Genetic and Environmental Risk Factors for Asthma
A Cotwin-control Study

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In complex diseases of genetic etiology such as asthma and atopy, it is difficult to differentiate causes of disease from consequences, and quantitate the importance of such causative factors. We examined possible risk factors for the development of wheezing and bronchial hyperresponsiveness in a cotwin-control study nested within a larger community-based twin-family study. In 62 monozygotic (MZ) twin pairs discordant for a history of wheezing, skin prick test to house dust extract was the most important discriminator, followed by sensitization to cat and cockroach allergens. In contrast, 62 dizygotic (DZ) discordant twin pairs differed additionally in sensitization to grass pollens and fungi. Markers such as serum haptoglobin, serum magnesium, and α-1-antitrypsin levels did not differ significantly between discordant twins. This MZ/DZ difference suggests that pollen allergy in asthmatics is more an epiphenomenon due to a genetic correlation between asthma and the allergic diathesis, whereas indoor allergens are likely to be direct environmental causes of asthma. Duffy DL, Mitchell CA, Martin NG. Genetic and environmental risk factors for asthma: a cotwin-control study.

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The evaluation of cause and effect for complex diseases like atopy is difficult. Genetically informative studies offer one route towards understanding etiology, even in cross-sectional data, because genotype always precedes phenotype in the pathway of causation. Among monozygotic (MZ) twins, the occurrence of a disease in one twin but not the cotwin must be due either to differential exposure to environmental risk factors for the disease, or represent stochastic developmental events such as those seen in the T-cell receptor repertoire (1). In the case of dizygotic (DZ) twins, intrapair differences may be environmental or genetic. Therefore, the matched twin case-control (cotwin-control) design offers one method of assessing the contributions of genetic and environmental factors to the development of disease, and has been applied successfully to other complex diseases (2). In the present report, we present results of such a study using Australian MZ and DZ twins discordant for wheezing and bronchial hyperresponsiveness.

We have examined a number of phenotypes that have been reported to be associated with asthma. These include skin test sensitization to 11 different aeroallergens (3, 4), and total serum Immunoglobulin E level (5). In the case of sensitization to house dust mite (HDM) allergen, the commonly accepted hypothesis is that exposure of genetically susceptible individuals to allergen leads to the development of sensitization, and subsequently to asthma (6–8). Evidence of temporal precedence and other criteria of causation is less strong for other allergens. Other factors that have been claimed to be associated with asthma include the serum levels of α-1-antitrypsin (9–11), haptoglobin (12, 13), and magnesium (14). These analytes would be expected to be lower in asthmatics than nonasthmatics. We also examined stature (since asthmatic children can experience growth retardation due to chronic illness and steroid therapy, and low birth weight can lead to both respiratory disease and short stature) and personal smoking history.

METHODS

Study Population

We recruited volunteer pairs of twins who were taking part in a larger project on the genetic and environmental determinants of asthma and allergic disease. Initially, as has been described elsewhere (15), all 5,967 adult pairs of twins registered in 1980 with the Australian National Health and Medical Research Council were sent an extensive questionnaire on health and lifestyle factors. This included an item screening for a history of "Asthma or wheezing." Those responding were rescreened in 1988, and a second cohort of 4,269 pairs of twins age 18 to 27 yr was sent a similar questionnaire in 1990 (Table 1). All 1,961 twin pairs from both cohorts where one or both twins reported wheezing were mailed a detailed respiratory symptoms questionnaire in 1991–1992. This instrument included an invitation for residents of major cities to take part in field testing.

