Indoor fungal diversity and asthma: a meta-analysis and systematic review of risk factors

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Abstract

Background: Indoor dampness increases the risk of indoor fungal growth, specifically the genera *Penicillium* and *Aspergillus*. These fungi are thought to increase the risk of asthma initiation, development and/or exacerbation. No systematic review to date has investigated this relationship.

Objective: The review aims to assess the relationship between exposure to indoor fungal species (specifically *Aspergillus* and *Penicillium*) and asthma outcomes in children and adults.

Methods: Ten databases were systematically searched on 18th April 2013 and limited to articles published since 1990. Reference lists were independently screened by two reviewers and authors contacted to identify relevant articles. Data were extracted from included studies meeting our eligibility criteria by two reviewers and quality assessed using the Newcastle-Ottawa scale designed for assessing case-control and cohort studies.

Results: *Cladosporium*, *Alternaria*, *Aspergillus* and *Penicillium* were found to be present in significantly higher concentrations in homes of asthmatic participants. The presence of these fungi increased the risk of current asthma by 36-48% compared to those exposed to lower concentrations of these fungi, as shown by random-effect estimates. *Cladosporium* and *Alternaria* increased the risk of current asthma when using sub-group analyses. Studies were of medium quality, showed medium-high heterogeneity, but evidence concerning the specific role of fungal species was limited.

Conclusion: Increased exposure to *Penicillium*, *Aspergillus*, *Cladosporium* and *Alternaria* species represents a health risk for asthmatic individuals. Sub-group analyses in our effect estimates suggest that *Cladosporium* and *Alternaria* were principally associated with an increased risk of asthma.

Systematic Review Registration Number

Prospero protocol registration number CRD42013004333, found here http://www.crd.york.ac.uk/PROSPERO/DisplayPDF.php?ID=CRD42013004333

Key message

Future studies should consider the adoption of a multidisciplinary approach utilizing both molecular and epidemiological tools to accurately determine the extent and timing of exposures to allergenic fungi to reliably assess potential health effects.

Key words: systematic review, damp, indoor fungi and allergic asthma

Abbreviations:

CE: Cell equivalent

CFU: Colony Forming Unit

EE: Effect Estimates

ERMI: Environmental Relative Moldiness Index

IAQ: Indoor air quality

NOS: Newcastle-Ottawa Scale

NR: Not reported

NS: Not significant

MSqPCR : Mold specific quantitative polymerase chain reaction

Introduction

Genetic factors alone cannot explain the high asthma prevalence rates in childhood[1] or adulthood[2] worldwide, or the variations between different regions comprising similar ethnicities[3]. This has led to a research focus on poor indoor air quality (IAQ) in the home environment. IAQ is likely to be compounded by efforts to alleviate climate change risks[4] resulting from reductions in property ventilation to reduce domestic carbon footprints and prevent heat loss. Inadequate ventilation increases the risk of elevated dampness[5], which currently affects around 16% of European dwellings[6]. Dampness raises the risk of fungal contamination and likelihood of developing asthma[7].

Human behaviors, socio-economic factors and the built environment have been shown to increase the fungal load found in house dust[8]. Old terraced houses (90+ years old) have been shown to increase concentrations of *Penicillium* and *Aspergillus* propagules, exceeding outdoor spores per m^3 of air per day in homes with no suspected damp or fungal contamination[9]. These fungi are also more frequently cultured from damp indoor home environments[10] and are of interest because they have been implicated in the onset of childhood asthma[11]. Variations in concentrations and diversity of fungal propagules (hyphae and spores) may regulate the risk of asthma initiation, development or exacerbation.

To our knowledge there has been no systematic review exploring the role of fungal diversity and risk of asthma in children and adult populations. This is complicated by the ubiquity of fungi and the fact more than 80 fungal genera have been shown to induce IgE-mediated Type I hypersensitivity in susceptible populations. These fungi primarily belong to the phyla Ascomycota, Basidiomycota and Zygomycota[12]. Systematically reviewing studies concerning the diversity and concentrations of indoor fungi and risk of asthma initiation and/or exacerbation provides an opportunity to assess associations and improve future health intervention work.

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90 **Objectives**

91 The review aims to assess the role of indoor fungal species (specifically those
92 belonging to the genera *Aspergillus* and *Penicillium*) on asthma outcomes (initiation,
93 development and exacerbation) in infants, children and adults. In doing so, we aimed to
94 investigate factors modifying the indoor concentration and diversity of fungi implicated with
95 increased risk of asthma, and to compare the strength and association with other reported
96 predictor variables such as known demographic and built environment risk factors.

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Materials and Methods

Search Strategy

Electronic searches were conducted on 18th April 2013 and limited to studies published after 1990, in accordance with our protocol (PROSPERO ref: CRD42013004333). In addition to electronic searches, author contacts and references of included studies were conducted in August 2013. The full search strategy was employed on all ten databases (listed our online repository Appendix E1) to identify eligible articles. The screening process was managed in Endnote version X5.0 (Thomas Reuters, USA)[13], and recorded using the PRISMA guidelines[14]. Articles were independently screened by two team members (RS & NB), and where there was disagreement a third reviewer (NJO) was consulted and any discrepancies were resolved through discussion.

Eligibility Criteria and Study Selection

Included articles were those reporting associations between the home environment, indoor fungal genera/species and risk of asthma (Figure 1). Forward and backward citation chasing was performed on all included studies, and authors contacted for additional relevant articles.

The populations investigated encompassed all ages (infants, children (aged <18) adults) and both sexes. Studies deemed eligible for the analysis comprised:

(i) original peer-reviewed articles publishing original data;

(ii) cohort, case-control studies, non-randomized and randomized controlled trials (RCT) (including cluster-randomized and cross over trials);

(iii) those published in 1990 or later;

(iv) investigations of the indoor home environment;

(v) assessments of indoor fungi, identified to the genus or species level;

(vi) those with outcomes: asthma ever and/or asthma symptoms in the last 12 months, including wheeze, whistling in the chest or a dry cough; doctor diagnosed, skin prick test, peak flow or spirometry; and asthma initiation / development, requiring newly diagnosed cases of asthma by a physician or doctor; and

(vii) those that provided a measure of risk for asthma, including the relative risk (RR) or odds ratio (OR) and confidence intervals (CI).

Data Extraction

Relevant participant and study characteristics were recorded using a standardized data extraction template (Appendix E2), which was subsequently used to populate data synthesis tables.

Quality Assessment

Two team members (RS & NB) assessed the quality of each study using the Newcastle-Ottawa Scale (NOS)[15], modified to reflect fungal exposure (see case-control form, Exposure point 1, Appendix E3). Included studies were independently scored out of 10, and 13 for case control and cohort studies, respectively, in accordance to the NOS standard procedure. Both team members (RS & NB) independently scored included articles and a final score was obtained by consensus. Journal article authors were contacted if data was missing.

