

Allergens, IgE, mediators, inflammatory mechanisms

Immunoblotting analysis of twin sera provides evidence for limited genetic control of specific IgE to house dust mite allergens

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Background: Although some studies have shown genetic control of specific IgE responses to purified grass allergens, studies with other allergens have not supported this. The extent of such control for house dust mite (HDM) (*Dermatophagoides pteronyssinus*) allergens is unclear.

Objective: We sought to determine the extent to which genetic factors control the specificity of IgE responses to individual HDM allergens by comparing the immunoblot patterns of IgE binding of serum from monozygotic and dizygotic members of a large cohort of Australian twins.

Methods: HDM proteins separated by sodium dodecylsulfate-polyacrylamide gel electrophoresis were immunoblotted with sera from 317 twin pairs in which at least one twin had at least a weak HDM skin test response. Concordance levels for IgE binding to the individual HDM components were compared in the subset of 142 pairs of twins in which both twins were allergic to HDMs (skin prick test wheal diameter, > 3 mm).

Results: Over all 36 blotted bands, the mean case-wise concordance was 41% for monozygotic twins and 17% for dizygotic twins. Of the components detected, only those of molecular weights 23 kd and 16 kd were significantly different between the groups ($p < 0.01$). Differences observed between the monozygotic and dizygotic twins could be partly explained by overall IgE hyperresponsiveness.

Conclusion: Evidence for genetic control of IgE responses to 36 IgE-binding HDM components from a large sample of twins showed significant differences in concordance for two components and nonsignificant differences for several others. In the monozygotic twins, concordance never exceeded 67% for any band, and most monozygotic individuals recognized

components their co-twin did not. Genetic control of overall atopy in monozygotic twins is far stronger than that controlling specific sensitization to HDM allergens. (*J Allergy Clin Immunol* 1998;101:491-7.)

Key words: IgE repertoire, atopy, twins, house dust mites, immunoblotting, genetic, gene, allergy

Identifying and understanding the mechanisms relating the clinical expression of many chronic diseases to specific genes may allow predictions of risk and provide targets for specific and novel interventions. Allergic diseases such as asthma and hay fever involve complex and poorly understood interactions between both genetic and environmental components. Environmental components known to increase risk include early and high levels of exposure to aeroallergens such as those of the house dust mite (HDM) (*Dermatophagoides pteronyssinus*). The independent or confounding roles of tobacco smoke, infections, and diet are also suspected. Elucidating the genetic components of asthma may provide a key to understanding the impact and influence of these environmental and lifestyle factors.

The first comprehensive study attempting to define and measure the inherited component of allergic diseases was conducted in the 1910s.¹ Subsequent family studies have favored autosomal recessive, dominant, and codominant models of inheritance.² More recent studies favor separate groups of genes associated with airways hyperreactivity³ and either total⁴⁻⁷ or specific IgE production.

Initially, there was strong support for genetic control of specific IgE production, which was consistent with demonstrations of immune responsiveness in animal models. These studies in geographically isolated, allergic cohorts showed that IgE production to some, but not all, purified allergens from ragweed⁸ and ryegrass⁹ pollens was strongly linked to specific HLA class II loci on chromosome 6. Although some subsequent studies have reported weak associations between HLA haplotypes and either allergic responsiveness or anergy, there is no consistent evidence for human immune response genes providing specific control of any of the many other available and purified

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Abbreviations used

HDM:	House dust mite
M _r :	Relative molecular mass
PBS:	Phosphate-buffered saline
SPT:	Skin prick test

allergens. The existence and importance of such links remains of obvious and great interest in our understanding of asthma.

Because most twins share a common environment in childhood, when the major allergic sensitization occurs, differences in the concordance of the specificity of allergic responses between identical and nonidentical twins provides a basis for determining the relative contribution of genetic factors in determining such responses. We compared the spectra of IgE responses to the blotted components with the most important regional indoor allergen, HDM, in a large group of Australian twins selected on the basis of a family or personal history of asthma.

METHODS**Twin cohort**

The twin cohort¹⁰ providing the serum, including details of the testing procedure,¹¹ has been described elsewhere. Skin testing was performed with HDM (*D. pteronyssinus*) extracts and standardized lancets from Hollister-Stier Laboratories (Hollister-Stier, Spokane, Wash.). The mean of the longest and perpendicular wheal size was recorded after 10 minutes. Zygosity was initially assigned on the basis of the questionnaire and then confirmed by genetic typing with nine microsatellite markers.