Testing Protocol

Field testing was carried out in respiratory function laboratories in seven centers around Australia (Brisbane, Sydney, Canberra, Melbourne, Geelong, Adelaide, and Perth). All testing was performed over spring and early summer of 1992, so most subjects were tested "in season" for both pollens and house dust mite. The twins were

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TABLE 1

CHARACTERISTICS OF MEMBERS OF THE AUSTRALIAN NATIONAL HEALTH AND MEDICAL RESEARCH COUNCIL TWIN REGISTER SCREENED FOR ASTHMA 1980–1989

Birth Cohort of Twins	Year of Survey	Number of Eligible Twin Pairs	Complete Pairs Responding		Crude Prevalence of Wheeze (%)
			(*n*)	(%)	
1880–1962	1980	5,967	3,808	64	13.2
	1988		2,990	50*	18.9
1963–1975	1989	4,269	1,575	39†	20.7

* Only respondents to the first questionnaire were resurveyed.
† The final response including abbreviated telephone interviews (no asthma item) was 56%.

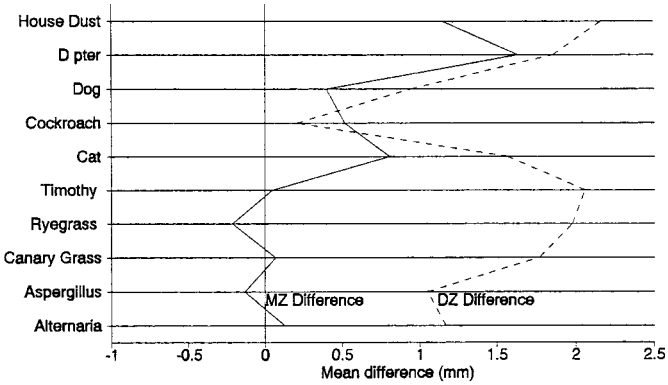

Figure 1. Differences in allergen response between twins discordant for ever wheeze (affected twin wheal size minus unaffected twin wheal size in mm).

asked to stop usual asthma and allergy medications for a set period before testing with the exception of inhaled steroids (β-agonist inhalers 6 h, theophylline preparations 24 h, most antihistamines 3 d, and astemizole 2 wk prior to testing). On arrival, weight and height were measured, and a brief set of questions were administered on smoking, recency of wheeze and lower respiratory tract infection, current medication use, and history of allergen hyposensitization therapy. Subjects then underwent epicutaneous allergen skin prick testing (SPT) with a panel of 11 allergens (cockroach mixture—German, Oriental, and American; house dust mixture; *Dermatophagoides pteronyssinus* (*D. pter*); cat fur and dander; dog fur; canary grass; timothy grass; Southern grasses mixture—Bermuda grass, orchard grass, red top, sweet vernal grass, timothy grass; perennial ryegrass; *Aspergillus* mixture; *Alternaria* mixture—all supplied by Hollister-Steier from the same production batch) as well as histamine (3.125 mg/ml) and glycerine controls. This was performed using a Hollister-Steier prick lancetter (Østerball standardized needle) on the volar forearm, the mean of the maximum and its perpendicular wheal diameters (to the nearest millimeter) being measured after 10 min. The mean wheal diameter for the negative control was subtracted from each allergen's for analysis.

Spirometry was performed using Vitalograph S wedge bellows spirometers (Vitalograph Ltd, Buckingham, UK). The calibration of all spirometers and absence of leaks was checked daily. Histamine inhalation challenge testing (HCT) was performed according to the brief protocol of Yan and coworkers (16), on all twins with a $FEV_1 \geqslant 60\%$ of predicted value. Each dose of histamine was administered via a hand-pumped DeVilbiss No. 45 nebulizer at approximately 2-min intervals until either a cumulative dose of 7.8 μmol of histamine was given or a decrease in FEV_1 to 80% of the post-saline-inhalation FEV_1 was observed. Any bronchoconstriction leading to a decrease of 10% or greater in FEV_1 was reversed at the termination of the protocol by terbutaline or salbutamol given via an aerosol with spacer. The response to histamine was expressed as the dose–response slope calculated from the linear regression of percentage drop in FEV_1 versus (untransformed) cumulative dose of histamine given (17), and bronchial hyperresponsiveness was defined as 20% fall in FEV_1 during the course of the protocol ($PD_{20} < 7.8$ μmol). Subjects who demonstrated a pretest FEV_1 below 60% of predicted underwent a brochodilator test. Either terbutaline or salbutamol was given via metered aerosol with a spacer. An increase in FEV_1 of 12% from baseline after 5 min was regarded as a positive result (18).

