

Sensitization to *Alternaria* and *Cladosporium* by the age of 4 years

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Summary

Of 1218 children born on the Isle of Wight in 1989/90, and followed for atopy at age 4 years, 981 were skin-prick tested with a battery of allergens. Of these 61 (6%) reacted positively to *Alternaria alternata* and *Cladosporium herbarum* (47 to *Alternaria*, 21 to *Cladosporium* and seven to both). Twenty-four (39%) were asymptomatic (latent atopy) of which 12 had a single positive reaction either to *Alternaria* or *Cladosporium*. Asthma was the most common disease in children sensitized to moulds. *Alternaria* sensitization correlated positively with clinical diagnosis of asthma ($P < 0.01$), eczema ($P < 0.001$) and rhinitis ($P < 0.05$). Likewise, *Cladosporium* sensitivity correlated with a diagnoses of asthma, eczema and rhinitis (all $P < 0.05$). Age of the house correlated with reported damp and lack of central heating (both $P < 0.001$), but not with sensitization to moulds. An association between the presence of damp or age of the house and mould allergy was confounded by 21 children moving house in the first 4 years. Exposure to pets, passive tobacco smoking and season of birth had no bearing on mould sensitivity. At 4 years of age *Alternaria* and *Cladosporium* were the third most common causes of sensitization, i.e. after house dust mite and grass pollen.

Keywords: *Alternaria*, *Cladosporium*, moulds, sensitization, skin-prick test

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Introduction

In recent years there has been interest in the role of sensitization to ubiquitous moulds such as *Cladosporium*, *Alternaria*, *Penicillium* and *Aspergillus* species as triggers for asthma. In a study from the upper Midwest of the United States of America *Alternaria* was linked to acute exacerbations of asthma culminating in respiratory arrest [1]. Studies from Western Europe and the United States of America show that sensitization to *Basidiomycetes* such as *Calvatia cyathiformis* and *Pleurotus ostreatus* is relatively common in subjects with respiratory allergic disease [2]. Furthermore, bronchial challenge with their extracts has been shown to induce bronchospasm in sensitized individuals [3]. Several reports on bakers' asthma have described allergy to alpha amylase obtained from *Aspergillus oryzae* [4]. Some atopic individuals have positive skin-prick tests

and/or serum specific IgE to several fungal antigens in the absence of crossreactivity, suggesting the existence of a possible linked, high responder status to fungal spores [5]. Most population studies did not look at the importance of an individual aeroallergen such as house dust mite, grass pollen, animal dander or moulds in the pathogenesis of atopic disorders after controlling for reactivity to other allergens. However, a recent large survey involving 4295 subjects showed an independent association of *Alternaria* sensitization to asthma and rhinitis [6]. In general, sensitization to moulds has been studied in subjects with atopic disorders. The true population prevalence of mould sensitization therefore remains unknown. This whole population study gives an estimate of sensitization to *Alternaria alternata* and *Cladosporium herbarum* by the age of 4 years and correlates it with asthma and atopy. It also assesses the influence of factors such as season of birth, the age of the house, reported damp and absence of central heating on sensitization to moulds.

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Materials and methods

Study population

Fifteen hundred and thirty-six babies born consecutively on the Isle of Wight between January 1989 and February 1990 were included prenatally in a birth cohort. A majority had cord blood IgE measured soon after birth. Family history of atopic disorders and environmental influences such as passive tobacco smoking, exposure to household pets and details of housing have been recorded. Findings of follow-up for the development of atopy at 1 and 2 years have been published [7,8]. Twelve hundred and eighteen children were available for review at 4 years of age.

