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Atopic Disease and Immunoglobulin E in Twins Reared Apart and Together

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Summary

Both genetic and environmental influences have been implicated in the etiology of atopic disease and in the determination of serum IgE levels. To quantify the relative contribution of these influences, we studied the prevalence of asthma and seasonal rhinitis, skin-test response, total serum IgE levels, and specific IgE, as measured by RAST, in a sample of MZ and DZ twins reared apart or together. Concordance rates for asthma, rhinitis, positive skin tests, and RAST were calculated. MZ twins, whether reared apart or together, showed a greater concordance than dizygotic twins reared apart or together. Maximum-likelihood tests of genetic and environmental components of the variation of total IgE levels revealed a substantial genetic component and a negligible contribution from common familial environmental effects.

Introduction

Family and twin studies have identified a substantial familial contribution to the etiology of atopic disease (Blumenthal and Bach 1983; Blumenthal and Amos 1987). Most previous studies have assigned an important role to genetic factors, but the precise modes of inheritance remain unresolved. Atopic disease appears to involve a complex immunologic reaction to a variety of environmental antigens. Although they are unable to identify the pattern of transmission, twin studies provide useful information concerning the relative importance of genetic and environmental factors (Martin et al. 1978).

The present investigation is the first to study rhinitis and bronchial asthma in twins reared apart since infancy. MZ twins reared apart offer the unique opportunity to examine the effects that different environments have on behavioral and medical characteristics of individuals with identical genotypes. Similarity among MZ twins reared together may result from common familial environmental factors, as well as from identical genes. In this present report, twins reared apart (MZ twins [MZA] and DZ twins [DZA]) are compared with MZ and DZ twins reared together (MZ twins [MZT] and DZ twins [DZT]) by using both conventional twin analysis and a biometric modeling approach (Eaves et al. 1979) to examine the heritability of bronchial asthma, seasonal rhinitis, serum IgE levels, and specific aeroallergen IgE responses.

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Material and Methods

The present study includes data collected at the University of Minnesota and at the Finnish National Public Health Institute. All participants were solicited without knowledge of the presence of atopic disease. Sixty-eight reared-apart twin pairs and two sets of reared-apart triplets were assessed as part of a comprehensive study evaluating medical and psychological differences between twins raised apart (Bouchard et al. 1990). Sixty-three reared-together twin pairs were located and recruited by utilizing the University of Minnesota Twin Registry (Lykken et al. 1990), as well as through birth certificates directly obtained from the Minnesota Department of Health. In addition to the Minnesota (MN) sample, data from 158 Finnish (F) reared-together twin pairs, obtained from Dr. Kimmo Aho of the Finnish National Public Health Institute (Helsinki), were evaluated (Sistonen et al. 1980). Descriptive data regarding zygosity, sex, age at testing,

Table 1

Descriptive Data for MZT/DZT and MZA/DZA

Variable	F		MN			
	MZT	DZT	MZA	DZA	MZT	DZT
No. of pairs	76	82	53	21	34	29[a]
Sex:						
Males	30	38	23[b]	5	15	11
Females	46	44	30	16	19	18
Age at assessment (mean ± SD years)	35 ± 13	38 ± 13	38.91 ± 12.89	43 ± 10.22	29 ± 11	30 ± 13
Length of separation[c] (mean ± SD years)	. . .	. . .	29.28 ± 14.53	37.85 ± 11.24	. . .	. . .
Age at separation (mean ± SD days)	. . .	. . .	113.40 ± 153.66	370.62 ± 498.29	. . .	. . .
Age range at assessment (years)	20–65	20–68	12–58	25–61	8–50	13–46

[a] Includes one set of DZ triplets.
[b] Includes two sets of MZ triplets, counted here as three pairs each.
[c] Data available for 51 MZA twin pairs and for 20 DZA twin pairs.

and age at separation for the twin groups are summarized in table 1. The F sample and the MN MZA/DZA sample were similar in age, although the MN MZT/DZT sample was somewhat younger than the others. There are more females than males in all groups.

