

Clinical Importance of *Alternaria* Exposure in Children

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The fungus *Alternaria* is known to be allergenic and is one of the most common fungi worldwide. We investigated the extent to which exposure to *Alternaria* increases the severity of asthma. We undertook a prospective cohort study in Australia of 399 school children who had positive skin tests to one or more aeroallergens. Airway responsiveness to histamine, wheeze, and bronchodilator use in 1 mo was measured five times between 1997 and 1999. Airway hyperresponsiveness was defined as $PD_{20}FEV_1 = 3.9 \mu\text{mol}$ histamine. Airborne concentrations of *Alternaria* spores were measured throughout the study, and mean daily concentrations over 1 mo ranged from 2.2 to 307.7 spores/ m^3 of ambient air. Using generalized estimating equations, we found that airway responsiveness, wheeze, and bronchodilator use increased significantly in association with increased spore concentrations and that the increase in airway responsiveness was greater in children sensitized to *Alternaria* than in other children ($p = 0.01$). The odds ratio for airway hyperresponsiveness in children sensitized to *Alternaria* was 1.26 (95% CI, 1.14 to 1.39) after an increase in mean exposure of 100 spore/ m^3/d over 1 mo. These results suggest that *Alternaria* allergens contribute to severe asthma in regions where exposure to the fungus is high.

Keywords: *Alternaria*; airway responsiveness; asthma; fungi; children

Fungi make up the largest proportion of airborne biologic particles (1, 2). Evidence of sensitivity to *Alternaria* and *Cladosporium* is commonly obtained by skin prick tests in clinical practice, but the implications of these allergies for respiratory illness have not been well characterized.

Alternaria is one of the most common fungal genera worldwide (1–3). Species parasitize a wide range of plants, and they are found in both urban and rural areas and are particularly prevalent in warm climates and regions where there is substantial cropping (4–7). The reported prevalence of sensitization to *Alternaria* in population samples of children ranges from less than 1% in Austria (8) to 50% in Arizona, USA (9). The major allergen (Alt a1) has been isolated from both the spores and the mycelia of strains of *Alternaria alternata* (10, 11). The spores, which range between 2 and 8 μm in diameter (12), are small enough to be inhaled and, under laboratory conditions, provoke respiratory symptoms and airway responsiveness in asthmatics (13).

Persons sensitized to *Alternaria* allergens are at least three times more likely to have asthma than the general population

(14, 15). Respiratory arrest in asthmatics sensitized to *Alternaria* is more likely to occur during harvesting seasons when airborne concentrations of *Alternaria* spores are high (5).

The effect of natural exposure to *Alternaria* spores on airway responsiveness and respiratory symptoms in a general population of allergic children has not been investigated. We undertook a prospective cohort study of allergic school children in two regions of New South Wales, Australia, where the prevalence of sensitization to *Alternaria* was known to be high (16). Our hypothesis was that symptoms and airway responsiveness would increase in association with an increase in airborne concentrations of *Alternaria* spores, and that this association would only be found in children sensitized to *Alternaria*.

METHODS

A prospective cohort study of 399 school children was conducted in two inland rural towns, Wagga Wagga and Moree, in southeastern Australia. The Human Ethics Committee at the University of Sydney gave approval for the study. Population samples of children in the towns had skin prick tests for allergy to eight allergens, including *Alternaria*, rye grass pollen, *Cladosporium*, *Dermatophagoides pteronyssinus*, and cat dander (17). All children in Moree and a random sample of children in Wagga Wagga with a positive skin prick test to *Alternaria* were selected for study ($n = 179$). In addition, a random sample of children from both towns with one or more positive skin prick tests to other allergens but not to *Alternaria* were selected ($n = 220$). The mean age was 9.1 yr (SD, 1.0) for children sensitized to *Alternaria*, and it was 9.1 yr (SD, 1.1) for the other children.

The children were assessed on five occasions in each town between August 1997 and June 1999. At each assessment, parents completed a questionnaire about respiratory symptoms in their child during the previous month (18), and lung function and airway responsiveness were measured in the children. Airway responsiveness was measured by a histamine bronchial provocation test (19). Dose-response ratio (DRR), which was the level of airway responsiveness, was calculated as the percent fall in FEV_1 at the last dose divided by the total dose of histamine administered ($FEV_1/\mu\text{mol}$) (20, 21). Subjects with a greater than 20% fall in FEV_1 at or before the maximum cumulative dose (3.9 μmol) were classified as having airway hyperresponsiveness (AHR).