Blood samples were collected and separated into sera and plasma, red cells, and buffy coats, which were stored for later processing. Total serum immunoglobulin E level (slgE) was estimated by an enzyme immunoassay (IMx Total IgE assay; Abbott Laboratories, Abbott Park, IL). Serum α-1-antitrypsin (AAT) levels were determined using standard immunoturbidometric assays performed on two nephelometers (Behring Nephelometer System, Behring Institute, Marburg, Germany; Beckman Array Nephelometer, Beckman Instruments, Fullerton, CA). Standard serum enzyme and electrolyte assays were performed on a Technicon CHEM1 (Tarrytown, NY).

Statistical Analysis

For the analyses presented we used only the same-sex pairs of twins where (*1*) one twin reported a history of ever wheezing ("Ever wheeze") and the other denied such; (*2*) one twin reported wheeze in the 12 mo prior to testing ("Recent wheeze") and the other denied ever wheezing; and (*3*) one twin reported ever wheezing and exhibited bronchial hyperresponsiveness on HCT ("Confirmed asthma") while the second twin denied ever wheezing and exhibited normal responsiveness to histamine. This gave three (nested) definitions of asthma increasing in stringency. Because twins have reported on symptoms on two or three occasions, we have taken a history of wheeze as present if reported on two occasions including at the time of testing, and absent if denied on all occasions.

Statistical analyses were performed using SAS 6.07 (19). These include *t* tests and signed rank tests of the significance of the intrapair differences in the independent variables (covariables), and of the difference between intrapair differences observed in the MZ and DZ same-sex twin groups. In the case of sIgE, because this variable is known to be log-normally distributed, the most appropriate analysis is of log [sIgE + 1], equivalent to testing the ratio of sIgE within the discordant pairs. Multivariate stepwise conditional logistic regression analyses have also been performed, examining interaction between covariables, and producing odds ratios as a summary of association size, though given the small sample size, the results of such multivariate techniques may not be robust.

The size of the intrapair difference of a variable in a twin pair discordant for a given trait is a measure of the correlation between the primary trait (asthma) and the covariable. The larger the intrapair difference for the covariable, the larger the correlation. If there is no environmental component to the covariance between the traits (whether external environment or "internal" bodily environment, i.e., physiological or immunological) then the MZ difference will be zero, but a significant DZ difference will be observed if a genetic correlation is present (20). In the case of a purely environmental correlation between disease and covariable, the MZ intrapair difference will be greater than the DZ intrapair difference. Other situations are more complex. In the case of asthma, where environmental and genetic determinants are roughly of equal importance (15), a greater DZ than MZ difference suggests that genes explain more of the correlation between the covariable and asthma than environment does, and equality of the MZ and DZ differences, a preponderance of environmentally mediated covariation.

RESULTS

A total of 863 subjects took part in the testing protocol (Table 2). Mean age was 36.4 yr, and age ranged from 19 to 76 yr. Two-thirds (64%) of the sample were women. The group contained 419 complete twin pairs. Taking the definitions of discordance described previously, there were 124 same-sex sets of twins discordant for ever wheeze, 94 pairs discordant for recent wheeze, and 78 pairs discordant for confirmed asthma.