Results Synthesis

Completed data extraction tables of included studies were used in an overarching narrative synthesis (Table 1). Seven studies (Salo, et al. [16], Araki, et al. [17], Dales, et al. [18], Jones R, et al. [19], Li and Hsu [20], Rosenbaum, et al. [21], Dharmage, et al. [22]) were included in meta-analyses using random-effect models. We had planned to prioritize studies rated more highly on NOS rating scale, however evidence located was all of a mid-range quality and so we did not weight studies in the analysis.

Outcomes

Three outcomes were included. Firstly, studies were grouped according to those reporting risk of increased fungal concentrations in asthmatic homes (analysis of indoor fungi in homes being occupied with one or more individuals with asthma). We then assessed fungal genera, total fungi and risk of asthma. Finally, potential predictor variables and risk of asthma were tabulated.

Meta-analyses were undertaken to explore the relationship between exposure to individual groups of fungi and current asthma using the 'generic inverse variance method' [23] to conduct random-effects meta-analysis[24] in Revman 5 (version 5.2.6)(Cochrane, Copenhagen). Logistic regression was used to calculate odds ratios (OR) and confidence intervals (CI) for adjusted and unadjusted data due to the inconsistency of reporting unadjusted data. We were unable to stratify by age, study design or outcome due to the limited number of studies and inconsistent reporting.

Heterogeneity was assessed using the I^2 statistic, where an I^2 of 0% to 40% was considered as low heterogeneity and ≥75% represented considerable heterogeneity[23]. No further analyses were conducted due to sample size limitations.

Results

Participant Characteristics of Included Studies

The searches revealed 17 studies meeting our eligibility criteria. Included studies were from 8 countries and included case-control, nested case control, cross-sectional and longitudinal design methodologies (Table 1). One author[17] provided additional analyses to be included in our results synthesis. Eight studies were based on populations living in the United States, the remaining were from the UK, Sweden, Taiwan, Columbia, Australia, Canada and China.

Thirteen included studies involved children (aged <18 years), two included adult populations and the remaining two included all age groups. Demographic variables (i.e. variations in the built environment and occupant behaviors) potentially modifying the risk of fungi and/or asthma were not consistently reported, preventing their inclusion into our analysis to address our secondary aim. Reported asthma outcome measures also varied (Table 1) and only two studies, Reponen, et al. [11] and Matheson, et al. [25], examined asthma development, which inhibited analyses concerning the role of fungal diversity in the initiation of asthma.

Study Design Characteristics of Included Studies

We included four cohort studies with follow up periods 1, 2 & 7 years and thirteen were cross-sectional, which included 9 case-control studies. Funding, recruitment and statistical analyses varied between studies (Table E2). The heterogeneity between study designs, the defined exposure and outcomes prevented the inclusion of all studies in our meta-analysis. For this reason the following are included in our narrative syntheses;

- Outcome 1 is the risk of fungi in asthmatic homes measured as cell equivalents per gram (CE/g) of house dust (Table E3) and colony forming units per meter cubed of air (CFU/m^3) (Table E4);
- Outcome 2 is the associated risk of asthma concerning exposure to groups of fungi, which included statistical analyses using rate ratios (Table E5a) and odds ratios (Table E6). The latter were included in our random-effects meta-analysis;
- Outcome 3 summarizes demographic predictor variables for asthma included in their analyses (Table E5b & E6e-f).

Outcome 1: Indoor Fungi Measured in Homes of Asthmatics

Three studies from the US assessed the risk of elevated fungal concentrations in asthmatic homes[11, 26, 27] using house dust samples and 'Mold Specific' qPCR (MSQPCR) to quantify concentrations of 36 fungi included in the ERMI[28]. Nine fungal genera (Table 2) were found to be present in significantly higher concentrations in asthmatic homes, though these were not consistent and concentrations varied considerably (Table E3). These findings were not consistent with studies utilizing air sampling to quantify fungal concentrations[29-32] (Table E4). Studies utilizing air sampling (Colony Forming Units per m^3 of air) used microscopy as opposed to qPCR to identify fungi to the genus level. Two studies showed a positive association between elevated fungal concentrations in homes of asthmatics compared to the control groups. This included *Penicillium* (496.8 versus 276.3 total CFU/m^3)[31], *Cladosporium* (5.18 versus 4.43 mean CFU/m^3), *Ulocladium, Acremonium* (3.32 versus 0 mean CFU/m^3) and total fungi (5.92 versus 5.19 mean CFU/m^3).[32]

Outcome 2: Fungal Exposure and Risk of Asthma

Investigations into specific groups of fungi and associated risk of current asthma were not consistent and limited our syntheses. Three studies assessed the potential risk of asthma by calculating prevalence or rate ratios (Table E5a). Herrera, et al. [33] reported an increased

probability (>50%) of respiratory symptoms (indicative of bronchial asthma) being associated with *Acremonium* spp. (PR 6.2 95%;CI 3.8-10.0). Gent, et al. [34] reported that the highest level of *Penicillium* (≥1,000 CFU/m^3) was associated with higher rates of wheeze (aRR 2.2 95%;CI 1.3-3.5) in the first year of life. Finally the summation of *Aspergillus ochraceus, Aspergillus uniguis* and *Penicillium variabile* were associated with the onset of asthma in children aged 7 (aRR 2.2 95%;CI 1.8-2.7)[11].

Eight studies used logistic regression to calculate odds ratios and confidence intervals to assess the risk of asthma associated with fungal exposure. In some cases, studies did not report unadjusted data (Table E6), which prevented the inclusion of raw data into our meta-analysis. We were unable to assess the risk associated with fungal species because identification was only made to the genus level for *Aspergillus, Penicillium, Cladosporium* and *Alternaria*, with the exception of one study[16]. Increased exposure to these fungi was associated with an increased risk of asthma in childhood and adult populations (Table 3), though this relationship was not consistently reported. Other fungi investigated included *Rhodotorula, Epicoccum, Acrodontium* and sterile fungi (those lacking asexual or sexual spore production), which were not associated with increased risk of residents having asthma (Table E6). Seven studies were included in random effects meta-analysis to assess the strength and direction of association concerning exposure to *Aspergillus, Penicillium, Cladosporium* and *Alternaria* and risk of current asthma (Table 4). We excluded data concerning the associated risk of asthma resulting from models investigating the associated level of risk with doubling fungal exposures[16, 25] because the methodology differed from other included data.

Outcome 2, Sub-group Analysis: Fungal Genera and Risk of Asthma

Random-effect estimates were calculated in combined models to investigate the role of fungal load, and then individual fungal genera. Effect estimates of each model were calculated with the number of included studies and I^2 statistic, indicating that included studies were subject to medium to high heterogeneity (Table 4). No associations were reported with the total fungal load found indoors (model 1) and models 2-4 suggest that fungi identified to the genus level increases the risk of current asthma. The combination of four prevalent indoor fungi *Cladosporium, Alternaria, Penicllium* and *Aspergillus* (Model 5) increased risk of current asthma by 48% in the unadjusted model and 36% in the adjusted model. Studies were subject to medium heterogeneity with I^2 statistic ranging from 61 to 67% (Table 4). Sub-group analyses suggests that the association was primarily due to elevated levels of *Cladosporium* and *Alternaria* (models 6-9), with no significant association with exposure to *Penicillium* and *Aspergillus* (Figures 2, 3 and Appendix E1). Further analyses showed that the findings may be driven by one study[16] demonstrating a strong association between *Alternaria alternata* and asthma. The fungal analysis of this study differed by the use of ELISA techniques to quantify concentrations of *Alternaria alternata* antigen in house dust. Analyses in these models excluded *Rhodotorla, Acrodontium* and *Epicoccum* because data concerning these fungi were not consistently reported.