Sera

Sera were obtained from 419 twin pairs. Of these, 317 pairs, in which at least one twin had any evidence of HDM allergy as determined by skin testing (defined here as a skin prick test (SPT) wheal diameter greater than 1 mm), were examined by immunoblotting because it was not known to what extent skin testing would be a reliable index of blot reactivity. However, for the statistical analysis of concordance of the blotting results, analysis was confined to the subgroup of 142 pairs (74 monozygotic, 68 dizygotic) in which both twins had unequivocal HDM allergy, regarded as a SPT wheal diameter greater than 3 mm each. Only five persons in the 419 pairs reported that they had received desensitization therapy.

Preparation of HDM allergen

Dried, clean HDM bodies (*D. pteronyssinus*) (kindly donated by the Commonwealth Serum Laboratories, Melbourne, Australia) were suspended in 0.01 mol phosphate-buffered saline (PBS) at 1 gm/10 ml, ground with a teflon/glass tissue grinder (Wheaton, Millville, N.J.) for 10 minutes, sonicated for 15 minutes, centrifuged (18,000g) for 30 minutes, and concentrated by ultrafiltration (10 kd cut-off) (Amicon, Beverly, Mass.). All operations were performed at 4°C or on ice. Aliquots (0.1 ml), at a final protein concentration of 15 mg/ml (BCA assay; Pierce Chemical Co., Rockford, Ill.), were stored at -20°C.

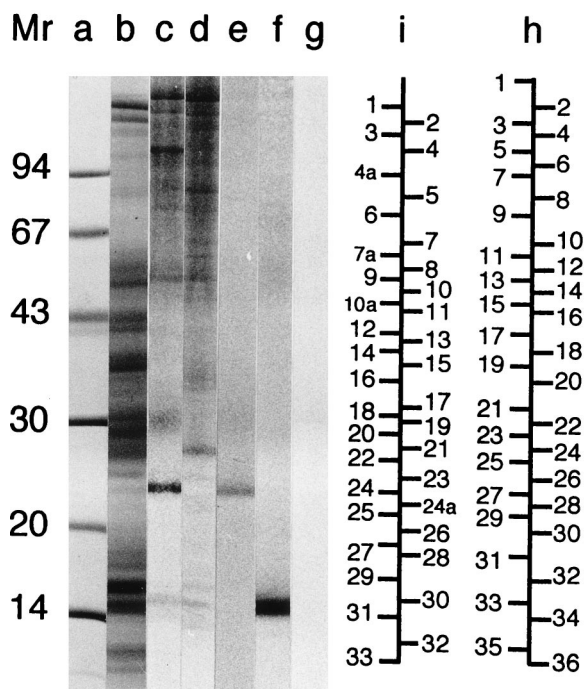


FIG. 1. Protein staining and immunoprobings of *D. pteronyssinus* mite body extract separated on nonreducing, gradient, sodium dodecylsulfate-polyacrylamide gel electrophoresis gels. Strips *a* and *b* show Coomassie Brilliant Blue staining of Mr markers and resolved HDM proteins. Strips *c* and *d* show reference pattern produced by two polyclonal rabbit sera with each blot as standards. Strips *e* and *f* show binding of monospecific antibodies to Der p 1 (Mr, 24 kd) and Der p 2 (Mr, 15 kd) on immunoblots, respectively. Normal rabbit serum control is shown in strip *g*. Strip *i* shows location of 37 rabbit immunoglobulin-binding components. Strip *h* shows location of 36 IgE-binding components in human sera.

Electrophoresis, transfer, and probing

Electrophoresis and electrotransfer were carried out with the equipment and reagents previously described,¹¹ with the exception that HDM extracts were not reduced by treatment with mercaptoethanol. Large gradient gels were each loaded with 750 µg HDM extract across 14 cm of the top, with low molecular weight markers (Pharmacia, Uppsala, Sweden) run in separate lanes down each side. Gels were run at 400V for 3.5 hours at 4°C and electrotransferred to 0.2 µm pore nitrocellulose membranes (Schleicher and Schuell, Dassel, Germany) by using a tank system at 80V for 3.5 hours at 4°C. Nitrocellulose sheets were reversibly stained with Ponceau S to confirm blot quality, photocopied, and stored damp at 4°C until used. Strips containing a 1 cm wide section of the resolved HDM proteins and the molecular weight markers were removed from each transfer and stained in Amido Black for reference.