Skin-prick testing

Nine hundred and eighty-one of the 1218 children seen at 4 years were given skin-prick tests (SPT) using extracts of house dust mite (*Dermatophagoides pteronyssinus*, HDM), grass pollen mix, cat and dog dander, cow's milk, egg, soya, wheat, cod, *Alternaria alternata*, *Cladosporium herbarum* and peanut (Soya, ALK Denmark; all others, Biodiagnostics, Allergopharma, Germany). The extract of *Alternaria* was standardized for its major allergen *Alt a 1* ($\approx 2.5 \mu\text{g/mL}$). The extract of *Cladosporium* lacked standardization; its manufacture however, utilized crossed immuno-electrophoresis to minimize batch to batch variability in the overall allergen content. Histamine (0.1% w/w, in 0.9% saline and 50% glycerine) and physiological saline were used as positive and negative controls, respectively. The test was read after 15 min and any reaction with a mean weal diameter at least 3 mm greater than the negative control was taken as positive.

Analysis of data

Details of environmental factors, family history and symptoms of atopic disease as well as the results of SPT were entered into a computer file (SPSS PC⁺ V4, Chicago, USA). Univariate associations between sensitivity to *Alternaria* and *Cladosporium* and atopic disorders were assessed using chi-square and Fisher's exact tests. For comparison all children negative to *Alternaria* and *Cladosporium* on SPT were taken as controls and, where appropriate, a comparison was also made to the findings for the whole cohort. Logistic regression analysis was carried out to estimate the importance of sensitization to *Alternaria* and *Cladosporium* in the pathogenesis of asthma and other atopic disorders after controlling for important hereditary, developmental and environmental influences. An association between mould sensitization, season of birth, age of the house, reported damp and absence of central heating was also estimated.

The controls for logistic regression analysis were defined as:

Hereditary and developmental factors

Family history	no atopy in first degree relatives
Sex	female
Cord blood IgE	< 0.5 KU/L
Birth weight	normal (> 2.5 kg)

Environmental factors

Smoking	no maternal smoking
Pets	no cat or dog in the house
Season of birth	spring or summer birth
Method of feeding	breast feeding (introduction to formula after age 3 months)

Results

Of 981 children who had SPT 61 (6%) reacted positively to moulds (48 reacted to *Alternaria*, 21 to *Cladosporium*, and seven to both; 35 (57.4%) male). Twenty-four of 61 (39%) had no symptoms and were classed as having latent atopy. Twelve (50%) of these had a single positive SPT either to *Alternaria* or *Cladosporium*. Latent atopy among children sensitized to moulds was more common than that estimated for the whole cohort (39% vs 8%, $P < 0.001$). Asthma (21 cases) was the most common atopic disorder in children sensitized to moulds, followed by eczema and allergic rhinitis. Atopic symptoms were not significantly worse in those sensitized to moulds. Only 12 of 135 (8.9%) children with 'asthmatic' wheeze and 22 of 186 (11.8%) with 'asthmatic' cough (three or more episodes per year, each lasting > 3 consecutive nights/days) were sensitized to moulds. Seventeen of 21 (81%) mould sensitized asthmatics were taking regular treatment of which 10 were on preventive (inhaled cromoglycate and/or corticosteroid) medication. Treatment figures for asthmatics not sensitized to moulds ($n = 160$) were similar (145 of 160 [90.6%] receiving regular treatment). The mean age of onset of asthma for mould sensitized children was similar to that for those sensitized to aeroallergens other than moulds (23.2 vs. 21.3 months). All atopic disorders except urticaria showed significant correlation with mould sensitization (Table 1). A majority of mould-sensitized children had multiple positive skin tests. There were statistically significant associations between positive SPT to *Alternaria* and/or *Cladosporium* and a positive reaction to HDM, cat and dog dander, and grass pollen mix. Table 2 lists the number of positive SPT reactions to other aeroallergens. Passive exposure to tobacco smoke during pregnancy or after birth did not increase mould sensitization. Sensitivity to *Alternaria* and

Table 1.

	<i>Alternaria</i> (n = 47)	<i>Cladosporium</i> (n = 21)	<i>Alternaria</i> &/or <i>Cladosporium</i> (n = 61)
Asthma	15 [†]	8	21 [‡]
Eczema	15 [†]	7	18 [‡]
Rhinitis*	7	4	10 [†]
Food intolerance*	4	4	7 [‡]
Any disorder	28 [‡]	11	35 [‡]

Chi-square with Yate's correction. * Fisher's exact test.