Burkard volumetric traps placed on the roofs of the local hospital were used to measure concentrations of *Alternaria* spores and pollen in the ambient air in the towns throughout the study period (22). Spore and pollen concentrations were expressed as number per cubic meter of air per 24 h.

The association between *Alternaria* exposure and presence and severity of asthma was examined using four respiratory outcomes: DRR, AHR, wheeze, and bronchodilator use. *Alternaria* and pollen exposures were defined as the mean daily airborne concentrations measured in each town for 30 d preceding the mid-date of each assessment period. Generalized estimating equations (GEE) were used to measure the association between outcomes and exposures (23, 24). All analyses were performed using the statistical program STATA.

Separate models relating *Alternaria* exposure to the respiratory outcomes were constructed for children sensitized to a specific allergen and children who were sensitized to other aeroallergens. We tested whether the relation between *Alternaria* exposure and the respiratory outcomes differed significantly between the groups of children. Possible confounders were placed in the models one at a time and removed if they did not have an important effect or substantially

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increased the standard error. Grass pollen could not be tested as a confounder because of the high correlation between *Alternaria* spore concentrations and grass pollen concentrations. The effects from exposure to *Alternaria* on the respiratory outcomes were calculated for an increase in mean daily airborne concentration of 100 spores/m³ over 1 mo.

RESULTS

Exposure to *Alternaria*, Pollen, House Dust Mites, and Meteorologic Factors

Mean daily airborne concentrations of *Alternaria* spores in Moree were higher than those in Wagga Wagga for most of the year. Concentrations were lowest during winter in both towns and were highest during summer. Mean daily airborne *Alternaria* spore concentrations for the 30 d preceding the mid-date of each of the five assessment periods ranged from 2.2 spores/m³ (range, 0 to 9) to 300.6 spores/m³ (range, 39 to 1,270.5) in Wagga Wagga, and from 14.3 spores/m³ (range, 0 to 55.5) to 307.7 spores/m³ (range, 3 to 916.5) in Moree. Mean spore counts over 3 d were strongly correlated with spore counts over 30 d ($r = 0.93$, $p < 0.001$). The spore concentrations were also strongly correlated with grass pollen concentrations ($r = 0.94$, $p < 0.001$) and with mean daily temperature for the previous month ($r = 0.78$, $p < 0.001$). There were moderate positive correlations between mean *Alternaria* spore concentrations and non-grass pollen ($r = 0.39$, $p < 0.001$) and house dust mite allergen concentrations ($r = 0.42$). Mean *Alternaria* spore concentrations were negatively correlated with mean relative humidity ($r = -0.51$, $p < 0.001$) and weakly positively correlated with mean rainfall ($r = 0.13$, $p < 0.001$).

Allergic Sensitization

Sensitization to *Cladosporium*, mixed grains, and cat dander were more common and sensitization to house dust mite was less common among the children sensitized to *Alternaria* than among non-*Alternaria* sensitized children (Table 1). The association was strongest between being sensitized to *Alternaria* and being sensitized to *Cladosporium* (kappa coefficient, 0.49; $p < 0.0001$). There was no difference between the two towns in the distribution of specific sensitization between *Alternaria*-sensitized children and non-*Alternaria*-sensitized children. Greater than 88% of the children who tested positive for allergic sensitization to *Alternaria* at the beginning of the study also tested positive at the final visit (kappa coefficient, 0.74; $p < 0.0001$).