Probing and autoradiography were carried out as described elsewhere.¹¹ In outline, 5 mm wide strips were blocked in 0.1% Tween-PBS for an hour, incubated with 10% atopic sera overnight, then incubated for 6 hours in ¹²⁵I-anti-IgE. All volumes were 1 ml. Sera from each twin pair were blotted with adjacent nitrocellulose strips, human fetal cord serum was used as a negative control, and all incubation steps had 3× 5-minute washing steps between them. Strips were dried before autoradiography, which was initially for 3 days and repeated for

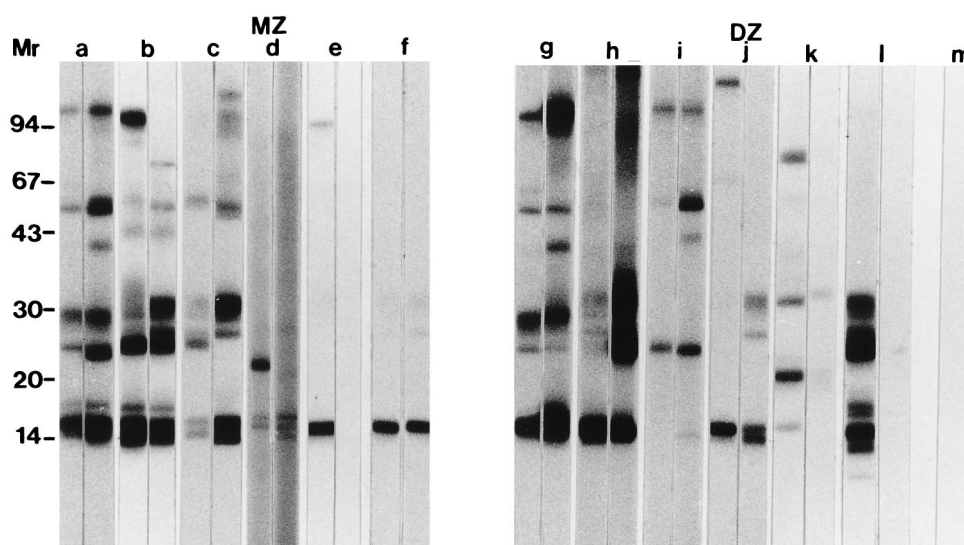


FIG. 2. Examples of patterns of IgE binding to *D. pteronyssinus* shown by six pairs of monozygotic sera (strips a through f) and six pairs of dizygotic sera (pairs g through l). Pairs are from different blots and patterns between pairs cannot be compared precisely. Negative control human fetal cord serum is shown in strip m.

shorter or longer times to maximize resolution of individual components. Scoring was performed blind as to zygosity.

Rabbit and murine monoclonal control sera

Four strips cut from each blot were incubated with two rabbit antisera raised against whole HDM bodies and monospecific anti-Der p 1 and anti-Der p 2 antisera¹² diluted 1:1000 in PBS-bovine serum albumin (0.5%) and Tween-20 (0.005% vol/vol). Specific binding was detected with alkaline phosphatase-labeled goat anti-rabbit immunoglobulin (Silenus, Melbourne, Australia) and a 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium staining system (Sigma Chemical Co.). Along with the protein-stained nitrocellulose strips, these immunostained strips were used to monitor for consistency between blots and to standardize the identification of IgE-binding HDM components on different blots.

Analysis of data

Each of 36 individual IgE-binding components identifiable on autoradiographs were initially scored visually as positive and graded 1+, 2+, 3+, 4+, or negative (-). For the analysis, however, only positive and negative categories were used. Because more dizygotic twin pairs were discordant for HDM sensitivity by SPT, for the analysis of specific reactivity, only the 142 pairs of twins in which both had an HDM SPT wheal diameter greater than 3 mm were used. With this criteria, 45% of sera were blot positive. A blot-negative result in this selected sample represents either a level of IgE undetectable by the present technique or sensitization to an epitope that is destroyed during blotting.

A number of different genetic analyses were performed. The simplest analysis is to examine concordance for presence or absence of a given band for each twin pair. If *C* is the number of pairs where both exhibit a positive band (+,+) and *D* is the number of pairs where only one twin exhibits a band (either +,- or -,+), the case-wise concordance is defined as:

$$\text{Case-wise concordance} = 2C/(2C + D).$$

The concordances were compared by Fisher's Exact test (two-tailed) and in view of the 36 tests performed, considered significant when *p* values were less than 0.01.