[†] $P < 0.01$, [‡] $P < 0.001$.

All other values $P < 0.05$.

Cladosporium was not related to the presence of pets in the house, exposure to cat and dog in the first 6 months of life, or to autumn or winter births. Although reported damp in the house was associated with a lack of central heating (27.5% vs 11.9%) as well as with age of the house (80.6 vs 59.5 years) (both $P < 0.001$), it was not a significant risk for sensitization to moulds. An effect of reported damp, age of the house and absence of central heating was confounded by the fact that 21 mould-sensitive children had moved house by the age of 4 years.

When compared with those with negative SPT to moulds, children sensitized to moulds often had a history of atopic illness in at least one first degree relative [49 of 61 (80%) vs 533 of 915 (58%), $P = 0.001$]. The duration of breastfeeding in children sensitized to moulds was similar to that for the whole cohort (16.3 ± 13.7 vs 14.9 ± 14.8 weeks). Likewise, there was no difference in the age of introduction to a milk formula. Cord blood IgE at birth had been measured in 54 of the mould sensitized children with 16 (29.6%) showing high (> 0.5 kU/L) levels ($P < 0.001$). Despite a statistically significant correlation with sensitization to moulds the predictive value of cord IgE had poor sensitivity (14.3%) and specificity (88%). Overall, 190 of 981 (19.4%) children had positive SPT to one or more of the aeroallergens. Cord blood IgE was available in 166 of these children and in 39 of 166 (23.5%) the levels were recorded as high.

Multifactorial analysis using logistic regression showed that family history of atopy (OR 2.59, CL 1.31–3.40) and a

cord blood IgE level at birth of > 0.5 kU/L (OR 2.99, CL 1.58–4.74) were significant for allergy to moulds. The results were similar for sensitization to any aeroallergen (including HDM, grass pollen, cat and dog dander, *Alternaria* and *Cladosporium*) except for the finding that more male children were sensitized (113 vs 77, OR 1.51, CL 1.06–2.14).

Discussion

Allergy to moulds is increasingly recognized as clinically relevant in patients with atopic disease such as asthma and allergic rhinitis. Efforts are being made to isolate and characterize specific antigens and epitopes from common moulds. In general, commercial extracts available for diagnosis and immunotherapy of fungal sensitivity lack standardization because of intrinsic differences between isolates as well as variations in extraction methods. There is a need for identification of major allergenic components of different moulds, and for their use in standardization of commercial extracts. Regional variability in the expression of allergens by various moulds and in the prevalence of sensitization to individual mould allergens, however, poses considerable difficulty. The outdoor air concentrations of certain mould spores such as *Alternaria* and *Cladosporium* peak in dry conditions. In contrast, ascospores and basidiospores rise after periods of rainfall and have therefore been implicated in late summer exacerbations of asthma in sensitized individuals. In response to the recent concern over clinically relevant sensitization to basidiospores and ascospores, the extracts of these spores will be added to SPT batteries used in the follow-up of these children.

Most studies of allergy to moulds have targeted groups of patients with a specific atopic disorder such as asthma, rhinitis or eczema [5,9]. A large population survey from the United States looked at sensitization to a number of inhalant allergens in 4877 white individuals between 6 and 24 years of age [6]. The results were stratified into three age ranges and revealed a clustering of positive reactions to *Alternaria* at around 5%. A prevalence of sensitization to *Alternaria* and/or *Cladosporium* of 6% in the population of children aged 4 years living on the Isle of Wight is very similar and may be taken as representative of the rural south of England. It has been estimated that as many as 30% of individuals with IgE-mediated aeroallergen reactivity may be positive to *Alternaria* on SPT [10]. In this birth cohort 47 of 190 (24.7%) children reacting to one or more aeroallergens were found to be positive to *Alternaria* and 21 (11%) reacted to *Cladosporium*. In contrast, sensitization to *Cladosporium* is more common in regions with a cold climate such as Scandinavia [5].

