

Brief Communications

The Relationship Between HLA Antigens and Bermuda Grass Hayfever

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Associations between atopic diseases and lymphocyte antigens seem to be possible, but not certain (for a review see McMichael and McDevitt 1977). Several studies (Levine *et al.* 1972, Marsh *et al.* 1973, Yoo *et al.* 1976, Bruce *et al.* 1976, Scholtz *et al.* 1976, Seignalet *et al.* 1976, Marsh and Bias 1977) have shown a certain association between atopy and different HLA antigens or haplotypes, within the same family, but without demonstrating a definite association with one specific antigen or haplotype. Rachelefsky and coworkers (1976) found a strong association between atopic asthma and B lymphocyte group 2, which was not paralleled by an association with the *HLA* complex. Since hayfever is the classical, representative atopic disease, we present here the HLA typing of 141 patients suffering from Bermuda grass hayfever. These patients comprise two groups: 86 of them were studied during the 1975 season; 65 during the 1976 season; and 10 during both seasons. The diagnosis of hayfever was based on a positive correlation between: (a) family history (several cases of proven atopy in three consecutive generations); (b) clinical picture (seasonal rhinitis, conjunctivitis, and asthma during the Bermuda grass blooming season only); and (c) positive skin tests to whole Bermuda grass extract, obtained from the Hollister-Stier Laboratory. The controls were pooled from data on a recently typed healthy Jewish population (Brautbar *et al.* 1977, Gazit *et al.* 1978a, b, c, Zamir *et al.* 1978) so as to match the stratification of the patients into individuals of Ashkenazi (Russia, Poland, and Roumania) and Sephardic (Marocco and Iraq) origin, in a total of 597 controls for 65 patients.

For HLA typing the standard N.I.H. technique was employed (Brand *et al.* 1970) and the lymphocytes were typed for 9 and 16 antigens at locus *A* and *B*, respectively, with 120 alloantisera obtained from the N.I.H. serum bank and from local preparations. The results in *both* groups (1975 and 1976) show an association with allele *B27*, at a significance of 1%, and this suggests more than a spurious correlation.

The data were subjected to a relative risk analysis. The Haldane (1955) relative risk evaluation uses the formula:

$$x = \frac{(a + \frac{1}{2})(d + \frac{1}{2})}{(b + \frac{1}{2})(c + \frac{1}{2})}$$

where a , b , c , and d are, respectively, the number of patients with and without the particular antigen and the number of controls with and without the antigen. The relative risk, x (more accurately, the adjusted odds ratio statistic), indicates approximately how many times more frequent the disease is in individuals carrying the antigen than in individuals lacking the antigen.

The large sample variance of $\log x$ is of the order

$$v = \frac{1}{a+1} + \frac{1}{b+1} + \frac{1}{c+1} + \frac{1}{d+1}$$

so that the standard deviation (SD) of $\log x$, is approximately $\sqrt{v}$. The chi-square values were calculated using the Woolf (1955) formula:

$$\chi^2 = \frac{(\log x)^2}{v}.$$

Combined estimates from different populations were obtained through the following equalities:

$$\bar{x} = \exp \left\{ \frac{\sum \log x/v}{\sum 1/v} \right\} \quad (\text{combined relative risk}),$$

$$\bar{\chi}^2 = \frac{(\sum \log x/v)^2}{\sum 1/v} \quad (\text{combined chi-square for association}), \text{ and}$$

$$\frac{\bar{\chi}^2 = \sum (\log x)^2}{v - \bar{\chi}^2} \quad (\text{combined chi-square for heterogeneity}).$$

Figure 1 descriptively summarizes the data. A visual examination supported by the relative risk computations shows no significant association in locus A and two significant associations in locus B , namely with $BW21$ and $B27$. The association with $BW21$ did not stand up in the 1975 patient group, and its significance is therefore likely spurious. The proportion of $B7$ alleles between controls and patients is inverse to the ratio found for $B27$. However, the difference between the number of patients and controls makes the association with $B7$ insignificant, since smaller SDs correspond to larger samples. In all of the individual typings for the two loci, there was only one blank. Since this accounted for less than 1% of the results, blanks were considered to be homozygotes. Further analysis of the data for the separate groups showed only one significant association, which is with $B27$ in the Ashkenazi group, whereas the association with $B27$ in the Sephardi group was insignificant. This is indicated in Table 1A, which also presents the weighted relative risk for the whole population ($\bar{x}$), the chi-square for heterogeneity ($\bar{\chi}_H$), and a weighted chi-square for association significance ($\bar{\chi}^2$).