Outcome 3: Residential Factors Modifying Risk of Asthma

Built environment and demographic risk factors were inconsistently reported, preventing their inclusion in our analyses (Tables E5b & E6e/f). Demographic and residential characteristics shown to modify the risk of asthma and/or wheeze are summarized (Table 5). Typical demographic risk factors reported included parental asthma, premature births, low SES and a pre-existing respiratory health problem (upper respiratory tract symptoms, pneumonia and rhinitis). Residential risk factors included the presence of fungal growth and

odor, though there were inconsistent findings. Other factors to consider include multi-family homes, elevated endotoxin, and use of humidifiers and levels of carpeting. No associations were reported with exposure to increased concentrations of VOCs, dampness, fungal ergosterol, HDM and heating system in use. Pet ownership investigated by two studies suggests a protective effect against the risk of asthma.

Risk of Bias of Individual studies

The NOS for included items (Table 1) indicated studies were of medium quality, suggesting the potential inclusion of bias. There is also the potential for the inclusion of reporting bias resulting from the inclusion of unadjusted and adjusted data into the random-effects models. Funnel plots present the variability between individual fungal groups (Figure E1) and the I^2 statistic (Table 4) suggests that there is medium to considerable heterogeneity, which suggests conservative effect estimates, with the exclusion of combined models for total fungi and *Alternaria* (I^2 ranging from 0 to <25).

Discussion

Risk of Fungi in Domiciles with Asthmatic Residents

The fungal genera *Aspergillus*, *Penicillium*, *Cladosporium*, *Ulocladium*, *Acremonium*, *Aureobasidium*, *Epococcum*, *Scopulariopsis*, *Trichoderma*, *Alternaria* and *Wallemia* were reported to be present in higher concentrations in homes of asthmatics. Identification to the genus level does not provide sufficient detail to assess the potential health outcomes resulting from increased exposure to known allergenic fungi present in higher concentrations at time of sampling. Development of the ERMI and use of MSqPCR[28] enables us to more reliably quantify fungal species present indoors[35]. *Aspergillus niger*, *Aspergillus unguis*, *Cladosporium cladosporioides*, *Aureobasidium pullans*, *Epicoccum nigrum*, and *Alternaria alternata* were found in higher concentrations in asthmatic homes in studies utilizing MSqPCR. These fungi are allergenic species that may induce Type I hypersensitivity[12]. It is not clear which factors regulate indoor fungal diversity and risk of asthma at the individual level, or how potential covariates that may modify the outcome.

Indoor Fungal Contamination and Asthma Initiation and/or Exacerbation

The majority of the included studies utilized cross sectional or case control study designs, which reduces our confidence in these results as it has also been found the relationship between moisture-related risk factors and asthma decreases in longitudinal analyses[36]. In an attempt to examine the role of fungi in asthma beyond exacerbation, two longitudinal studies have enabled the investigators to assess the effect of fungal diversity prior to the initiation of asthma. Birth cohorts at risk of atopy showed a two-fold increased risk of higher rates of infant wheeze[34] and the onset of childhood asthma[11] associated with exposure to species of *Penicillium* and *Aspergillus*. *Cladosporium* increased the risk of developing a new asthma attack in the last 12 months by 50% in adults[25]. There was limited

evidence of sufficient quality demonstrating how indoor fungal diversity and concentrations regulates the risk of developing asthma.

Our meta-analysis was primarily restricted to exposure to fungi identified to the genus level. This method of identification may underestimate occupant fungal exposures because only a small number of fungal spore types can be identified, and it is difficult to differentiate between significant genera such as *Penicillium* and *Aspergillus*[37]. *Penicillium*[21, 22], *Apsergillus*[19], *Cladosporium*[20, 22] and *Alternaria*[16] increased the risk of asthma by 36 to 48% in our effect estimates. Sub-group analyses and effect estimates suggests association results from exposures to increased concentrations of *Cladosporium* and *Alternaria*. The strong association with *Alternaria* results from the inclusion of one study,[16] which had a large sample size (N=2,456) compared to other studies and utilized ELISA to quantify concentrations of *Alternaria alternata* antigen. This study supports the adoption of such diagnostic assays and a large sample size in future investigations into fungal exposure and asthma.

Heterogeneity between studies explains some of the inconsistent findings, including sample size, age ranges and outcome definitions. This is likely to be compounded by variations in the adopted sampling methodologies (air CFU/m^3 versus dust CFU/g sampling) due to their poor correlation in estimating potential exposures[38] and differences in fungal identification techniques.[37, 39] Resultant health risks depend on the timing and extent of exposure to other groups of fungi, as well as ambient indoor conditions, growth substrates and levels of dampness,[5] which cannot be ascertained from the included studies.

Focusing on four commonly reported fungi fails to account for other species shown to induce Type I hypersensitivity[12], therefore the potential level of risk associated with other fungi cannot be discounted. It is also not clear from the evidence reviewed here how fungal diversity and risk of asthma may be modified by residential characteristics and the influx of

outdoor fungal spora, which regulates the indoor fungal profile.[5] *Penicillium, Apsergillus, Cladosporium* and *Alternaria* sporulation rates have considerable daily and seasonal variability, and combined with the adoption of different sampling techniques[40, 41]add another level of complexity. Indoor fungal concentrations used to calculate ERMI values have also been shown to be heterogeneously distributed across the USA[42]. These factors introduce another layer of uncertainty that cannot be explained from the evidence included in this review. The evidence reviewed suggests that exposure to increased concentrations of these four fungal groups represent a respiratory risk for asthma sufferers, but the evidence is not conclusive when assessing species diversity and the risk of asthma. It is still yet unknown how exposure to fungi influences the initiation of asthma.

Synthesis with Existing Knowledge

Combined random-effect estimates of 36% and 48% are similar to the meta-analyses of Fisk WJ, et al. [43] who reported an approximate 30-50% increase risk of asthma outcomes. Two cohort studies have demonstrated that exposure to increased fungal contamination and risk of atopy increases the risk of asthma development in childhood[44] and adult[45] populations. A recent systematic review reported a significant association with increased exposure to fungal odor (random-effects model; EE 1.7 95%;CI 1.2-2.5) and the development of asthma[7]. Fungal diversity and concentrations of *Penicillium, Aspergillus, Cladosporium* and *Alternaria* varies considerably between different populations[32, 46, 47]. This is likely to regulate asthma outcomes in different populations given that variations in residential characteristics regulates fungi found in US[8] and UK[9] homes.