TABLE 1. CHARACTERISTICS OF STUDY SAMPLES AT RECRUITMENT

Characteristic	Sensitized to <i>Alternaria</i> (n = 179)		Sensitized to Other Allergens (n = 220)	
	%	(95% CI)	%	(95% CI)
Male	37.4	(30.3–45.0)	44.1	(37.4–50.9)
Wheeze in the preceding year	43.0	(35.7–50.6)	39.7	(33.1–46.5)
Percent predicted FEV ₁ *	99.3	(97.5–99.9)	98.2	(96.6–99.8)
Specific allergic sensitization				
House dust mite	53.1	(45.5–60.6)	75.0	(68.7–80.6)
Rye grass pollen	62.0	(54.5–69.1)	45.5	(38.7–52.3)
<i>Cladosporium</i>	49.2	(41.6–56.7)	2.3	(0.7–5.2)
Cat dander	25.1	(19.0–32.2)	12.3	(8.2–17.4)
Wheat whole grain	7.8	(4.3–12.8)	6.8	(3.9–11.0)
Grain mill dust	35.2	(28.2–42.7)	7.3	(4.2–11.5)
Cotton	1.7	(0.3–3.9)	1.4	(0.3–4.8)

* Reference values (42).

The Association between Airway Responsiveness and *Alternaria* Spore Concentrations

Children sensitized to *Alternaria* were more likely to have airway hyperresponsiveness than were other allergic children (Figure 1). The difference between *Alternaria*-sensitized children and other allergic children in the proportion of children with AHR increased with increasing spore concentrations.

There was a significant difference between *Alternaria*-sensitized children and other allergic children in the association between *Alternaria* spore concentrations and presence of AHR (Table 2). The presence of AHR was significantly associated with spore concentrations in *Alternaria*-sensitized children, whereas it was not in other allergic children. The effect from an increase of 100 spores/m³air/d over 1 mo is shown in Table 2. None of the potential confounders had a strong effect on the association between airway responsiveness and *Alternaria* spore concentrations, and unadjusted measures of the associations are reported. The odds for AHR after an increase in mean exposure of 300 spores/m³/d over 1 mo almost doubled for children sensitized to *Alternaria* compared with other allergic children (odds ratio, 1.99; 95% CI, 1.45 to 2.70).

The relation between severity of airway responsiveness measured by DRR and *Alternaria* spore concentrations also differed significantly between children sensitized to *Alternaria* compared with children sensitized to other allergens ($p = 0.01$). After an increase in mean exposure of 100 spores/m³air/d over 1 mo, DRR increased by a factor of 1.14 (95% CI, 1.09 to 1.19) in *Alternaria*-sensitized children and 1.06 (95% CI, 1.02 to 1.10) in the other allergic children.

It was not possible to directly test the effect of confounding by grass pollen exposure because of the strong correlation between grass pollen and *Alternaria* spore concentrations. However, there was no difference between rye-grass-sensitized and other allergic children in the association between *Alternaria* spore concentrations measured by DRR ($p = 0.76$) or presence of AHR ($p = 0.45$). There was also no difference between rye-grass-sensitized and other allergic children in the association between grass pollen concentrations and DRR ($p = 0.84$) or presence of AHR ($p = 0.68$). Finally, there was a significant difference between *Alternaria*-sensitized children and other allergic children in the association between grass pollen con-

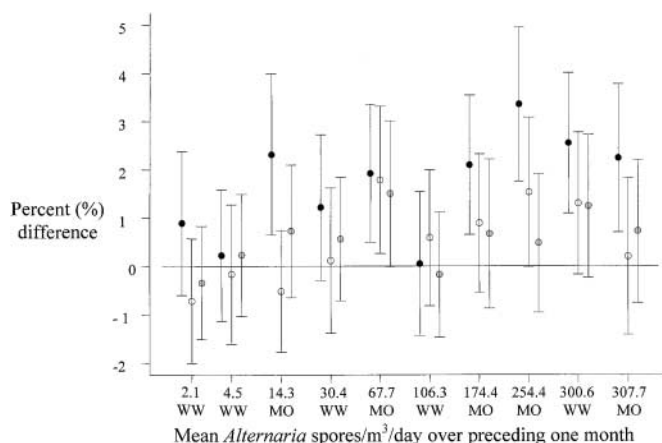


Figure 1. Difference between children sensitized to *Alternaria* and children sensitized to other allergens in proportion to airway hyperresponsiveness, wheeze, or using bronchodilator against airborne concentrations of *Alternaria*. Airway hyperresponsiveness (closed circles). Wheezed some or more days each week during the previous month (open circles). Used bronchodilator four or more times a week during the previous month (shaded circles). WW = Wagga Wagga; MO = Moree.