Zygosity was determined by using serological analysis and anthropometric methods. MN twins were classified as MZ if they were concordant for eight red blood cell antigens and for ten serum protein types and red blood cell enzymes. Corroborating evidence for zygosity was obtained from observations of shape and attachment of earlobes, eye color, cranial measurements, and the z-score of fingerprint ridge counts (Lykken 1978). The calculated probability of a misdiagnosis of zygosity was less than .001 when this procedure was made. Similar genetic markers were used in evaluating the F twins (Sistonen et al. 1980).

Each twin of a pair underwent a history and physical examination, phlebotomy, and skin testing at the same time. Seasonal rhinitis was defined as symptoms of nasal congestion, a clear nasal discharge, sneezing, and eyes watering and itching that increased in severity during a known pollen season. Bronchial asthma was defined as reversible airway disease, historically characterized as intermittent episodes of coughing and wheezing and/or evidence of reversible airway obstruction as documented by pulmonary functions studies.

Skin Testing

Intradermal titrations were performed by using standard methodology (Norman 1983) with dilutions of 10^{-1}–10^{-12} of separate stock solutions containing 125 μg *Amb a* I/ml, 140 μg *Amb a* III/ml, 75 μg *Amb a* V/ml, and 100 μg *Lol p* I/ml. Single-dose prick skin testing was done using *Ambrosia artemisiifolia, Aspergillus fumagatus, Alternaria teni, Phleum pratense,* and *Lolium perenne* solutions in concentrations (w/v) of 1/5,000 and 1/500. The *P. pratense* and *L. perenne* were obtained from Greer Laboratories (Lenoir, NC), and *Aspergillus fumagata* and *Alternaria teni* were obtained from Hollister-Steir Laboratories (West Haven, CT). *Amb a* I and *Amb a* III were prepared by using modifications of the procedures of King (1972) and Underdown and Goodfriend (1969). Antigen *Amb a* V was prepared by using the method of Lapkoff and Goodfriend (1974). Crude ragweed was prepared and made to a 1/500 and 1/5000 weight/volume solution. All the prepared test antigens were evaluated for purity against known standards by using immunodiffusion and specific antisera acrylamide gel, electrophoresis, histamine release assays, and radioallergosorbent inhibition tests performed in our laboratories. Histamine sulfate (1/10,000 [0.275 mg/cc]) was used for a skin-test-positive control. The dilutant served as a negative control. A positive skin-test response was defined either as a mean wheal diameter of 5 mm or greater for the titration intradermal testing using a titer of 10^{-3} or less or as a concentration of 1/500 or less for prick testing. Ten MZA pairs, two DZA pairs, and one DZT pair were not skin tested.

IgE Level

The majority of twins were studied by using a double-antibody radioimmunoassay (Gleich et al. 1971). The PRIST (Phadebas Pharmacia Diagnostics,

Uppsala) was used to determine the IgE levels in the remaining twins (Kjellman et al. 1976). The two methods were comparable, as indicated by a nonsignificant two-tailed *t*-test. As a result, the IgE values obtained by the two methods were pooled.

Specific Serum IgE Levels

Specific IgE antibodies for *Ambrosia artemisiifolia, P. pratense,* and *Alternaria teni* were measured by using the radioallergosorbent test (RAST). Results are reported in arbitrary units, calculated as percentage of activity, in comparison with that in pooled sera of nonallergic controls as defined by negative allergic history and nonreactive skin tests ([patient's counts per minute/control counts per minute] × 100). A positive response was defined as being one in which the activity was more than 400% of that in nonallergic controls.