Exposure to *Cladosporium* and *Alternaria* increased risk of asthma in our effect estimates, which may be due to asthma severity being associated with *Cladosporium*[25, 48] and *Alternaria*[49, 50]. It is not clear how the risk of asthma and severity of symptoms may be modified in sensitized populations, which is important to consider given that the development

of allergic asthma (presence of IgE antibodies) in adults have been associated with *Aspergillus fumigatus and Cladosporium*[51]. *Penicillium* is frequently cultured from damp indoor home environments and has been associated with asthma severity[52] peak flow variability[53] and asthma morbidity[54] when present in low concentrations[55]. The lack of association between exposure to *Penicillium* and *Aspergillus* and current asthma in meta-analyses may be due to the limitations discussed above. These are important fungi to consider in future work because they dominate damp indoor environment where propagule concentrations exceed those in their natural outdoor environments[5] and have been implicated in the initiation of childhood asthma[11]. Damp appears to be a high risk of having fungal growth present both in the US and European scenarios.

There is insufficient evidence to support targeted intervention work to lower exposures to high risk fungi in the general public, in order to reduce symptoms or the initiation of disease. It is accepted that fungal sensitization is associated with an increased risk of asthma[56]. Fungal diversity and concentrations of different fungal groups appear to modify asthma outcomes in atopic and non-atopic individuals. However, this may also be the result of the inhalation of different indoor/outdoor fungal propagules that regulates fungal sensitization and asthma severity[57]. This is likely to be influenced by a high aeroallergen load[58], which may have opposing health effects[59]. Work to date is inhibited by the lack of species identification. The adoption of a multidisciplinary approach and consistent sampling methodologies are required to accurately measure the timing and extent of exposures to microbial agents and other indoor/outdoor aeroallergens. This should be combined with a protocol for identifying the appropriate sampling period[60], along with clearly defined outcomes for asthma initiation (long-term) or exacerbation (short-term) and epidemiological techniques to investigate the etiology of asthma at a population level.

Strengths and Limitations of the Systematic Review

This assessment of the fungi and asthma literature has undergone a structured systematic review with all phases of this systematic review conducted in accordance to our published protocol. A number of limitations exist and we have tried to account for them by synthesizing our findings (Tables E7a-c). Our analyses were limited by the quality, reporting inconsistencies and limited number of peer reviewed studies investigating the role of fungal diversity and risk of asthma. The included studies had relatively small sample sizes giving low power to our analyses and prevented the stratification by age, exposure and outcome definitions. This assumes that asthma in children and adults is the same disease with the same pathways of pathogenesis. They showed medium to high heterogeneity and were of medium quality meaning that our findings may include reporting bias. Finally, we were unable to conduct further analyses to explore potential bias associated with the heterogeneity between studies due to the small number of included studies.

Conclusions

There is insufficient evidence to make any conclusion concerning the risk of asthma initiation by fungi, but exposure to *Penicillium, Apsergillus, Cladosporium* and *Alternaria* species may influence asthma outcomes. Sub-group analyses in our effect estimates suggest that *Cladosporium* and *Alternaria* were principally associated with an increased risk of asthma. Adoption of a holistic approach to the complex disease of asthma in atopic and non-atopic populations, with the understanding that multiple exposures are potentially involved and should be measured will lead to better study design and capture of sufficient data to allow a more measured view. This remains challenging as it will be expensive to achieve at the population level. We recommend that future studies should consider the adoption of a multidisciplinary approach utilizing both molecular and epidemiological tools to accurately estimate the extent and timing of exposures to reliably assess potential health effects.

Supporting Information

Available from the online repository: Appendices E1-E3, Tables E1-E7 and Figure E1

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422

423 **Conflict of Interest**

424 We declare that none of the authors involved in writing this paper have any conflict of
425 interests with respect to the content of this article.

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Figure 1.0 Diagram of the Systematic Search and Included Studies