Ten of the patients examined in the 1975 study reappeared for the 1976 study. Using the same controls, the only consistent result between the two studies is an association with the $B27$ allele in the pooled population. This is summarized in Table 1B, which also presents relative risk, x , and $\bar{\chi}^2$ values for each group separately and for the whole population. These data indicate a 1% significant association between hayfever and $B27$. No conclusion regarding different strengths of association in the two different Jewish groups could be validated.

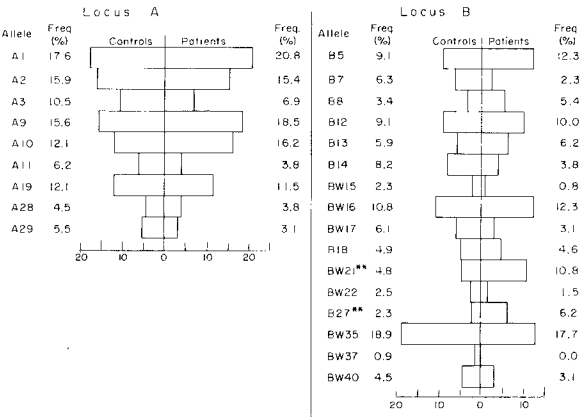


Fig. 1. Allelic frequencies in patients (65) and controls (597). (**) = Associations significant at the 1% level

Table 1A. Relative Risk, χ^2 Computations, and Combined Estimates for Alleles *B27* and *BW21*, with the 1976 Patients

Patient Population	Association with <i>B27</i>		Association with <i>B21</i>	
	<i>x</i>	$\chi^2_{(1)}$	<i>x</i>	$\chi^2_{(1)}$
Ashkenazi population	4.5	10.1 ^a	2.9	5.5
Sephardi population	1.6	.5	2.5	4.9
Pooled population	2.9	7.5 ^a	2.6	9.7 ^a
Combined estimates	$\bar{x} = 3.2$	$\bar{\chi}^2_{(1)} = 9.0$ $\bar{\chi}^2_H = 1.6$	$\bar{x} = 2.6$	$\bar{\chi}^2_{(1)} = 10.5$ $\bar{\chi}^2_H = .1$

^a Significant at the 1% level

Table 1B. Relative Risk, χ^2 Computations, and Combined Estimates for Allele *B27*, with the 1975 Patients of the Pilot Study

Patient Population	Association with <i>B27</i>	
	<i>x</i>	$\chi^2_{(1)}$
Ashkenazi population	1.8	1.1
Sephardi population	2.6	3.7
Pooled population	2.2	4.0 ^a
Combined estimates	$\bar{x} = 2.2$	$\bar{\chi}^2_{(1)} = 4.6$ $\bar{\chi}^2_H = .2$

^a Significant at the 5% level

Table 2. Allelic Frequencies in Patients and Controls Subdivided into Ashkenazi and Sephardi Groups

Locus A										
Patient Group	Alleles									
	A1	A2	A3	A9	A10	A11	AW19	A28	A29	Total
Ashkenazi patients	12	10	5	14	18	4	5	3	3	74
Ashkenazi controls	102	126	54	110	104	44	85	43	38	705
Sephardi patients	15	10	4	10	3	1	10	2	1	56
Sephardi patients	108	64	71	76	41	30	59	11	28	488

^a Controls differ in their distribution, but patients do not, suggesting that the origin variable is inconsequential

To obtain larger counts we initially pooled together the origins of the individuals so that we had a patient population and a control population consisting of a mixture of Ashkenazi and Sephardi individuals (see Fig. 1). This pooling is validated by our choice of a 55 : 45 ratio of Ashkenazi and Sephardi, as controls matching the population stratification of the patients.