Among the 62 discordant ever wheeze MZ pairs (Table 3,

TABLE 2

CHARACTERISTICS OF 863 TWINS TAKING PART IN CLINICAL PHASE OF STUDY

	Frequency of Reported Wheeze at Time of Testing			
Descriptive Variable	In Last Week	In Last Year	Less Recently	Never
Male sex, % (n)	26 (47)	35 (68)	41 (89)	39 (106)
Median age, yr (range)	33 (19–76)	32 (21–70)	35 (21–75)	33 (20–76)
Current smoker, % (n)*	28 (51)	21 (42)	24 (51)	21 (56)
$PD_{20} \geq 7.8$ μmol HIS, % (n)	69 (123)	66 (129)	41 (90)	24 (64)
Geometric mean sIgE, IU/ml	107	85	69	41
Positive† SPT ryegrass, % (n)	45 (81)	54 (106)	51 (110)	30 (81)
Positive SPT *D. pter*, % (n)	66 (119)	71 (139)	64 (139)	44 (120)
Positive SPT cockroach, % (n)	31 (56)	34 (66)	27 (58)	21 (56)
Positive SPT cat, % (n)	47 (84)	46 (91)	33 (71)	20 (53)
Total number of subjects	179	196	217	271

* Current at time of screening questionnaire.
† Mean skin wheal diameter 3 mm or more greater than negative control.

Figure 1), significant differences were noted for domestic aeroallergens such as *D. pter*, house dust mixture, cockroach, cat and dog hair/epithelia. Total serum IgE level only weakly differed between the affected and unaffected twin. No significant differences were observed for either the grass pollens, fungi or a number of biochemical markers such as serum AAT, haptoglobin level, and a number of electrolyte and enzyme levels, most notably serum magnesium.

In the 62 discordant DZ twin sets, the indoor allergens were significant associates of wheezing, and wheal size differences tended to be larger than those observed in the MZ twins, though not statistically significantly so. The fungal and grass allergen wheal differences were much larger than those of the MZ twins, and the MZ-DZ difference was highly significant. Although the Wilcoxon pair-difference test of the MZ-DZ sIgE difference was not significant, the *t* test of the difference for log transformed sIgE was ($t = 2.37$, $p = 0.020$).

In conditional logistic regression in the MZ "ever wheeze" discordant group using all the skin test results, only house dust mixture could be entered into the model (entry criterion $\alpha = 0.15$, model $\chi^2_1 = 17.26$, residual $\chi^2_8 = 3.34$). The odds for wheezing increased 1.76 (95% confidence limit 1.25–2.48) for every 1-mm increase in house dust mixture wheal size. Expressing skin reactivity as present (wheal diameter 3 mm or greater) or absent (diameter under 3 mm), the same regression analysis included cat and house dust in the final model (model $\chi^2_2 = 11.52$; residual $\chi^2_7 = 3.66$; house dust odds ratio = 3.22, 95% confidence interval 1.04–10.01; cat OR = 2.88, 0.77–10.75). Smoking, in all analyses, whether analyzed as approximate pack-years smoked, quantity smoked daily at time of screening, or smoking on day of testing was not a significant risk factor.

Similar analyses for the DZ "ever wheeze" discordant group suggested the significant covariates to be allergy to house dust mixture—leading to an increase in the odds of wheeze of 1.9 per mm increase in wheal diameter, *D. pter* (1.2), *Alternaria* (1.6), *Aspergillus* (1.8), and cockroach, where the adjusted odds ratio was opposite in direction to the crude effect at odds of 0.5 per mm increase in wheal. A combined analysis found the MZ and DZ difference in odds ratios to be significant for the *Aspergillus* and cockroach mixtures (with χ^2_1 equal to 9.3 and 8.1, respectively).