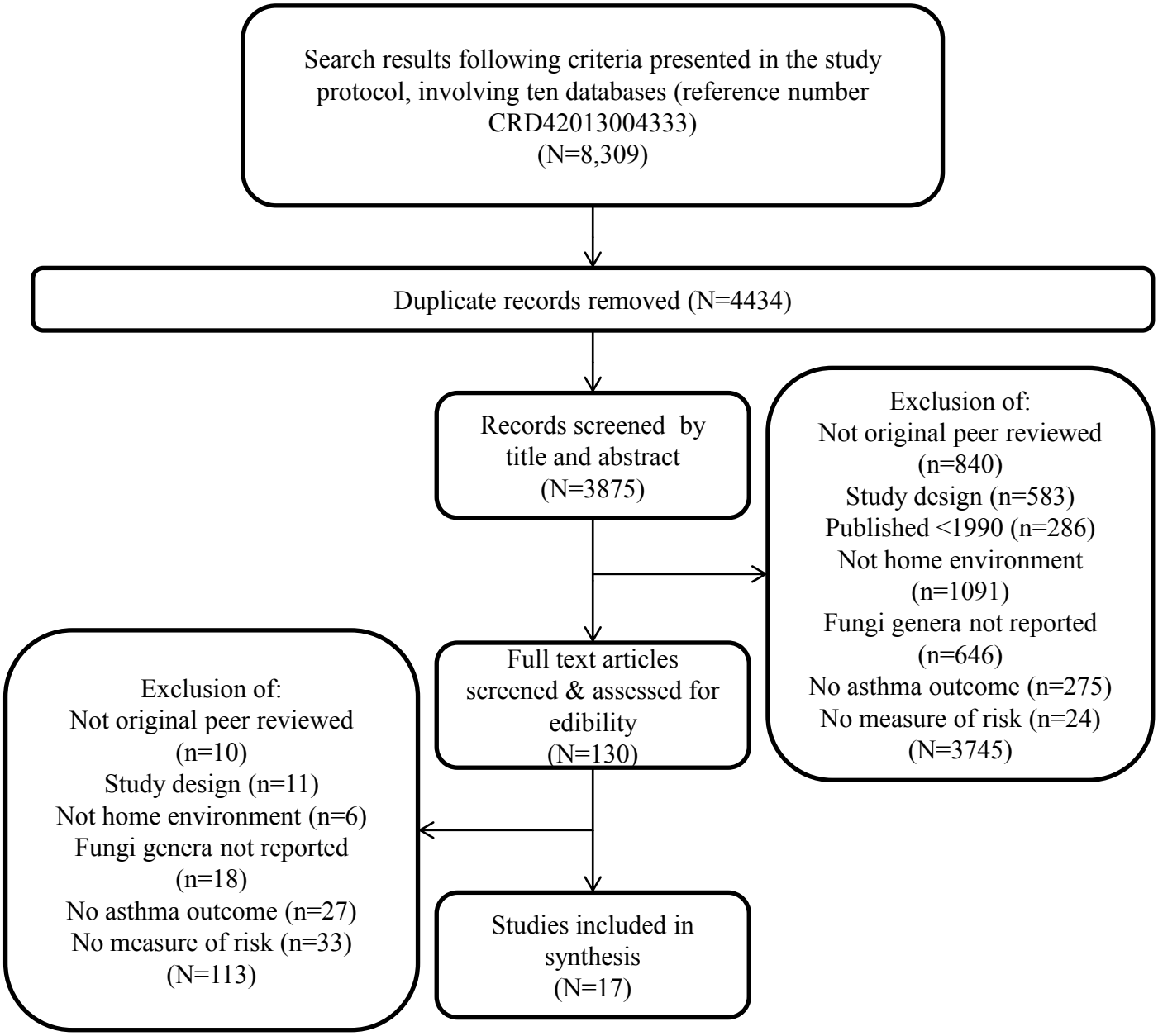


Table 1 Summary of participant characteristics of included studies

Author, year & Country	Country	Study population	Study design	Study size	Follow-up years	Exposure measurement	Definition of asthma	Final quality score
Vesper, et al. [1]	USA	Children, mean age 6.8 years	Case Control	60 cases, 22 controls	N/A	Air and dust sampling (mg/g) (ERMI)	Homes with an asthmatic child	4/10
Strachan, et al. [2]	UK	Children aged 6-7 years	Case Control	34 cases, 54 controls	N/A	Air sampling (CFU/m^3)	Wheeze in <12 months and Bronchial lability >10%	5/10
Holme, et al. [3]	Sweden	Children, aged 1-6 years	Nested Case Control	198 cases, 202 controls	N/A	Air sampling (CFU/m^3)	Asthma status defined by medical examination	12/20
Vesper, et al. [4]	USA	Children aged 9-12 years	Case Control	28 cases, 83 controls	N/A	House dust by vacuum CE / mg dust (ERMI)	Parental self-reported use of asthma medication	6/10
Su, et al. [5]	Taiwan	Children aged 10-12 years	Case Control	23 cases, 12 controls	N/A	Air sampling (CFU/m^3)	Adult self-reported child being diagnosed by a physician	6/10
Meng, et al. [6]	USA	Children aged 2-18 years	Case Control	88 cases, 85 controls	N/A	Air sampling (CFU/m^3)	Persistent asthma defined by National Heart, Lung and Blood Institute	4/10
Gent, et al. [7]	USA	Infants age <1 year	Cohort, Longitudinal	819	3 in 1 year	Air sampling (CFU/m^3)	Respiratory symptoms of wheeze and persistent cough, defined by yearly symptom counts	5/13
Herrera, et al. [8]	Columbia	Children aged 7 years	Cross Sectional	678	N/A	Air sampling (CFU/m^3)	Self-reported via questionnaire	4/10
Reponen, et al. [9]	USA	Children aged 7 years	Birth Cohort	69 cases, 220 controls	1 & 7	House dust sampling (ERMI)	Parental self-reports and spirometry	6/13
Matheson, et al. [10]	Australia	Adults aged 20-45 years	Longitudinal	360	2	Air sampling (CFU/m^3)	Wheeze <12 month plus bronchial hyper-reactivity to methacholine & clinical activity	7/13
Salo, et al. [11]	USA	All ages	Cross Sectional	2456	N/A	Dust sampling (mg/g)	Dr diagnosed asthma and allergy, symptoms in last year and medication use	7/10
Araki, et al. [12]	Japan	All ages	Case Control	609	N/A	Air sampling (CFU/m^3)	Self-reported questionnaire for receiving medical treatment for bronchial asthma	7/10
Dales, et al. [13]	Canada	Children aged 10 year	Cross Sectional	400	N/A	Self-reported & house dust samples collected	Self-reported questionnaire of current & diagnosed asthma	5/10
Jones R, et al. [14]	USA	Children aged 3-17	Nested Case Control	50 cases, 59 controls	N/A	Air sampling (CFU/m^3)	Self-reported questionnaire and clinical interview	8/10
Li and Hsu [15]	China	Children aged 7-15 years	Case Control	46 cases, 26 controls	N/A	Air sampling (CFU/m^3)	Asthma status defined by American Thoracic Society's criteria	5/10
Rosenbaum, et al. [16]	USA	Infants age <1 year	Birth Cohort	39 cases, 64 controls	2	Air sampling (CFU/m^3)	Diagnosis of wheeze defined by primary care provider and medication use	7/13
Dharmage, et al. [17]	Australia	Adults aged 20-44 years	Cross Sectional	485	N/A	Air sampling (CFU/m^3)	Wheeze <12 month plus bronchial hyper-reactivity to methacholine & clinical activity	6/10

Table 2 Results Synthesis – Outcome: Risk of Fungi in Asthmatic Homes

	Fungi measured as Cell Equivalents per gram of house dust								
Study	*Aspergillus niger* *Aspergillus ochraceus* *Aspergillus unguis*			*Penicillium* group 2 *Penicillium spinulosum* *Penicillium variabile*			*Cladosporium sphaerospermum* *Cladosporium cladosporioides 1* *Cladosporium cladosporioides 2*		
	Case	Control	P value	Case	Control	P value	Case	Control	P value
Vesper, et al. [1] GM CE/g	NR 1895.46 3831.60	NR 2117.95 1881.66	NR 0.79 0.32	2604.09 710.90 1050.69	654.48 3600.06 1033.93	**0.08** **0.01** 0.92	4714.39 177704.3 16155.37	8172.98 544160.00 50671.42	**0.03** **0.00** **0.01**
Vesper, et al. [4] Median CE/mg	67 40 3	24 24 2	**0.01** 0.09 **0.02**	16 ** 27	11 ** 14	0.49 ** 0.39	16 325 7	9 370 10	0.10 0.59 0.70
Reponen, et al. [9] GM CE/g	13.7 6.8 2.6	5.7 2.0 1.0	**<0.05** **<0.05** **<0.05**	- 1.1 12.6	- 0.9 4.0	NS NS **<0.05**	137.2 2099.3 28.1	70.5 1349.2 27.7	NS NS NS
	Aureobasidium pullulans			*Epicoccum nigrum*			*Scopulariopsis brevicaulis*		
Vesper, et al. [1]	417991.0	727917.3	**0.02**	407868.70	920578.1	**0.00**	1179.00	480.64	**0.04**
	Trichoderma viride			*Alternaria alternata*			*Wallemia sebi*		
Vesper, et al. [1]	1602.96	284.82	**0.01**	16452.45	55594.45	**0.00**	18954.01	8442.97	**0.05**

