other" DR were combined in rounds 2 and 3.

The observed haplotype-sharing frequencies were tested by a χ^2 test against the 1/4, 1/2, 1/4 distribution, which is the expected one if the disease is not linked to HLA. Both the Caucasian and the Chinese data deviate significantly ($P < .005$) from these expectations, indicating the presence of a disease gene in or near the HLA region in both populations.

The data were also tested against the expectations from recessive and dominant (additive) models. In order to correct for possible ascertainment bias, sibships that had more than two affected individuals were weighted using the three schemes outlined by Motro and Thomson (1985) and then were combined with the pairs. Neither recessive nor dominant modes of inheritance could be rejected unequivocally for either population. However, the Chinese data are closer to the dominant expectations than to the recessive expectations with an estimated allele frequency of .04 (under the assumption of incomplete single ascertainment.) The Caucasian data, on the other hand, are more compatible with a recessive model with an estimated allele frequency of .5. The haplotype-sharing values suggest the presence of some sporadic cases in the Caucasian data set (the characteristic effects of sporadics on the haplotype-sharing distribution are discussed by Hodge [1981] and Louis et al. [1983]).

Discussion

In agreement with the previously established association between DR3 and Graves disease, we found the effect of this allele to be the most significant among all DR alleles that were tested. When patients were classified according to the presence or absence of eye disease, the frequency of DR3 was found to be significantly increased in both groups.

Next to DR3, the most notable effect was that of DR5. We found a significant decrease in the frequency of DR5 in the collective cohort of Graves patients, as well as in the subgroup that had eye disease. The group that did not have eye disease showed only a marginal decrease in DR5 frequency. This is the first time that the significance of the DR5 effect has been demonstrated statistically—and independently of the effect of DR3 (i.e., the decrease in DR5 is not solely due to the increase in DR3).

Graves patients with eye disease did not show any significant deviations in DR frequencies, other than the increase in DR3 and the decrease in DR5, when compared with the controls. Patients without eye disease, on the other hand, showed a statistically significant increase in the frequency of DR4 when they were compared with controls. The predispositional effect of DR4 in this group was noted to be next to that of DR3 and was clearly stronger than the protective effect of DR5. Frecker et al. (1988), using the OR method, found a significantly lower frequency of DR4 in Graves patients who had eye disease than in those who did not have eye disease, and they suggested that DR4 plays a role in protection against eye disease. However, an alternative hypothesis that must be considered is that DR4 has a predispositional effect associated with Graves disease without eye disease. This possibility is favored by the present study, which shows both that DR4 is increased over population frequencies in patients without eye disease and that the frequency of DR4 in patients with eye disease is not different from that in the general population, once the data are corrected for the effects of the DR3 and DR5 associations. These results indicate that DR4 is associated with predisposition to Graves disease without eye disease but is neutral in regard to susceptibility to Graves disease with eye disease.

In summary, the positive association between DR3 and susceptibility to Graves disease is confirmed; DR5 is found to be associated with protection against Graves disease; and DR4 is found to be associated with predisposition to the subtype of Graves disease that does not involve eye disease.

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