Statistical Analysis

Concordance rates for each subgroup of the MN sample (i.e., MN MZA, MN DZA, MN MZT, and MN DZT) were computed for asthma, seasonal rhinitis, skin-test responses, and antigen-specific IgE serum levels. Concordance was studied only for those pairs in which at least one member was positive. IgE levels were log transformed and then were corrected for age and sex by using regression analysis (McGue and Bouchard 1984). Intraclass correlations were computed for the age-and-sex-adjusted log-transformed serum IgE levels for the combined male and female MN MZA, MN DZA, MN MZT, MN DZT, F MZT, and F DZT samples.

In order to obtain a summary estimate of the extent to which genetic and environmental factors contributed to variation in log IgE levels, biometric analyses were performed by using the procedures described by Tellegen et al. (1988). In brief, the expected mean squares between and within each twin type were expressed in terms of a biometric model that assumed that as many as three components contributed to overall variability; these components were G, the variance proportion due to additive genetic factors; E_1, the variance proportion due to nonfamilial environmental factors; and E_2, the variance proportion due to familial environmental factors.

Three separate biometric models were fitted to the data by using the method of maximum likelihood; these models were (1) a general model that included all three variance components, (2) a reduced model that assumed no genetic factors whose fit could be compared to the fit of the general model, to assess the statistical significance of genetic influence, and (3) a reduced model that assumed no familial environmental factors whose fit could be compared to the fit of the general model, to assess the influence of being reared together.

Results

Asthma was detected in 7.7% of the MN MZA/DZA sample and in 6.6% of the MN MZT/DZT sample. Seasonal rhinitis was detected in 27.4% of the MN MZA/DZA group and in 31.7% of the MN MZT/DZT group. Pairwise concordance rates for asthma and seasonal rhinitis for each subgroup are reported in table 2. The sample sizes were small, and none of the MZ/DZ differences in concordance rates reach significance at the 95% level, with the exception of the MZA asthma concordance value.

The means and SDs, by sex, for serum IgE (International Units [IU]) and log IgE are displayed in table 3. They were calculated by assuming that the twins were unrelated individuals. Means, means squares, and intraclass correlations for age-and-sex-adjusted log

Table 2

Pairwise and Probandwise Concordance Rates for Seasonal Rhinitis and Asthma in MN MZA/DZA and MN MZT/DZT

	Seasonal Rhinitis				Asthma			
Twin Type	No. Concordant	No. Discordant	% Concordant Pairwise	% Concordant Probandwise	No. Concordant	No. Discordant	% Concordant Pairwise	% Concordant Probandwise
MZA.....	9	12	43	60	4	1	80*	89*
DZA.....	2	5	28	44	0	2	0	0
MZT.....	8	10	44	62	2	1	33	80
DZT.....	3	8	27	43	0	2	0	0

* Significant at the 95% level.

Table 3

Means and SDs for Serum IgE

Group (*N*)	IgE		
	Mean	SD	Geometric Mean
Males (102)	314.8	1949.9	65.8
Females (163)	84.3	465.2	32.0
Total (265)	173.0	1288.5	42.0
	ln IgE		
Males (102)	4.172	.702	. . .
Females (163)	3.466	.521	. . .
Total (265)	3.738	.613	. . .

Note.—Data are expressed as International Units.

Table 4

Intraclass Correlations of log(IgE) Levels

Twin Type (no. of sets)	Intraclass Correlation	95% Confidence Interval
MN:		
MZA (49)	.640*	.442–.779
DZA (21)	.486*	.087–.751
MZT (34)	.422*	.106–.661
DZT (27)	.258	−.123–.573
F:		
MZT (76)	.560*	.384–.696
DZT (82)	.365*	.152–.530

Note.—Data are expressed as International Units.
* Significant at .05.

(IgE) levels are shown, by twin type, in table 4. These age-and-sex-adjusted scores were scaled to have, in the total sample, means of 0 and SDs of 1. The mean and variance did not statistically vary across twin types. All correlations, with the exception of the MN DZT, were significantly greater than zero. Because the intraclass correlation assumes a normal distribution, we were unable to compute them for RAST, as extremely high values precluded normalization even with log transformation.