* missing data

Table 3 Summary Table of Commonly Reported Fungi & Risk of Asthma

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes								
Study	Analysis	*Aspergillus*		*Penicillium*		*Cladosporium*		*Alternaria*	
		unadjusted	adjusted	unadjusted	adjusted	unadjusted	adjusted	unadjusted	adjusted
Salo, et al. [11] 2 fold increase in concentration	<3.90 3.90-6.27 ≥6.28 µg/g All ages Children <18 Adults >18	Not reported		Not reported		Not reported		1.0 1.60 (0.90-2.77) 1.84 (1.21-2.93) Not reported Not reported Not reported	1.0 1.52 (0.90-2.55) **1.84 (1.18-2.85)** **1.31 (1.05-1.64)** 1.47 (0.83-2.62) 1.25 (0.99-1.58)
Araki, et al. [12]	>GM CFU/m^3	0.83 (0.53-1.29)	0.73 (0.45-1.21)	1.44 (0.89-2.33)	1.43 (0.84-2.42)	0.84 (0.59-1.20)	0.87 (0.59-1.28)	Not reported	
Dales, et al. [13]	Detectable limits CFU/g		0.92 (0.35–2.44) 0.50 (0.25-1.00)	Not reported			0.46 (0.18–1.21) 0.69 (0.33-1.41)		1.90 (0.55–6.59) 2.00 (0.85-4.74)
Jones R, et al. [14] Viable counts Total counts	≥85th percentile CFU/m^3 Spores/m^3	2.81 (1.00-7.90)	**6.1 (1.37-27.19)[1]** 0.54 (0.10-2.92)[2]	0.49 (0.19-1.31) 0.70 (0.27-1.82)[3]	0.35 (0.11-1.17) 0.94 (0.31-2.83)[3]	1.37 (0.52-3.56) 1.93 (0.73-5.14)	1.19 (0.39-3.60) 2.37 (0.77-7.26)	Not reported	
Li and Hsu [15]	Summer Winter		1.55 (0.71-3.36) 0.69 (0.28-1.73)		0.61 (0.21-1.81) 0.56 (0.17-1.84)		**1.88 (1.07-3.30)** **4.14 (1.17-14.67)**		
Rosenbaum, et al. [16]	Not detected v high	3.00 (1.07-8.39)	1.58 (0.43-5.79)	7.88 (2.30-26.99)	**6.18 (1.34-28.46)**	2.74 (0.98-7.66)	2.28 (0.41-12.67)	1.18 (0.41-3.41)	0.96 (0.27-3.45)
Dharmage, et al. [17]	Highest quartile	Not reported			**3.9 (1.1-14.3)**		**8.5 (1.6–44.3)**	Not reported	
Matheson, et al. [10]	CFU/m^3	Not reported		Not reported			0.96 (0.80-1.16)[4] 1.11 (0.91-1.37)[5] **1.52 (1.08-2.13)[6]**	Not reported	

Individual analyses in studies:

- [1] without family history of asthma ; [2] with family history of asthma ; [3] model for *Aspergillus* and *Penicillium* combined (Jones 2011), [4] effect of doubling allergen or fungal exposure on the risk of developing current asthma ; [5] Effect of doubling exposure to allergens or fungi on the remission of current asthma ; [6] effect of doubling allergen or fungal exposure on the risk of developing attack of asthma in last 12 months (Matheson 2001)

Adjusted models in each study:

- Salo, et al. [11] adjusted model for age, sex, race, education, smoking, and sampling season. NB other adjusted models provided and all showing positive associations in the 3rd quartile. Analysis for 2 fold increase (children <18 years) has fewer observations because of missing values. Araki, et al. [12], adjusted for gender, age, tobacco smoking exposure, renovation history, wall-to wall carpeting, dampness index, and hay-fever. Dales, et al. [13], adjusted for child's age, parental illness, passive smoking, and dust mites. Jones R, et al. [14], adjusted for age and one or more family members with asthma. There was a strong interaction between an elevated level of *Aspergillus* and one or more family members with asthma. Therefore, separate models were generated for individuals with and without a family member with asthma. Li and Hsu [15], adjusted for age, parental education, number of household smokers, and use of gas stove for cooking. Rosenbaum, et al. [16], adjusted for season of visit, maternal smoking during pregnancy, any smoker in the home, day care center or non-relative care, endotoxin. Dharmage, et al. [17] adjusted for potential confounders – Socio-demographic factors, current smoking, parental asthma/allergy, medication use, and the season during which the participant was investigated. Matheson, et al. [10], adjusted for season of sampling and smoking status. Analysis provided for asthma attack in the last 12 months, atopy and doctor diagnosed asthma

Table 4 Summary Effect Estimates and heterogeneity Scores of Results Synthesis

Model in sub-group analysis	Unadjusted synthesis of outcome: asthma			Adjusted synthesis of outcome: asthma		
	No. of studies included in analysis	Summary Effect Estimates for pooled unadjusted data (95%;CI)	I^2	No. of studies included in analysis	Summary Effect Estimates for pooled adjusted data (95%;CI)	I^2
Model 1 - Total fungi	3	0.98 (0.53-1.82)	25%	3	0.86 (0.46-1.59)	1%
Model 2 – identified & unidentified fungi *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhodotorula*, *Acrodontium*, *Epicoccum**, Sterile, Basidiomycetes, Hyaline unknown & Dark unknown	4	**1.40 (1.07-1.82)**	54%	7	**1.29 (1.02-1.62)**	50%
Model 3 – fungi, including non-sporulating *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhodotorula*, *Acrodontium*, *Epicoccum**, Sterile	4	**1.47 (1.09-1.97)**	61%	7	**1.34 (1.05-1.71)**	54%
Model 4 – fungi, excluding non-sporulating *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhodotorula*, *Acrodontium*, *Epicoccum**	4	**1.51 (1.10-2.07)**	64%	7	**1.34 (1.04-1.73)**	64%
Model 5 – four most commonly reported fungi *Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*	4	**1.48 (1.03-2.14)**	67%	7	**1.36 (1.02-1.82)**	61%
Model 6 – *Aspergillus*	3	1.74 (0.66-4.60)	76%	5	0.98 (0.59-1.63)	54%
Model 7 – *Penicillium*	3	1.66 (0.48-5.70)	83%	5	1.19 (0.56-2.54)	67%
Model 8 – *Cladosporium*	3	1.29 (0.64-2.59)	61%	6	**1.96 (1.13-3.41)**	66%
Model 9 – *Alternaria*	2	**1.71 (1.11-2.63)**	0%	3	**1.77 (1.22-2.56)**	0%