Different patterns in concordances in monozygotic and dizygotic twins are expected under different genetic hypotheses. For example, if a character (i.e., IgE specific to a given band) is completely genetically determined, is Mendelian recessive, and is present in one twin, then monozygotic twins are expected to be completely concordant and dizygotic twins concordant in one-quarter of cases. If genes have no effect on HDM allergy, but early household environmental levels of exposure were critical, then a similar level of concordance would occur in twins, with a high prevalence of sensitization in those twins with high exposure. If sensitization is completely random in nature, one would expect both concordances to be equal to the population risk.

Additionally, more complex statistical analyses were undertaken to adjust twin concordance for the effects of covariates. Logistic regression adjusting for the total number of bands the subject reacted with was performed with SAS PROC LOGISTIC (SAS Institute Inc., Cary, N.C.) by using all pairs in which a proband is positive for that band. This is similar to the exact test used above, except it adjusts for the number of positive bands. This was performed with SAS 6.09 PROC FACTOR (SAS Institute Inc.). We have also used the Mantel-Haenszel procedure to compare monozygotic versus dizygotic concordances across all 36 allergens simultaneously. In addition, principal components analysis of the binary correlations between 36 components was performed to adjust for overall IgE responsiveness. The relationship between monozygotic:dizygotic concordance and band frequency was compared by chi-squared analysis.

RESULTS

The complexity of the resolved *D. pteronyssinus* components on sodium dodecylsulfate-polyacrylamide gel electrophoresis gels is shown by Coomassie Brilliant

TABLE I. Percent frequency of band occurrence and number of concordant and discordant bands

M _r (kd)*	Band no.	MZ % frequency†	MZ number of bands		MZ case-wise concordance	DZ % frequency†	DZ number of bands		DZ case-wise concordance	Comparison Fisher's Exact p-value‡
			Concordant bands	Discordant bands			Concordant bands	Discordant bands		
130	1	0.8	0	1	0	1.4	0	2	0	
115	2	4.1	1	3	0.4	3.4	0	4	0	1
110	3	4.1	1	3	0.4	4.7	0	5	0	0.444
105	4	4.9	1	5	0.286	4.1	0	4	0	1
100	5	22.9	6	12	0.5	18.2	4	10	0.444	1
97	6	0.8	0	1	0	0.7	0	0	0	
94	7	4.9	1	3	0.4	6.1	1	5	0.286	1
78	8	6.6	2	4	0.5	5.4	1	3	0.4	1
69	9	5.7	3	1	0.857	1.4	0	1	0	0.4
60	10	1.6	1	0	1	3.4	0	2	0	0.333
56	11	6.6	2	2	0.667	3.4	0	3	0	0.429
54	12	14.8	1	13	0.133	13.5	2	10	0.286	0.58
51 (4)	13	28.7	10	10	0.667	23.0	2	14	0.222	0.034
49	14	19.7	6	8	0.6	15.6	1	6	0.25	0.337
47	15	0.8	0	1	0	1.4	1	0	1	1
44	16	2.4	1	1	0.667	4.1	1	3	0.4	1
40	17	4.9	1	5	0.286	2.7	0	4	0	1
37	18	1.6	0	1	0	2.0	0	3	0	
36	19	3.3	1	1	0.667	0.7	0	1	0	1
34	20	1.6	0	2	0	2.0	0	2	0	
33	21	0.8	0	2	0	1.4	0	0	0	
30 (3)	22	38.5	12	13	0.649	37.8	6	26	0.316	0.042
29 (3)	23	39.3	11	14	0.611	39.9	8	25	0.390	0.163
28	24	10.7	3	3	0.667	9.5	0	12	0	0.029
26	25	13.9	1	11	0.154	17.6	0	16	0	0.423
25	26	14.8	2	9	0.308	16.9	4	9	0.471	0.649
24 (1)	27	31.9	7	15	0.483	32.4	4	19	0.296	0.314
23	28	28.7	8	12	0.571	25.0	1	20	0.091	0.009
22	29	1.6	0	2	0	6.1	0	5	0	
21	30	3.3	2	0	1	2.7	0	4	0	0.067
18	31	1.6	0	3	0	4.1	0	3	0	
16	32	27.9	9	10	0.643	18.9	1	20	0.091	0.002
15 (2)	33	73.8	25	22	0.695	71.6	18	34	0.514	0.15
14 (5)	34	48.4	14	20	0.583	34.5	4	23	0.258	0.046
12	35	1.6	0	2	0	1.4	0	2	0	
11	36	4.9	1	5	0.286	2.0	0	8	0	0.429

Percent frequency of band occurrence for 270 individuals (122 monozygotic, 148 dizygotic) with positive blot results. Number of concordant and discordant bands for the 36 IgE-binding HDM components in a subset of 142 pairs (74 monozygotic pairs, 68 dizygotic pairs) in which both twins were allergic to HDMs but did not all have positive blot results.