TABLE 2. EFFECT OF INCREASE IN EXPOSURE BY 100 *ALTERNARIA* SPORES/m³/d OVER ONE MONTH*

Outcomes	Sensitized to <i>Alternaria</i>		Sensitized to Other Allergens (not sensitized <i>Alternaria</i>)		p Value for Difference in Odds Ratio
	Odds Ratio	(95% CI)	Odds Ratio	(95% CI)	
Airway hyperresponsiveness	1.26 [†]	1.14–1.39	1.03	0.97–1.19	0.04
Wheeze on some or more days each week during the previous month	1.17 [‡]	1.03–1.32	1.02	0.90–1.16	0.14
Bronchodilator used four or more days each week during the previous month	1.11 [‡]	1.03–1.27	1.02	0.90–1.15	0.39

* The numbers of observations used in analyses for airway responsiveness were 750 for *Alternaria*-sensitized children and 877 for children sensitized to other allergens. The numbers of observations used in the analyses for wheeze and bronchodilator use were 661 for *Alternaria*-sensitized children and 706 for children sensitized to other allergens.

[†] p < 0.01 relative to reference group (no wheeze or no bronchodilator use).

[‡] p < 0.05 relative to reference group (no wheeze or no bronchodilator use).

centrations and DRR (p = 0.01), although not for the presence of AHR (p = 0.15).

An increase in mean exposure of 100 *Alternaria* spores/m³/d over 1 mo was associated with a 1.15-fold increase in DRR (95% CI, 1.09 to 1.22) in children sensitized to *Cladosporium*, which was significantly higher than the effect measured in other children (p = 0.02). There were negative interactions by house dust mite sensitization with *Alternaria* spore concentrations and AHR (p = 0.002) and DRR (p = 0.02). Children sensitized to house dust mite were less likely to have increased airway responsiveness as *Alternaria* spore concentrations increased.

The Association between Wheeze and *Alternaria* Spore Concentrations

The proportion of children with wheeze tended to increase with increasing spore concentrations, but the effect was not specific for children sensitized to *Alternaria* (Figure 1 and Table 2). There also was no interaction by sensitization to any other allergens in the association between wheeze and spore concentrations, although the interaction with grass pollen sensitization was nearly significant (p = 0.05). The odds ratio for wheeze on some days or more associated with an increase in mean exposure of 100 spores/m³/air over 1 mo for children sensitized to *Alternaria* combined with children sensitized to other allergens was 1.10 (95% CI, 1.00 to 1.20). None of the potential confounders had a strong effect on the association between wheeze and *Alternaria* spore concentrations.

The Association between Bronchodilator Use and *Alternaria* Spore Concentrations

The proportion of children using bronchodilator tended to increase with increasing spore concentrations, but the effect was not specific for *Alternaria*-sensitized children (Figure 1 and Table 2). There was no interaction by sensitization to *Alternaria* or any other allergens in the association between bronchodilator use and *Alternaria* spore concentrations for any frequency categories of bronchodilator use (Table 2). The odds ratio for using bronchodilator four or more times a week associated with an increase in mean exposure of 100 spores/m³/air over 1 mo for children sensitized to *Alternaria* combined with children sensitized to other allergens was 1.06 (95% CI, 0.97 to 1.17).

DISCUSSION

This is the first study to examine the effects of natural exposure to *Alternaria* in both symptomatic and nonsymptomatic children using an objective measure of airway abnormality. By conducting a long prospective study, we found that the severity of airway hyperresponsiveness, which is a fundamental component of current symptomatic asthma, increased with airborne *Alternaria* spore concentrations and that the effect was significantly

greater in *Alternaria*-sensitized children. We also found that wheeze increased in periods of high spore concentrations, although this effect was not specific for children sensitized to *Alternaria*.

Standard methods were used to measure allergic sensitization, symptoms, medication use, and airway responsiveness. We found the strongest association between *Alternaria* spore concentrations and airway responsiveness. Airway hyperresponsiveness is a highly specific indicator for asthma and asthma exacerbations, airway inflammation and long-term asthma (25–28). There may have been errors in parental recall of wheeze and bronchodilator use, which could have masked a specific effect related to *Alternaria* sensitization.