*Only unadjusted data available

Table 5a Summary of Demographic Variables and Risk Factors for Asthma

Predictor variable	Outcome: Asthma 95%:CI	
	un adjusted	adjusted
Parent /s with asthma	**1.7 (1.3-2.1)** [3]	**1.40 (1.10-1.78)** [1] **2.6 (1.4-5.0)** [2] **1.4 (1.1-1.8)** [3]
Mother has allergies		1.23 (0.97-1.58)[1]
Low education level: <12 years ≤high school	**3.47 (1.18-10.19)**[7]	**1.87 (1.25-2.80)** [1]
Income: referent >$40,000 $20,000-$40,000 <$20,000		**1.4 (1.02-1.8)** [3] **1.4 (1.1-1.8)** [3]
Maternal smoking, pregnancy	1.47 (0.66-3.27)[7]	
Smoking in the home	1.63 (0.67-3.93)[7]	0.88 (0.62-1.25) [1]
Health insurance: referent private Medicaid	**6.69 (1.45-30.82)**[7]	
Male vs female		**1.60 (1.26-2.02)** [1] 1.1 (0.8-1.4) [3] 2.16 (0.96-4.85)[7]
Season of birth; winter Spring Summer Fall	1.00 1.67 (0.50-6.61)[7] **4.52 (1.44-14.20)** [7] 1.40 (0.35-5.55) [7]	
Prematurity		**3.4 (1.7-6.50)**[2]
Mothers age at delivery, years: referent <20 20-29 >30	 1.21 (0.373.89)[7] 2.20 (0.57-8.47)[7]	
Mothers marital status, not married	1.64 (0.64-4.21)[7]	
Ever breast fed	0.46 (0.20-1.03)[7]	
Attended day care/non-relative care	0.57 (0.24-1.35)[7]	
Race: White Black/other	1.00 1.56 (0.69-3.50) [7]	
Ethnicity: Non-Hispanic Hispanic	1.53 (0.47-4.94)[7]	
Positive SPT response to any aeroallergen		**1.7 (1.3-2.1)**[3]
Upper respiratory tract symptoms		**2.5 (1.7-3.7)** [3]
Pneumonia		**4.0 (2.5-6.4)**[2]
Allergic rhinitis		**1.9 (1.1-3.1)**[2]

1 Adjusted Rate Ratio, socio economic factors & housing characteristics increased infant symptom days for wheeze [7]
2 Prevalence Ratios, analyses of >50% probability of Respiratory symptoms indicative of bronchial asthma [8]
3 Adjusted Rate Ratio for Model 2, asthma predictors at age 7 years for 289 subjects [9]
4 Odds Rations for relationship between asthma and environmental variables, with adjusted models including gender, age, tobacco smoke exposure, renovation history, wall-to-wall carpeting, dampness index and hay fever [12]
5 Odds Rations for associations between asthma and home dampness/fungi [15]
6 Odds Ratios, fungal exposure and risk of wheeze for self-reported fungi [a] and bedroom being monitored [b 2]
7 Odds Ratios for risk of wheeze in first year of life [16]
8 Odds Ratios, effect of doubling allergen and risk of developing new current asthma [a] and remission of clinical outcomes for current asthma [b 10]

Table 5b Summary of Residential Characteristics and Risk Factors for Asthma

Predictor variable	Outcome: Asthma 95%:CI	
	un adjusted	adjusted
Multifamily home		**1.50 (1.10-2.02)** [1]
Visible fungi	1.23 (0.94-1.61)[1]	
	3.70 (2.22-6.15)[6a], 3.25 (1.60-6.60)[6b]	- 1.02 (0.39-2.69)[5]
Fungi severity index (No. observations) 1-2	0.90 (0.35-2.29)[7]	
Stuffy odor	1.02 (0.36-2.85)[7]	**3.19 (1.08-9.42)[5]**
	1.32 (0.58-3.02)[7]	
Self-reported dampness		1.46 (0.55-3.85)[5]
Water damage		0.70 (0.27-1.86)[5]
Flooding		1.18 (0.27-5.17)[5]
Water Leaks		1.18 (0.90-1.55) [1]
Dampness	1.32 (0.54-3.22)[7]	1.01 (0.34-3.01)[5]
Ergosterol		1.06 (0.67-1.69)[8a], 1.08 (0.67-1.75)[8b]
House dust mites		1.7 (1.0-3)[2]
		1.07 (0.64-1.81)[4]
Der p1 floor		1.24 (0.88-1.73)[8a],
Der p1 bed	0.95 (0.60-1.49) [4]	0.93 (0.70-1.25)[8b]
		0.85 (0.57-1.27)[8a],
		0.84 (0.58-1.20)[8b]
Pet ownership		**0.4 (0.2-0.9)** [2]
Cat allergen		**0.6 (0.4-0.9)** [3]
Pet cat		
Pet dog	0.77 (0.30-2.03)[7]	
Cat Allergen Fel d1 floor	1.55 (0.66-3.65)[7]	0.65 (0.40-1.08)[8a], 0.89
Endotoxin >100 EU/mg dust	**2.62 (1.12-6.13)[7]**	
Presence of cockroaches	1.93 (0.76-8.46)[7]	
Formaldehyde	1.81 (0.44-7.36)[4]	1.15 (0.26-5.08)[4]
29 combined VOCs	0.86 (0.16-4.64) [4]	1.19 (0.19-7.36)[4]
Sampling season: Referent summer		1.0[1]
Fall		1.00 (0.73-1.38) [1]
Winter		0.87 (0.59-1.29) [1]
Spring		0.81 (0.57-1.15) [1]
Season of fungal sample collect: referent winter		
Spring	0.86 (0.30-2.46) [7]	
Summer	1.49 (0.51-4.42) [7]	
Fall	**3.76 (1.02-13.92)[7]**	
Family moved during study	1.15 (0.50-2.61)[7]	
Humidifier use		**1.41 (1.11-1.79)** [1]
	1.30 (0.47-3.61)[7]	
Dehumidifier use		0.83 (0.61-1.13) [1]
Heating system: Referent forced air		1.0[1]
Steam/hot water		0.89 (0.68-1.15) [1]
Electric		1.30 (0.93-1.82) [1]
Other		0.43 (0.15-1.19) [1]
Living room carpeted/with rug	**0.38 (0.16-0.88)[7]**	

1 Adjusted Rate Ratio, socio economic factors & housing characteristics increased infant symptom days for wheeze [7]
2 Prevalence Ratios, analyses of >50% probability of Respiratory symptoms indicative of bronchial asthma [8]
3 Adjusted Rate Ratio for Model 2, asthma predictors at age 7 years for 289 subjects [9]
4 Odds Rations for relationship between asthma and environmental variables, with adjusted models including gender, age, tobacco smoke exposure, renovation history, wall-to-wall carpeting, dampness index and hay fever [12]
5 Odds Rations for associations between asthma and home dampness/fungi [15]
6 Odds Ratios, fungal exposure and risk of wheeze for self-reported fungi [a] and bedroom being monitored [b 2]
7 Odds Ratios for risk of wheeze in first year of life [16]
8 Odds Ratios, effect of doubling allergen and risk of developing new current asthma a and remission of clinical outcomes for current asthma[10]