In Table 2 the data are presented separately for the Ashkenazi and Sephardi groups. The Hardy-Weinberg equilibrium approximation (see Cavalli-Sforza and Bodmer 1971), implying a random union of gametes, provides a test of whether the patient population has the same allelic distribution as the control population. The χ^2 values for the 2×9 contingency table, corresponding to locus A, and the 2×16 contingency table corresponding to locus B, are reported in Table 3A, without separating the Ashkenazi and Sephardi groups. A stronger association of hayfever allergy with locus B rather than with locus A is clearly noted.

In our pooled population the condition variable (patient versus control) is therefore independent of the origin variable (Ashkenazi versus Sephardi). Combined estimates computed using the formulas developed by Woolf (1955) show that such collapsing indeed results in insignificant discrepancies between the estimates computed for the pooled (collapsed) population and the combined estimates (see Tables 1A and 1B). For the conditions permitting the collapse of a variable (under a general mode) see Bishop and coworkers (1975).

When the allelic composition of the Ashkenazi and Sephardi patient groups was investigated, no significant difference in allelic composition was found between them. This observation was true of both locus A and locus B (Table 3B). We therefore suggest that the relation of hayfever to HLA is unaffected by the origin variable. In

Table 3A. χ^2 Values for the Patient-Control Versus Allelic Contingency Table

	χ^2	P^b	df
Locus A	5.4	.71	8
Locus B	27.3 ^a	.02	15

^a Significant at the 5% level

^b P = Probability ($\geq \chi^2$)

Locus <i>B</i>												
Alleles												
<i>B5</i>	<i>B7</i>	<i>B8</i>	<i>B12</i>	<i>B13</i>	<i>B14</i>	<i>BW15</i>	<i>BW16</i>	<i>BW17</i>	<i>B18</i>	<i>BW21</i>	<i>BW22</i>	<i>B27</i>
10	1	3	6	2	5	1	12	2	5	6	2	6
45	28	28	50	30	86	11	101	63	38	24	17	14
6	2	4	7	6	0	0	4	2	1	8	0	2
64	47	13	59	40	12	16	28	10	20	33	13	13

Table 2—continued

Locus <i>B</i>			
Alleles			
<i>BW35</i>	<i>BW37</i>	<i>BW40</i>	Total
12	0	1	74
135	8	28	706
11	0	3	56
91	3	26	488

Table 3B. χ^2 Values for the Ashkenazi-Sephardi Versus Allelic Contingency Tables in the Patient and Control Populations

	Patients		Controls	
	χ^2	<i>P</i>	χ^2	<i>P</i> ^b
Locus <i>A</i>	14.3	.07	45.8	.00 ^a
Locus <i>B</i>	19.4	.15	135.5	.00 ^a

^a Significant at the 0.1% level^b *P* = Probability ($\geq \chi^2$)

contrast to the patient population, a strong difference in HLA composition was found between the Ashkenazi and Sephardi control groups. These results indicate that further investigations are necessary.

The degree of correlation that we found was no stronger than that found by other researchers between hayfever and HLA antigens other than *B27*, and was similar to correlations that have been reported between many other diseases and HLA antigens. The correlation that we observed between Bermuda grass hayfever and HLA-*B27* is of particular interest because of the well-known relationship between HLA-*B27* and diseases with an immunopathological background (see, e.g., Nijenhuis 1977). It seems reasonable to assume that an *Ir*-like gene is linked to the *I*-region equivalent HLA-*D* locus, rather than to HLA-*A* or HLA-*B* (McMichael and McDevitt 1977). The data reported here support this assumption, since no association with HLA-*A* was found, and since the genetic map indicates that *B* is closer to *D* than is *A*. Finally, our results are compatible with the hypothesis that there is an association between HLA and atopy, and do not preclude the possibility of an epistatic relationship rather than pure genomic linkage.

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