TABLE 3

DIFFERENCES (AFFECTED TWIN VALUE MINUS UNAFFECTED TWIN VALUE) IN VALUE OF COVARIATES IN MZ AND DZ TWINS DISCORDANT FOR "EVER WHEEZED"*

Trait	MZ Mean Difference D_{MZ}	p Value Wilcoxon Test $D_{MZ} = 0$	DZ Mean Difference D_{DZ}	p Value Wilcoxon Test $D_{DZ} = 0$	p Value Wilcoxon Test $D_{MZ} = D_{DZ}$
Alternaria	+0.13 mm	0.66	+1.17 mm	0.001	0.020
Aspergillus	−0.13 mm	0.65	+1.05 mm	0.0004	0.008
Canary grass	+0.07 mm	0.99	+1.77 mm	0.0001	0.015
Perennial ryegrass	−0.21 mm	0.85	+1.98 mm	0.0005	0.015
Timothy	+0.05 mm	0.66	+2.06 mm	0.0003	0.020
Cat hair/epithelia	+0.81 mm	0.001	+1.57 mm	0.0001	0.066
Cockroach	+0.52 mm	0.009	+0.21 mm	0.40	0.76
Dog	+0.40 mm	0.04	+0.94 mm	0.0001	0.088
D. pter	+1.64 mm	0.0002	+1.86 mm	0.002	0.66
House dust	+1.15 mm	0.0001	+2.17 mm	0.0001	0.24
Histamine	+0.30 mm	0.06	−0.07 mm	0.78	0.13
AAT	−0.05 U/ml	0.88	+0.01 U/ml	0.85	0.90
Haptoglobin	−0.06 mM	0.65	+0.18 mM	0.06	0.09
IgA	−0.00 U/ml	0.89	+0.30 U/ml	0.19	0.35
IgE	+7 U/ml	0.016	+96 U/ml	0.003	0.42
Pack-years†	+0.53 Pkyr	0.49	+0.92 Pkyr	0.71	0.79
Height	−0.29 cm	0.68	−0.8 cm	0.28	0.64

* Affected twin listed reported a history of wheeze, while the unaffected twin denied ever wheezing. There were 48–62 MZ pairs where both values were present, and 52–62 DZ pairs.
† Approximate pack-years of cigarettes smoked based on duration in years and average.

Tightening the definition of affected to recent wheeze led to a very similar pattern of responses in 48 pairs of MZ and 46 pairs of DZ twins, with a net increase in the size of the intrapair differences for most variables. The smallest group for comparisons comprised those twins discordant for bronchial hyperresponsiveness and a history of wheeze (Table 4). For the discordant MZ twins (36 pairs), the largest difference was noted for SPT wheal size for *D. pter* and house dust mixtures. Sensitization to indoor allergens such as cats, dogs, and cockroach also remained a significant risk factor. Sensitization to grass pollen was not a statistically significant factor. The difference between the affected and unaffected twin for *Aspergillus* wheal size was large enough to be mildly significant. In the DZ twins as before, allergic sensitization to grass pollens was highly significantly associated with bronchial hyperresponsiveness, significantly more so than in the MZ twins. The wheal size difference for *D. pter* was equal in the MZ and DZ groups, as were those for cockroach and dog allergens. Although cat sensitization was a significant predictor in the MZ twins and DZ twins, a significantly larger wheal diameter difference was seen in the DZ twins.

DISCUSSION

The nature of the relationship between allergic sensitization to specific allergens and asthma can either be a direct causative one, with allergen exposure causing asthma in susceptible individuals, or an indirect one, where the genetically determined atopic diathesis causes both asthma and expression of sensitization to ubiquitous aeroallergens. We would interpret the present results as implying that the first mechanism is the most likely for allergens such as house dust, cat, and cockroach, and the second for allergens such as the grass pollens and molds. This is not to say that appropriate exposure to grass pollen (21) and mold (22, 23) will not precipitate attacks in predisposed asthmatics, but that they are less important factors in the development of asthma.

A number of other lines of evidence tend to support this view. In cross-sectional studies of Australian children (24), house dust mite sensitization was found to have the strongest independent association with current asthma (wheeze plus bronchial hyperresponsiveness at testing). Exposure to high domestic levels of house dust mite allergen is associated with increased risk of developing asthma (6), and avoidance of exposure leads to decreased frequency of wheezing in asthmatics (7). Importantly, one British longitudinal study (8) has shown a relationship between level of exposure to house dust mite at age 1 and asthma at age 11.