We used a proxy measure for true personal exposure to *Alternaria* in that we estimated that airborne concentrations from one fixed point in the towns would be a valid measure of inhaled dose. However, personal exposure to *Alternaria* is likely to vary significantly from estimates of exposure at a fixed point and between individual subjects (29). There would have also been some error in the measurement of exposure because the allergen rather than the spores per se induce an allergic response. Airborne concentrations of *Alternaria* allergens have been reported as closely related and as unrelated to airborne *Alternaria* spore concentrations (30, 31). The most likely effect of the measurement errors is that we have underestimated the strength of the relation between *Alternaria* spore concentrations and the respiratory outcomes (32, 33).

It is possible that exposure to other allergens whose airborne concentrations were closely correlated with *Alternaria* could explain some of the observed effects. Grass-pollen concentrations were closely correlated with spore concentrations but we found no evidence of an increase in airway responsiveness or bronchodilator use that was specific to children who were sensitized to rye grass. The association with wheeze frequency may have been related to rye-grass sensitization status as the test of interaction was borderline-significant. There was evidence of a specific relation with airway responsiveness in children sensitized to *Cladosporium*. We did not measure airborne concentrations of *Cladosporium* and are therefore unable to determine whether this relation exists because of a close correlation between *Alternaria* and *Cladosporium* spore concentrations or because of the strong association between being sensitized to *Alternaria* and also being sensitized to *Cladosporium*. We conclude that the observed association between *Alternaria* spore concentrations and airway responsiveness can be attributed to fungal exposure. However, the apparent association with wheeze frequency may be attributable to other confounding factors, possibly grass pollen exposure.

The association observed between airway responsiveness and *Alternaria* spore concentrations was based on mean airborne concentrations over 1 mo for 10 mo in different seasons over 2 yr. The effect of changes in allergen exposure on level

of airway hyperresponsiveness is known to extend for several weeks, and therefore the averaging period of 4 wk may be appropriate for measuring the effect of *Alternaria* exposure on this feature of asthma (34, 35). As we expected increased airway responsiveness to be associated with more frequent symptoms and need for bronchodilator, we believed that it was appropriate to measure these outcomes in the same context. However, because of the limited number of measurements of exposure, we are unable to describe the nature of the dose-response relation in detail, and the period of exposure necessary to cause an adverse respiratory outcome may be less than 1 mo. A significant association has been measured between daily airborne *Alternaria* spore concentrations and daily asthma symptoms using time series analysis in a cohort study of 19 asthmatics living in Southern California (36).

In regions where exposure to *Alternaria* is high, sensitization to the fungus is more strongly associated with asthma than sensitization to other allergens (9, 15). The airborne concentrations of *Alternaria* that we measured in Moree and in Wagga Wagga, and have been measured in other hot dry inland regions, are orders of magnitude higher than concentrations measured in cold temperate climates (37–39). In Arizona, children are more likely to have persistent asthma if they are sensitized to *Alternaria* than if they are sensitized to house dust mite (9). In coastal New South Wales, where airborne concentrations of *Alternaria* are lower than they are in Moree and Wagga Wagga, children with asthma are more likely to be sensitized to house dust mite than they are to *Alternaria* (15, 16, 22, 40).

We also found that the proportion of children sensitized to *Alternaria* who had airway hyperresponsiveness was approximately twice that of other allergic children, even during periods of "low" airborne concentrations of *Alternaria*. A study in France has found that sensitization to *Alternaria* is more strongly associated with reports of severe asthma than with reports of mild asthma (41).

In conclusion, we found that increases in respiratory symptoms and airway responsiveness were associated with increases in airborne concentrations of *Alternaria*. In addition, the association between spore concentrations and airway responsiveness was significantly stronger in children who were sensitized to *Alternaria*. This suggests that exposure to *Alternaria* is an important cause of more severe asthma in regions when exposure to the fungus is high. Further research is needed to more closely define the dose-response relation between *Alternaria* exposure and asthma and to develop effective environmental and therapeutic interventions.