Biometric analysis using the MN MZT/DZT twins versus the F MZT/DZT twins were performed. Comparisons of the IgE correlations (*a*) between the MN MZT and F MZT samples and (*b*) between the MN DZT and F DZT samples revealed no significant differences: for MN MZT versus F MZT, $\chi^2 = 0.53$, df = 1, $P = .47$; for MN DZT versus F DZT, $\chi^2 = 0.24$ df = 1, $P = .62$. The serum IgE correlations from the F and MN MZT/DZT twin groups were, therefore, combined to yield greater power in the remaining analyses. The three-variance-component biometric model fits the data extremely well ($\chi^2 = 5.99$, df = 6, $P = .42$), with the following mean ± standard error maximum-likelihood estimates: G = .564 ± .050; E_1 = .436 ± .050; and E_2 = 0 (estimated at a boundary value of zero).

The results do not support the existence of familial environmental effects, because the MZA/DZA twins, who did not share a familial environment, were more similar than the MZT/DZT twins, who did. Deleting the G component from the model produced a significant increase in the χ^2 statistic ($\chi^2 = 46.20$, df = 1, $P < 10^{-5}$), indicating that genetic influences are highly significant, as could be expected, given the substantial correlation observed in the MZA sample.

Concordance rates were analyzed either for at least one positive response by skin test and RAST (table 5) or for a positive response to each specific antigen as measured by the skin test (table 6) and RAST (table 7). A trend for greater concordance was seen in MZ twins as compared with DZ twins, but the sample sizes were too modest to achieve statistical significance.

Table 5

Concordance Rates for Presence of at Least One Positive Response

	No. of Sets		
	+ +	+ −	% Concordance + +
Skin test:			
MN MZ (10 pairs not done)	16	13	55
MN DZA (two pairs not done)	5	5	50
MN MZT (all pairs done)	16	7	70
MN DZT (one pair not done)	5	13	28
RAST:			
MN MZA	10	10	50
MN DZA	0	6	0
MN MZT	4	4	50
MN DZT	2	4	33

Discussion

The present study of unselected MZT/DZT twins and MZA/DZA twins provides a unique approach to evaluating the genetics of atopic disease. MZA/DZA twins enable direct examination of genetic effects, without the confounding factor of a common rearing environment. The prevalences of asthma and seasonal rhinitis, in both the MN MZA/DZA and the MN MZT/DZT groups, are within the ranges reported in population studies by other investigators (Davis 1976; Smith 1988). Prevalence values in the MZT/DZT group are quite similar to those in the MZA/DZA group and do not differ statistically. Evaluation of the concordance of allergic disease in twins has resulted in conflicting results. The pairwise concordance rates are .25–.88 in MZ twins and .09–.63 in the DZ twins (Blumenthal and Bonini 1990). Differences in results may reflect methodological problems. Given that our twins were selected without regard to presence of atopic disease, we did not have a large number of atopic twins from which more powerful conclusions regarding heritability of asthma and seasonal rhinitis might have been generated. Our data suggest that common environment has very little effect on the development of asthma and rhinitis. Because of the small sample size utilized in the present study, these results should be viewed with caution.

It is apparent from family and twin studies that serum IgE levels have a heritability in the range of 50%–84% (Blumenthal and Bonini 1990). This has also been found to be the case for other major classes of immunoglobulins studied in MZA/DZA twins (Kohler et al. 1985). A major gene is implicated, but the precise mode of inheritance is not yet well established (Blumenthal and Bonini 1990). Family studies suggest a great deal of genetic heterogeneity (Blumenthal et al. 1981; Blumenthal and Amos 1987). For total serum IgE levels, nearly all twin studies have revealed greater concordance for MZ twins than for DZ twins, but estimates of heritability were often different. The magnitude of the heritability index depends, in part, on the type of statistical analysis employed (Blumenthal and Bonini 1990).