The most common cause of sensitization at 4 years was house dust mite [117 children (11.9%)] followed by grass

Table 2.

House dust mite	26 (42.6%)	Grass pollen mix	19 (31.1%)
Cat	17 (27.9%)	Dog	9 (14.7%)

All $P < 0.001$.

Table 3.

	<i>Alternaria</i> &/or <i>Cladosporium</i> (n = 61)	Any aeroallergen (n = 190)
Family history of atopy	2.59 (1.31–3.40) [‡]	1.50 (1.04–2.17) [‡]
Male sex	–	1.51 (1.06–2.14) [‡]
High cord blood IgE	2.99 (1.58–4.74) [‡]	2.46 (1.05–3.85) [‡]

Adjusted odds ratios (confidence limits).

[†] $P < 0.05$, [‡] $P < 0.01$

Other factors evaluated but found not to be significant were birthweight, parental smoking, exposure to pets, autumn/winter birth, formula feeding, maternal and paternal allergy.

'Any aeroallergen' includes *Dermatophagoides pteronyssinus*, grass pollen mix, *Alternaria alternata*, *Cladosporium herbarum*, and cat and dog epithelia.

pollen mix [77 children (7.8%)]. Taken together, *Alternaria* and *Cladosporium* were the third most common source of sensitization, i.e. more than cat allergen [57 cases (5.8%)].

A number of studies show that sensitivity to *Alternaria* and *Phoma betae* may co-exist [5–11]. A clustering of sensitization to *Alternaria*, *Cladosporium* and *Aspergillus fumigatus* within certain individuals has also been described [12]. These observations suggest a specific genetic susceptibility to mould sensitivity. Although there was a trend towards clustering of sensitization to *Alternaria* and *Cladosporium* in the children born on the Isle of Wight, no conclusions could be drawn because of the small numbers affected.

Latent atopy to moulds is not well recognized. This may simply be due to the recruitment, in previous studies, of individuals with symptoms of atopy [5,10,11,12]. This whole population study highlighted this phenomenon with a large minority (39.5%) of mould sensitized children exhibiting latent atopy to *Alternaria* and *Cladosporium* at 4 years. One study of asthmatic subjects found those monosensitized to *Alternaria* to be younger suggesting that sensitization to *Alternaria* may precede sensitization to pollens [13]. This observation could not be confirmed in this study as *Alternaria* was not part of the SPT batteries used at previous follow-ups. Nevertheless, if one accepts this theory, then it is plausible that those children with current latent atopy to *Alternaria* and *Cladosporium* may become symptomatic as they develop multiple aeroallergen sensitivity later in life.

Sensitization to moulds has been linked to asthma, rhinitis and eczema [1,5–7,13] with seasonal exacerbations of asthma occurring during periods of high mould levels [1]. In this study, exacerbations of asthma or rhinitis could not

be correlated with seasonal peaks in the levels of mould spores as data on spore counts were not available. Furthermore, the duration of allergic respiratory tract symptoms was short in the majority. Bronchial challenge of sensitized asthmatics with fungal spores has been shown to produce a marked response [14] and this technique may be useful in evaluating the clinical relevance of sensitization. One population survey found that reactivity to *Alternaria* increased the risk of asthma 5 times as compared with a two- to threefold increase from sensitization to other aeroallergens [6]. The number of children sensitized to *Alternaria* in this study was small for making such comparisons.

A poor predictive value of cord blood IgE for the development of atopy was reported at the age of 1 year [15]. Cord IgE, though significantly associated with sensitization to moulds at age 4 years on both univariate and multivariate analysis, remains a poor predictor for atopy and cannot be recommended as a routine investigation in the newborn.

In conclusion, at the age of 4 years, *Alternaria* and *Cladosporium* are the third most common cause of sensitization on the Isle of Wight. A considerable proportion of mould-sensitized children can be asymptomatic. Allergy to moulds may be clinically relevant and tends to cluster within individuals.

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