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References

- Horner WE, Helbling A, Salvaggio JE, Lehrer SB. Fungal allergens. *Clin Microbiol Rev* 1995;8:161–179.
- D'Amato G, Spiekma FTM. Aerobiologic and clinical aspects of mould allergy in Europe. *Allergy* 1995;50:870–877.
- Kauffman HF, Tomee JFC, Van der Werf TS, de Monchy JGR, Koeter GK. Review of fungus-induced asthmatic reactions. *Am J Crit Care Med* 1995;151:2109–2116.
- Calvo MA, Guarro J, Suarez G, Ramirez C. Air-borne fungi in Barcelona city (Spain): I. A two year study (1976–1978). *Mycopathologia* 1980;71:89–93.
- O'Hollaren MT, Yunginger JW, Offord KP, Somers MJ, O'Connell EJ, Ballard DJ, Sachs MI. Exposure to an aeroallergen as a possible precipitating factor in respiratory arrest in young patients with asthma. *N Engl J Med* 1991;324:359–363.
- Mitakakis TZ, Ong EK, Stevens A, Guest D, Knox BR. Incidence of *Cladosporium*, *Alternaria* and total fungal spores in the atmosphere of Melbourne (Australia) over three years. *Aerobiologic* 1997;13:83–90.
- Takahashi T. Airborne fungal colony-forming units in outdoor and indoor environments in Yokohama, Japan. *Mycopathologia* 1997;139:23–33.
- Riedler J, Eder W, Oberfeld G, Schreuer M. Austrian children living on a farm have less hay fever, asthma and allergic sensitisation. *Clin Exp Allergy* 2000;30:194–200.
- Halonen M, Stern DA, Wright AL, Taussig LM, Martinez FD. *Alternaria* as a major allergen for asthma in children raised in a desert environment. *Am J Respir Crit Care Med* 1997;155:1356–1361.
- Yunginger JW, Jones RT, Nesheim ME, Geller M. Studies on *Alternaria* allergens. III. Isolation of a major allergenic fraction (Alt-1). *J Allergy Clin Immunol* 1980;66:138–147.
- Fadel R, David B, Paris S, Guesdon JL. *Alternaria* spore and mycelium sensitivity in allergic patients: *in vivo* and *in vitro* studies. *Ann Allergy* 1992;69:329–335.
- Chelkowski J, Visconti A. *Alternaria*: Biology, Plant Diseases and Metabolites. Amsterdam: Elsevier Science Publishers B.V., 1992.
- Licorish K, Novey HS, Kozak P, Fairshirer RD, Wilson AF. Role of *Alternaria* and *Penicillium* spores in the pathogenesis of asthma. *J Allergy Clin Immunol* 1985;76:819–825.
- Gergen PJ, Turkeltaub PC. The association of individual allergen reactivity with respiratory disease in a national sample: data from the second National Health and Nutrition Examination Survey, 1976–80 (NHANES II). *J Allergy Clin Immunol* 1992;90:579–588.
- Peat JK, Tovey E, Mellis CM, Leeder SR, Woolcock AJ. Importance of house dust mite and *Alternaria* allergens in childhood asthma: an epidemiological study in two climatic regions of Australia. *Clin Exp Allergy* 1993;23:812–820.
- Peat JK, Toelle BG, Gray EJ, Haby MM, Belousova E, Mellis CM, Woolcock AJ. Prevalence and severity of childhood asthma and allergic sensitisation in seven climatic regions of New South Wales. *Med J Aust* 1995;163:22–26.
- Pepys J. Skin testing. *Br J Hosp Med* 1975;14:412–417.
- Usherwood TP, Scrimgeour A, Barber JH. Questionnaire to measure perceived symptoms and disability in asthma. *Arch Dis Child* 1990;65:779–781.
- Yan K, Salome C, Woolcock AJ. Rapid method for measurement of bronchial responsiveness. *Thorax* 1983;38:760–765.
- O'Connor G, Sparrow D, Taylor D, Segal M, Weiss S. Analysis of dose response curves to methacholine. An approach suitable for population studies. *Am Rev Respir Dis* 1987;136:1412–1417.
- Peat JK, Salome CM, Berry G, Woolcock AJ. Relation of dose response slope to respiratory symptoms in a population of Australian school-children. *Am Rev Respir Dis* 1991;144:663–667.
- Mitakakis TZ, McGee P. Reliability of measures of spores of *Alternaria* and pollen concentrations in air over two towns in rural Australia. *Grana* 2000;39:141–145.
- Zeger SL, Liang K-Y. Longitudinal data analysis for discrete and continuous outcomes. *Biometrics* 1986;42:121–130.
- Zeger SL, Liang K-Y, Albert PS. Models for longitudinal data: a generalised estimating equation approach. *Biometrics* 1988;44:1049–1060.
- Toelle BG, Peat JK, Salome CM, Mellis CM, Woolcock AJ. Toward a definition of asthma for epidemiology. *Am Rev Respir Dis* 1992;146:633–637.
- Juniper EF, Kline PA, Vanzieleghe MA, Ramsdale EH, O'Byrne PM, Hargreave FE. Effect of long-term treatment with an inhaled corticosteroid (budesonide) on airway hyperresponsiveness and clinical asthma in nonsteroid-dependent asthmatics. *Am Rev Respir Dis* 1990;142:832–836.
- Sont JK, Han J, van Krieken JM, Evertse CE, Hooijer R, Willens LNA. Relationship between the inflammatory infiltrate in bronchial biopsy specimens and clinical severity of asthma in patients treated with inhaled steroids. *Thorax* 1996;51:496–502.
- Ulrik CS. Outcome of asthma: longitudinal changes in lung function. *Eur Respir J* 1999;13:904–918.
- Mitakakis TZ, Tovey ET, Xuan X, Marks GB. Personal exposure to allergenic pollen and mould spores in inland New South Wales, Australia. *Clin Exp Allergy* 2000;30:1733–1739.
- Agarwal MK, Swanson MC, Reed CE, Yunginger JW. Immunochemical quantification of airborne short ragweed, *Alternaria*, antigen E, and Alt-I allergens: two-year prospective study. *J Allergy Clin Immunol* 1983;72:40–45.
- Barnes C, Schreiber K, Pacheco F, Landuyt J, Hu F, Portnoy J. Comparison of outdoor allergenic particles and allergens levels. *Ann Allergy Asthma Immunol* 2000;84:47–54.