For MZA and MZT twins, total IgE correlations were very similar, suggesting that the effects that rearing environment has on IgE levels might not be as large as previously thought. If familial environment were of primary importance, the MZT twins should show a higher correlation than do MZA twins, an effect that is not observed. It can be safely assumed that MZA and MZT twin pairs share similar prenatal environments; perhaps the intrauterine environment plays a significant role in the determination of total IgE levels. This might explain, in part, the lack of a significant difference in resemblance between the MZA and MZT twins.

Twin studies suggest that genetic influences on serum levels of specific antibodies to allergens may be less pronounced than are environmental influences. Wutrich et al. (1988) studied total IgE and specific IgE as measured by RAST in 57 twin pairs. They concluded that the overall regulation of total IgE is largely heritable but that specific IgE levels are influenced mainly by environmental effects. Bahna et al. (1983) and Bonini et al. (1983) have reported similar findings.

We investigated the specific immune response, as measured by RAST and skin testing, to *Ambrosia artemisiifolia, Amb a* I, *Amb a* III, *Amb a* V, *Lolium perenne, Aspergillus fumigatus, Alternaria teni,* and *Phelum pratense,* in MZA and DZA twins. When the

Table 6

Concordance Rates for RAST, by Antigen

Antigen	No. of Individuals Tested		No. of Positive Individuals		Concordance Status[a]							
					MZA		DZA		MZT		DZT	
	MZA/DZA	MZT/DZT	MZA/DZA	MZT/DZT	C	D	C	D	C	D	C	D
Ambrosia artemisiifolia	140	123	10	13	0 (0%)	9	0 (0%)	1	2 (28%)	5	1 (33%)	2
Phleum pratense.....	140	123	27	9	5 (26%)	14	0 (0%)	3	2 (100%)	0	1 (25%)	3
Alternaria tenia.....	140	123	2	0	0 (0%)	2	ND	ND	ND	ND	ND	ND

[a] C = + + (concordant); D = + − (discordant); ND = not done.

Table 7

Concordance Rates for Skin Tests, by Antigen

Antigen	No. of Individuals Tested		No. of Positive Individuals		Concordance Status[a]							
					MZA		DZA		MZT		DZT	
	MZA/DZA	MZT/DZT	MZA/DZA	MZT/DZT	C	D	C	D	C	D	C	D
Ambrosia artemisiifolia	120	118	4	14	1 (50%)	1	0 (0%)	1	2 (28%)	5	1 (25%)	3
Lolium perenne.....	120	114	19	9	5 (45%)	6	1 (50%)	1	2 (50%)	2	0 (0%)	3
Aspergillus fumgatus.....	113	112	12	29	2 (40%)	3	2 (66%)	1	6 (46%)	7	2 (25%)	6
Alternaria teni	113	112	24	27	6 (43%)	8	0 (0%)	4	5 (56%)	4	2 (18%)	9
Phleum pratense.....	113	112	28	32	6 (38%)	10	1 (20%)	4	7 (39%)	11	1 (17%)	5
Median % concordance..					43%		20%		56%		18%	

[a] C = + + (concordant); D = + − (discordant).

RAST data were analyzed for positive concordance rates, no significant differences were noted either when comparing MZA twins with DZA twins or when comparing MZT twins with DZT twins. Similar findings were noted when the skin tests were analyzed. These results collectively indicate that sensitivity to particular antigens may be influenced more by environmental factors than by genetic factors.

Twin studies have revealed that, for atopic disease as well as for total IgE levels, MZ twins are more concordant than are DZ twins. It is apparent from the literature and from the present twin study that the ability to produce IgE as reflected by total serum IgE levels has a large heritable component. The mode of inheritance is yet to be established and will probably best be elucidated by family studies. The lack of significant differences, between MZT and MZA twins, in the variables measured suggests that familial environment may play a minor role in the development of atopic disease.

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