MZ, Monozygotic; DZ, dizygotic.

*Numbers in parentheses indicate defined HDM allergens (Der p 1, 2, 3, 4, and 5) as determined by monospecific antibody and/or M_r and frequency distribution.

†Percent occurrence of each component for all blot-positive monozygotic ($n = 122$) or dizygotic ($n = 148$) sera.

‡ $p < 0.01$ considered significant (in bold), $p < 0.05$ shows trend (in italics).

Blue protein staining in Fig. 1, *strip b*. Two rabbit polyclonal antisera raised to HDM reacted with multiple components (Fig. 1, *strips c* and *d*), numbered in *strip i*. The allergens Der p 1 (relative molecular mass (M_r), 24 kd; component 33) and Der p 2 (M_r, 15 kd; component 27) were identified by monospecific rabbit antisera (Fig. 1, *strips e* and *f*). Normal rabbit serum failed to react (Fig. 1, *strip g*).

In total, 36 IgE-binding components of HDM extract were detected with sera from 317 pairs of twins, one of whom had, at some level, a positive SPT response to HDM. The numbering is shown in Fig. 1, *strip h*. Fig. 2

shows selected examples of autoradiographs for monozygotic pairs (*strips a* to *f*) and dizygotic pairs (*strips g* to *l*). The frequency of recognition of the HDM components by monozygotic and dizygotic twins was almost identical (Table I). In the combined data, components of M_r 100, 51, 30/29, 24 (Der p 1), 23, 16, 15 (Der p 2), and 14 kd were each bound by more than 50% of blot-positive sera.

Among all 317 pairs in which at least one had an SPT wheal diameter of 1 mm or greater, monozygotic twins were more likely to have positive concordant SPT responses (defined as exhibiting a wheal diameter to HDM

TABLE II. Skin prick test positivity to HDMs in a total cohort of 317 monozygotic and dizygotic twin pairs

Zygosity	Neither > 3.0 mm	One > 3.0 mm	Both > 3.0 mm	Total
MZ pairs	24	38	74	136
DZ pairs	19	94	68	181
Total	43	132	142	317

Twin pairs were selected on the basis that at least one of each pair had a SPT wheal diameter ≥ 1 mm. Table shows where neither, one, or both members exhibited a skin test wheal to *D. pteronyssinus* greater than 3 mm in diameter.

DZ, Dizygotic; MZ, monozygotic.

allergen > 3 mm) than were dizygotic pairs (54% vs 37%, $p < 0.0001$) (Table II). In this group monozygotic twins were more likely to be concordant at a greater number of bands than dizygotic twin pairs ($p = 0.017$ on chi-square for linear trend) (Table III). Monozygotic twins were also more likely to exhibit the same number of bands as their co-twin when compared with dizygotic twins (monozygotic: $r = 0.55$, $p < 0.0001$; dizygotic: $r = -0.09$, $p = 0.49$).

Individuals with larger SPT wheal diameters were more likely to recognize a greater number of allergen bands by blotting (Kendall's tau-B = 0.39, $p < 0.0001$) and there was a strong correlation between SPT diameter and the proportion of individuals who had positive blot results (Fig. 3).

Concordance and discordance for each of the 36 bands for the monozygotic and dizygotic groups among the 142 pairs tested that included both members who had positive SPT responses (wheal diameter > 3 mm) is shown in Table I. Concordance for a single band never reached 100%. The maximum monozygotic concordance (excluding rare bands seen in fewer than four individuals) was 69% for component 33 (a 15 kd component identified with Der p 2) compared with 51% for dizygotic twins (not significantly different).