A causative relationship between exposure to cat and cockroach allergens and the development of asthma is less well documented. Allergy to cockroach has been increasingly recognized as common in asthmatics, and is possibly associated with more severe disease (25). Sensitization to cockroach was less frequent in this study than that reported in one U.S. study (25), though higher than another (26), in which sensitization to cockroaches and to cats was a risk factor for acute hospital presentation with asthma. Allergy to cats is associated with asthma in Australian (24) and New Zealand children (3), though not in U.S. adults (4). Demonstration of an association between exposure to cats and the development of asthma (again as opposed to exacerbation of existing asthma) has been complicated by the fact that sensitized individuals often have removed cats from their homes (27). Our results would again suggest sensitization to cat allergens is a marker or mediator of an environmental cause of asthma.

In the case of grass pollens, bronchial hyperresponsiveness in pollen-sensitized asthmatics rises following pollen season and declines afterwards. However, fewer urban asthmatics are sensitized to grass pollen than are to house dust mite, as in the present study (Table 2), and upon adjustment for other allergies (by multiple logistic regression), the association between grass pollen sensitization and asthma disappears (3, 4, 28), while that with hayfever remains significant. Our results are in agreement with these findings, suggesting that sensitization to grass allergens is not among the proximate causes of asthma.

An alternative hypothesis to explain the findings of the cur-

TABLE 4

DIFFERENCES (AFFECTED TWIN VALUE MINUS UNAFFECTED TWIN VALUE) IN VALUE OF COVARIATES IN MZ AND DZ TWINS DISCORDANT FOR WHEEZE ASSOCIATED WITH BRONCHIAL RESPONSIVENESS*

Trait	MZ Mean Difference D_{MZ}	p Value Wilcoxon Test $D_{MZ} = 0$	DZ Mean Difference D_{DZ}	p Value Wilcoxon Test $D_{DZ} = 0$	p Value Wilcoxon Test $D_{MZ} = D_{DZ}$
Alternaria	+0.54 mm	0.090	+1.44 mm	0.0002	0.23
Aspergillus	+0.65 mm	0.037	+1.51 mm	0.0001	0.15
Canary grass	+0.86 mm	0.11	+2.96 mm	0.0001	0.029
Perennial ryegrass	+1.08 mm	0.098	+2.95 mm	0.0001	0.057
Timothy	+1.05 mm	0.09	+3.50 mm	0.0001	0.037
Cat hair/epithelia	+1.05 mm	0.0021	+2.42 mm	0.0001	0.0047
Cockroach	+0.97 mm	0.0026	+0.88 mm	0.016	0.76
Dog	+0.79 mm	0.0017	+1.33 mm	0.0001	0.24
D. pter	+2.97 mm	0.0001	+3.04 mm	0.0001	0.70
House dust	+1.69 mm	0.0002	+2.17 mm	0.0001	0.46
Histamine	+0.07 mm	0.81	+0.23 mm	0.22	0.52
AAT	−0.23 U/ml	0.026	−0.05 U/ml	0.72	0.41
Haptoglobin	+0.01 mM	0.89	+0.09 mM	0.57	0.66
IgA	+0.06 U/ml	0.38	+0.51 U/ml	0.03	0.14
IgE	+47 U/ml	0.0019	+137 U/ml	0.0003	0.13
Pack-years†	−1.12	0.59	+1.94	0.73	0.75
Height	−0.64 cm	0.47	−0.4 cm	0.69	0.92

* The affected twin reported a history of wheeze and exhibited bronchial hyperresponsiveness at time of testing. The unaffected twin denied wheezing and had normal bronchial responsiveness. There were 29–36 MZ pairs where both values were present, and 31–42 DZ pairs.