32. Hatch M, Thomas D. Measurement issues in environmental epidemiology. *Environ Health Perspect* 1993;101(Suppl):49–57.
33. Armstrong BK, White E, Saracci R. Principles of exposure measurement in epidemiology. Oxford: Oxford University Press; 1995.
34. Boulet L, Cartier A, Thomson NC, Roberts RS, Dolovich J, Hargreave FE. Asthma and increases in nonallergic bronchial responsiveness from seasonal pollen exposure. *J Allergy Clin Immunol* 1983;71:399–406.
35. Platts-Mills TA, Tovey ER, Mitchell EB, Moszoro H, Nock P, Wilkins SR. Reduction of bronchial hyperreactivity during prolonged allergen avoidance. *Lancet* 1982;2(8300):675–678.
36. Delfino RJ, Zeiger RS, Seltzer JM, Street DH, Matteucci RM, Anderson PR, Koutrakis P. The effect of outdoor fungal spore concentrations on daily asthma severity. *Environ Health Perspect* 1997;105:622–635.
37. Srivastava AK, Wadhvani K. Dispersion and allergenic manifestations of *Alternaria* airspora. *Grana* 1992;31:61–66.
38. Sneller MR, Hayes HD, Pinnaas JL. Frequency of airborne *Alternaria* spores in Tucson, Arizona over a 20-year period. *Ann Allergy* 1981;46:30–33.
39. Hjelmroos M. Relationship between airborne fungal spore presence and weather variables. *Cladosporium* and *Alternaria*. *Grana* 1993;32:40–47.
40. Bass D, Morgan G. A three year (1993–1995) calendar of pollen and *Alternaria* mould in the atmosphere of south western Sydney. *Grana* 1997;36:293–300.
41. Neukirch C, Henry C, Leynaert B, Liard R, Bousquet J, Neukirch F. Is sensitization to *Alternaria alternata* a risk factor for severe asthma? A population based study. *J Allergy Clin Immunol* 1999;103:709–711.
42. Quanjer PH, Borsboom GJJM, Brunekreef B, Zach M, Forche G, Cotes JE, Sanchis J, Paoletti P. Spirometric reference values for white European children and adolescent. *Pediatr Pulmonol* 1995;19:135–142.