By Fisher's Exact test, significant monozygotic:dizygotic differences in case-wise concordance, suggestive of genetic determination, were observed for two components: 28 ($p = 0.009$) and 32 ($p = 0.002$). The difference in concordance for another four bands (numbers 13, 22, 24, and 34) reached a p less than 0.05 level of significance (Table I). Overall, the mean monozygotic concordance was 41%, and the mean dizygotic concordance was 17%. In the regression analysis, which adjusts for the number of positive bands, the only significant monozygotic:dizygotic differences were for band 26 ($p = 0.06$), band 28 ($p = 0.07$), and band 32 ($p = 0.05$). No other bands were significant even at the 10% level. A Mantel-Haenszel test applied to all 36 bands simultaneously suggested that monozygotic twins overall were significantly more concordant than dizygotic twins (chi-square = 39.6, $df = 1$, $p < 0.0001$). The Breslow-Day test for heterogeneity among the monozygotic:dizygotic con-

TABLE III. Percentage of twin pairs (monozygotic and dizygotic) positive for the same component(s) in the cohort in which both members exhibited a SPT wheal diameter to *D. pteronyssinus* greater than 3 mm in diameter

Number of bands concordant	Percentage of MZ ($n = 74$ pairs)	Percentage of DZ ($n = 68$ pairs)
0	51.3	64.7
1	13.5	17.6
2	6.8	5.9
3	8.1	1.5
4	2.7	5.9
5	6.8	1.5
6	4.1	1.5
7	2.7	0
8	2.7	0
9	0	1.5
14	1.3	0

Significantly more components were concordant in monozygotic than dizygotic twins ($p = 0.017$).

MZ, Monozygotic; DZ, dizygotic.

cordance differences was not significant (chi-square = 32.4, $df = 27$, $p = 0.217$). If this test was significant, it would be strong evidence that genes controlling specific IgE to particular allergens existed. When the monozygotic:dizygotic ratio was compared with frequency of recognition of the band (to determine if less common bands were under genetic control) there was no significant trend (chi-squared = 3.4, $df = 4$).

By principal components analysis, the first principal component extracted from correlations between the HDM components was strongly correlated with the total number of components ($r = 0.97$). The second principal component is, by construction, completely uncorrelated with the first component, and thus represents correlations between the individual HDM allergens not explained by total number of HDM allergens recognized. The monozygotic correlation for the second component was 0.54, significantly higher than that for dizygotic twins (0.20, $p = 0.01$). This suggests there are some genetic factors different from those acting on the total band count.

DISCUSSION

Studies of allergy and asthma in this sample of twins have previously confirmed that atopy is highly heritable.^{10, 13} Furthermore, because sensitization to HDMs is the most common marker of atopy in urban Australian populations, this strong genetic effect extends to *D. pteronyssinus* skin prick testing (Table II). The focus of this study was to attempt to detect other genetic effects that control sensitization to particular major or minor *D. pteronyssinus* allergens. We found that genetically identical individuals (monozygotic twins) sensitized to HDMs can differ substantially in terms of the specific allergens to which they respond. We concluded that allergen-specific immune response genes may exist, but