† Approximate pack-years of cigarettes smoked based on duration in years and average daily use on a six-point scale from "Nil" to "Over 40 per day."

rent study must have a mechanism that allows: (*1*) asthmatics overall to have higher rates of sensitization to pollen and mite allergens than nonasthmatics as a group; and (*2*) the nonasthmatic identical twin to have a smaller HDM wheal but not a smaller wheal to pollens compared with the asthmatic cotwin. One possibility is that asthmatics become sensitized disproportionately to those allergens that (because of particle size) are deposited in the inflamed/primed lower airway (so asthma *causes* sensitization). The fact that these MZ twins were reared in the same household, so that it seems unlikely that one twin would have been exposed to higher environmental levels of mite allergens than the other might be seen as indirect support for this suggestion.

The necessity for adjustment for sensitization to other allergens in such analyses arises from correlations between allergens. These may arise from (*1*) a common underlying genetic predisposition to allergy; (*2*) an adjuvant or permissive effect of sensitization to one particular allergen, possibly at a critical period of development of the immune system; (*3*) sharing of allergic epitopes, such as that seen in cross-reactivity of grass antigens (29, 30); (*4*) occurrence of exposure to a number of allergens in the same environment, such as house dust mite, mold, and cat allergens in house dust. The pattern of correlations seen suggests it is appropriate to group allergen responses into indoor, where the fourth mechanism may explain the clustering, and outdoor, where the latter two mechanisms would be of equal importance.

The association between sIgE and asthma as well as bronchial hyperresponsiveness has been interpreted as evidence that allergic processes underlie most if not all asthma (5, 31). A significant difference in sIgE level was observed in the discordant MZ twins, which implies an environmental or phenotypic (e.g., physiological) relationship between sIgE and asthma, while the larger DZ difference suggests genetic covariation is also present. This mixed picture might have been expected given our conclusions reached about sensitization to different types of allergen. That is, specific IgE might be either raised to an indoor allergen and so be environmentally correlated with asthma, or produced in response to the other allergens which are genetically correlated with asthma.

A relationship between serum haptoglobin level has been noted with bronchial hyperresponsiveness and asthma in several studies (12, 13). In the latter study, serum haptoglobin was decreased in individuals with a history of wheeze, and increased in smokers, those exhibiting bronchial hyperresponsiveness and those with diminished FEV_1. Such an association with wheezing or asthma was not detected in the present study. The association between α-1-antitrypsin phenotype (Pi type) and asthma has also been reported on a number of occasions (9–11). Heterozygotes carrying an M or S allele have lower levels of α-1-antitrypsin activity, and so one might expect a DZ but not MZ difference. Again, no such difference was detectable. Britton and coworkers (14) have recently reported a protective effect of increased dietary magnesium intake against wheezing and BHR. Although the correlation between serum and intracellular magnesium levels is not great (32), one might expect a homologous relationship. This was not detected in the present study or in the conventional case-control study of de Valk and coworkers (32). Finally, the height difference between the twins is in the expected direction, but does not reach statistical significance.

This study does suffer from shortcomings. Because asthma is moderately heritable, only a small number of MZ twins in our study were discordant for the trait. This means that conclusions based on the absence of a twin difference for a risk factor may represent a Type II error. Furthermore, when the causative pathway between two phenotypes is multiplex, for example where both genetic and environmental relationships exist, or the same risk factor operates differently at different ages, then the study results will be difficult to interpret. For example, twins may both be sensitized to grass pollen by the time of testing, but the asthmatic twin may have become sensitized at a critically earlier age than the unaffected cotwin.

We conclude by saying that the cotwin-control design is, with caveats, a powerful approach to examining the nature of the associations between asthma and risk factors. A likely interpretation of our results is that for urban asthmatics (possibly an important qualification [24]), sensitization to outdoor allergens such as grass pollens is not a risk factor for disease, but merely a marker of the atopic diathesis, while sensitization to house dust mite is more likely to be an intermediate step between allergen exposure and the development of asthma.

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