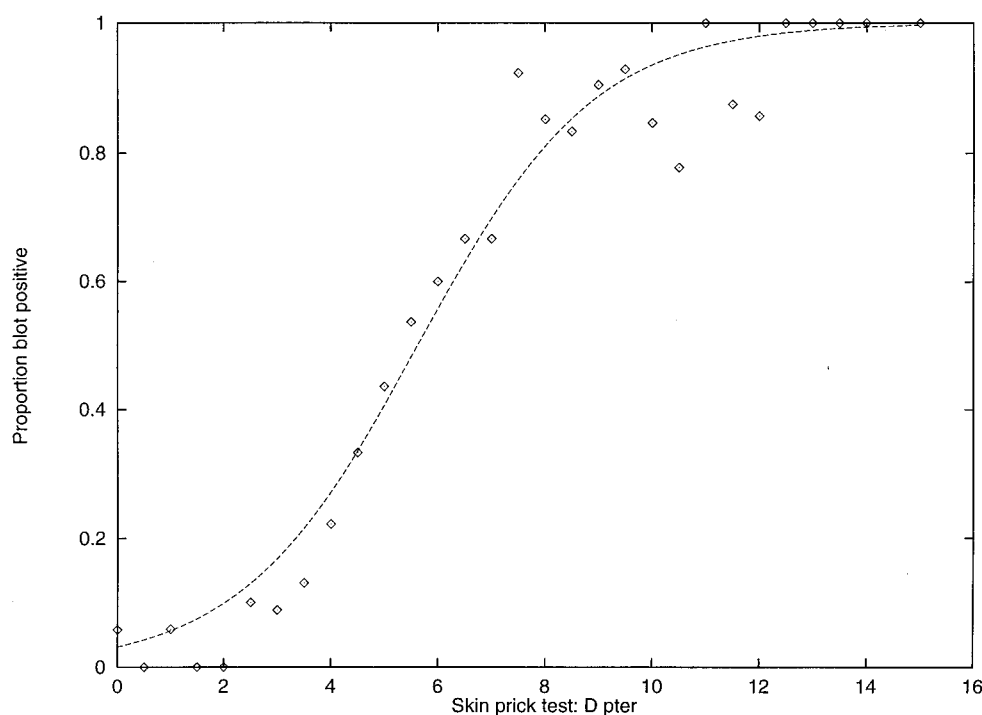


FIG. 3. Correlation between SPT wheal diameter and proportion of immunoblot positivity for *D. pteronyssinus* (*D pter*).

their effects are much smaller in magnitude than those controlling overall nonspecific IgE hyperresponsiveness.

In interpreting our results, it is important to note that monozygotic twins were more concordant for sensitization to most specific allergen components than dizygotic twins. Such a finding suggests genetic factors are at work. Precisely because this increased monozygotic concordance was general, rather than specific to any major allergen or specific band, we interpret this as caused by the effects of global or pleiotropic atopy genes.

The monozygotic concordance for a trait, such as individual allergen sensitization, places an upper boundary on the role that genetics can play in the determination of that trait. In the case of HDM sensitization, the mean monozygotic case-wise concordance for specific bands was only 41%, and in almost all HDM-positive monozygotic twin pairs there was some discordance in the individual HDM components recognized. In fact, monozygotic concordance never exceeded 67%.

In the comparison of case-wise concordance, only two IgE-binding components, bands 28 (23 kd) and 32 (16 kd), showed *p* values of less than 0.01, a value we would regard as significant in view of the multiple statistical tests being performed. Band 26 was also weakly significant after adjustment by regression analysis for the total number of bands to which a twin is sensitive.

We found that monozygotic twins tended to be more concordant than dizygotic twins in both the identity and number of allergens recognized. This could be explained by the monozygotic twin being more concordant for IgE

hyperresponsiveness, rather than by genes controlling the response to particular allergens, such as those encoding HLA or T-cell receptors.¹⁴ We tested these two alternative explanations by using two approaches.

The first method was to adjust concordance for specific bands versus the total number of HDM components expressed as a proxy for overall atopy. This logistic regression analysis found only three components (bands 28, 30, and 33) that provided statistically significant evidence of additional genetic determination as described above. This procedure was not powerful, owing to the relatively small numbers displaying sensitization to any particular band, and the parameters for even this simple model were not estimable in many cases.

The second method, principal components analysis, also suggested the presence of genetic factors acting independently of those causing the overall correlation in atopic status. The second component was not associated with any particular band, however, and may merely reflect residual confounding caused by atopy genes.

The combination of both these lines of evidence suggests that some components are under specific genetic control. There was also a trend, with exceptions, towards greater concordance for the lower molecular weight components among the monozygotic pairs. This is consistent with observations that genetic control can be demonstrated for ragweed pollen allergens of lower, but not of higher, molecular weights,⁹ possibly because

fewer IgE-binding epitopes per allergen molecule are involved. However, a hypothesis that commonly recognized (major) allergens may invoke more promiscuous immune responses outside genetic control, whereas allergens that are seldom recognized may be regulated by tighter genetic control, was not supported by comparisons of monozygotic:dizygotic concordance ratios plotted against band frequency.

It seems unlikely that individual differences in environmental factors alone would account for the large, observed differences in IgE specificity, particularly in the HDM-allergic monozygotic twins. The hypothesis that discordant allergen exposure to HDM allergens developed after the twins stopped sharing environments would be difficult to test as differences in longitudinal exposure are not known. In Australia the opportunity for exposure to discordant HDM allergens is low (compared with grass pollen exposure) because the single dominant species is *D. pteronyssinus*, and exposure to other HDM species, which share some common determinants (e.g., *Euroglyphus maynei*, *Dermatophagoides farinae*, *Blomia* spp.), is rare.¹⁵ Significant differences in individual exposures to other arthropods that share allergic determinants with HDM is also unlikely.

The analytical approach of protein blotting has advantages and limitations. Such a method of analysis enables a wide spectrum of IgE-binding components of HDM to be analyzed simultaneously with a small sample of serum. The main limitation is that some IgE-binding epitopes are lost, with the effect that fewer sera would be recognized as being blot positive and there would be a different and lower frequency of IgE binding detected as compared with assays that use individual allergens in their native conformation. These effects were more apparent in sera from individuals with a smaller SPT wheal and, as Fig. 3 demonstrates, the majority of the sera were blot positive. The high level of discordance shows the binding was not nonspecific. There is no basis for assuming that the undetected epitopes are more likely to be under genetic control than those that are detected. The second limitation is that identification of components is based on their migration distance in a gel. Molecular weight calculations based on recombinant sequences suggest overlapping molecular weights for some HDM allergens, and bands as resolved here by their physicochemical properties may not always correspond precisely to allergens characterized from cloning studies. With studies involving the blotting of hundreds of sera, there is the possibility for misclassification of individual components. In this study the resolution between twin pairs was maintained by blotting pairs on adjacent strips. The alternative analytical route of creating separate assays for monocomponent native or recombinant allergens might have been more ideal, but would be logistically formidable and limited to a few components.

In summary, these results confirm strong genetic influences on atopy, but provide limited support for genetic control of IgE responses to specific HDM allergens, suggesting that most epitope-specific responses are shaped by nongenetic factors. In view of the level of discordance observed between monozygotic twins and the similar prenatal, infant, and childhood environments shared by most twins, these nongenetic factors are more likely to be random developmental events in the immune system, rather than differences in allergen exposure.

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REFERENCES

1. Cooke RA, Vander Veer A. Human sensitization. *J Immunol* 1916;1:201-37.
2. Blumenthal MN, Bonini S. Immunogenetics of specific immune responses to allergens in twins and families. In: Marsh DG, Blumenthal MN, editors. Genetic and environmental factors in clinical allergy. Minneapolis: University of Minnesota Press; 1990. p. 132-42.
3. Postma DS, Bleecker ER, Amelung PJ, Holroyd KJ, Xu J, Panhuysen CI, et al. Genetic susceptibility to asthma—bronchial hyper-responsiveness coinherited with a major gene for atopy. *N Engl J Med* 1995;333:894-900.
4. Cookson WO, Young RP, Sandford AJ, Moffatt MF, Shirakawa T, Sharp PA, et al. Maternal inheritance of atopic IgE responsiveness on chromosome 11q. *Lancet* 1992;340:381-4.
5. Shirakawa T, Li A, Dubowitz M, Dekker JW, Shaw AE, Faux JA, et al. Association between atopy and variants of the beta subunit of the high-affinity immunoglobulin E receptor. *Nat Genet* 1994;7:125-30.
6. Marsh DG, Neely JD, Breazeale DR, Ghosh B, Freidhoff LR, Ehrlich Kautzky E, et al. Linkage analysis of IL4 and other chromosome 5q31.1 markers and total serum immunoglobulin E concentrations. *Science* 1994;264:1152-6.
7. Meyers DA, Postma DS, Panhuysen CI, Xu J, Amelung PJ, Levitt RC, et al. Evidence for a locus regulating total serum IgE levels mapping to chromosome 5. *Genomics* 1994;23:464-70.
8. Levine BB, Stember R, Fotino M. Ragweed hay fever: genetic control and linkage to HLA haplotypes. *Science* 1972;178:1201-3.
9. Marsh DG, Meyers DA, Bias WB. The epidemiology and genetics of atopic allergy. *N Engl J Med* 1981;305:1551-9.
10. Duffy DL, Martin NG, Battistutta D, Hopper JL, Mathews JD. Genetics of asthma and hay fever in Australian Twins. *Am Rev Respir Dis* 1990;142:1351-8.
11. Sluyter R, Tovey ER, Duffy DL, Britton WJ. Limited genetic control of specific IgE responses to ryegrass pollen allergens in Australian twins. *Clin Exp Allergy* 1998. In press.
12. Tovey ER, Baldo BA. Comparison by electroblotting of IgE-binding components in extracts of house dust mite bodies and spent mite culture. *J Allergy Clin Immunol* 1987;79:93-102.
13. Duffy DL, Healey SC, Martin NG. Path analytic, sub-pair linkage and co-twin control studies of asthma and atopy [abstract]. *Am J Hum Genet* 1994;55(suppl):332.
14. Moffatt MF, Schou C, Faux JA, Cookson WOCM. Germline TCR- α restriction of immunoglobulin E responses to allergen. *Immunogenetics* 1997;46:226-30.
15. Tovey ER, Peat J, Marks G, Mahmic A, Woolcock A. Distribution of mite allergen Der p I in Australian communities [abstract]. *J Allergy Clin Immunol* 1995;739:325.