Figure 2 Unadjusted Model for Indoor Fungi and Risk of Asthma

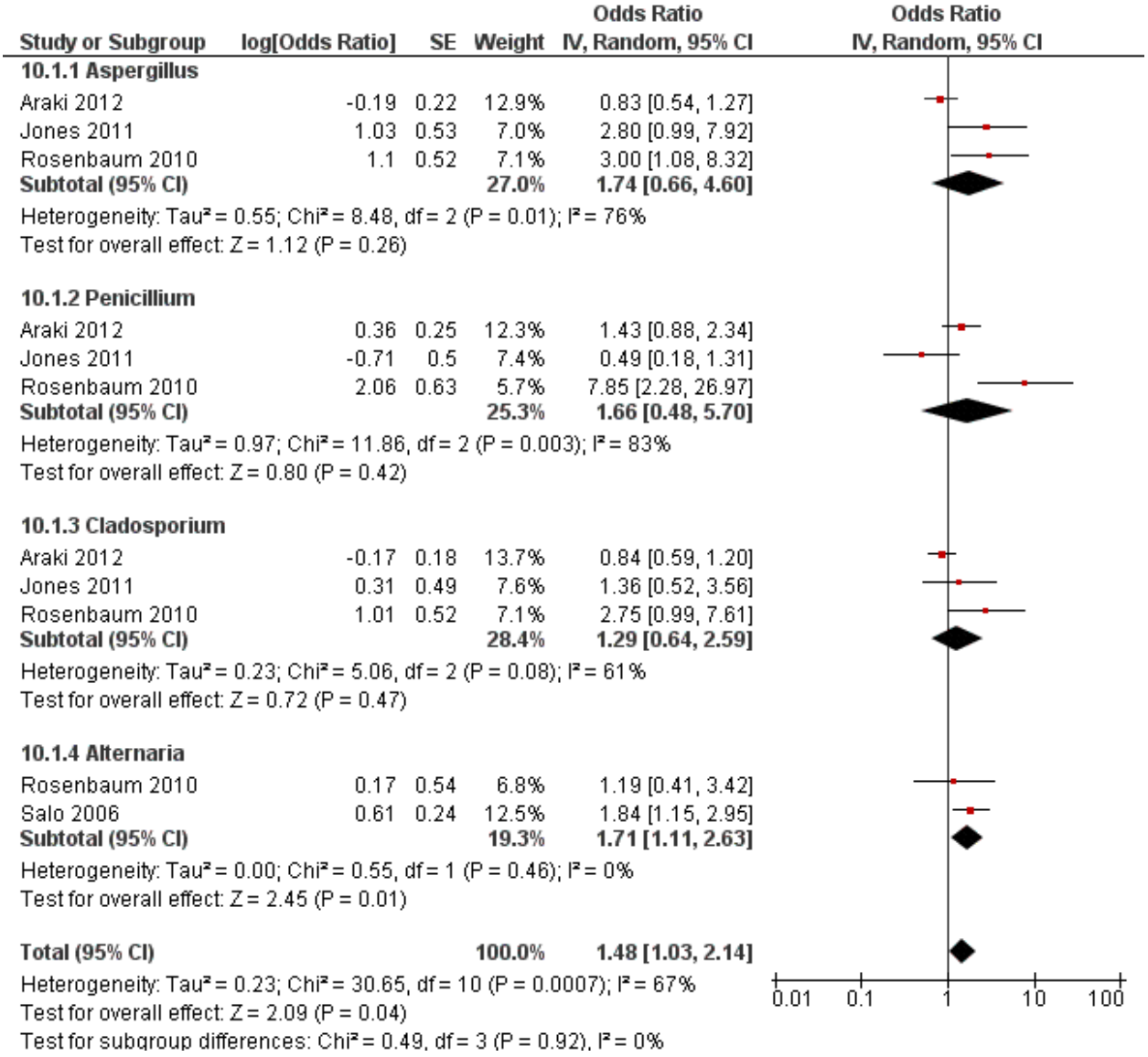


Figure 3 Adjusted Model for Indoor Fungi and Risk of Asthma

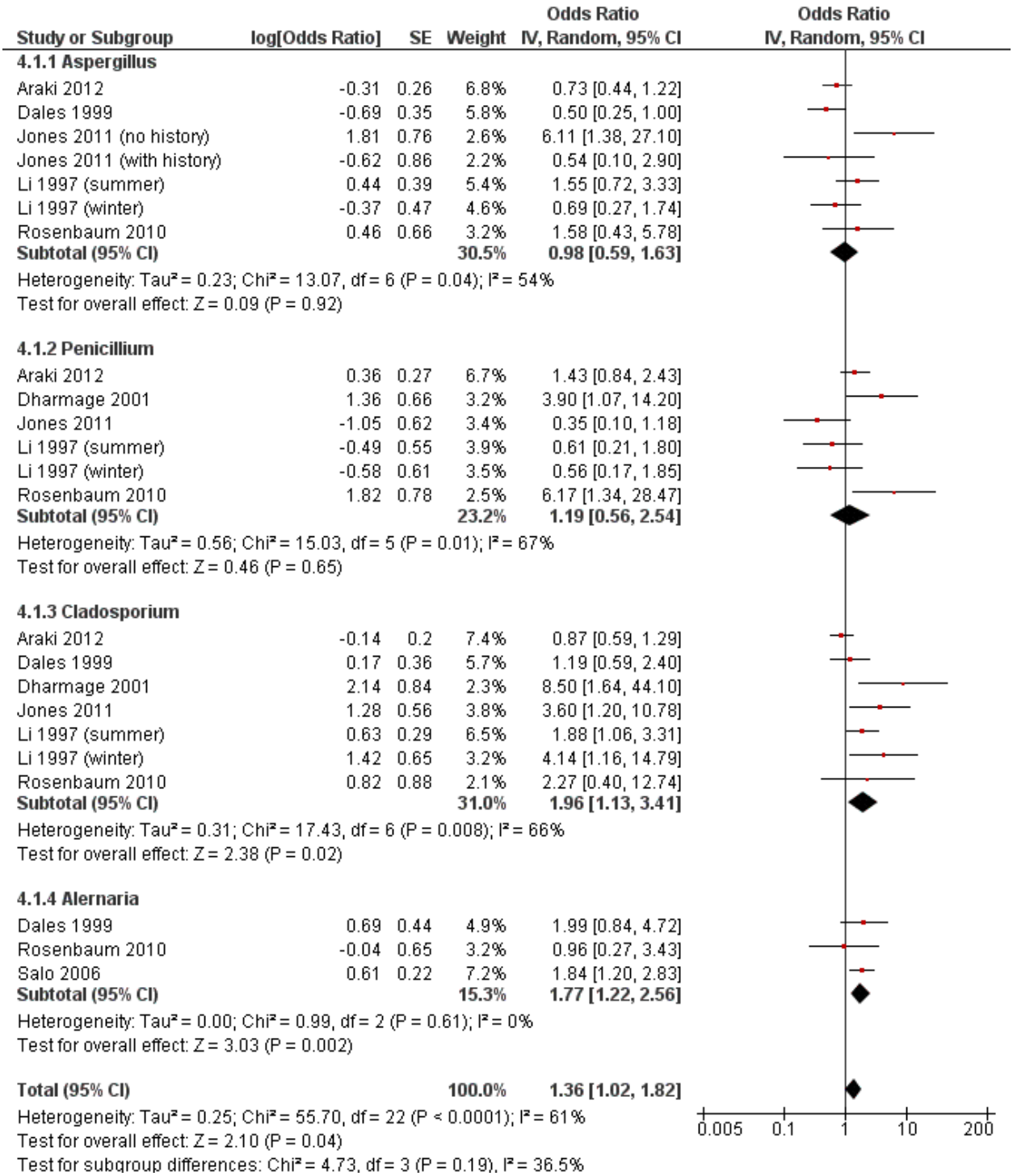


Figure E1a Unadjusted Model for Fungi and Asthma

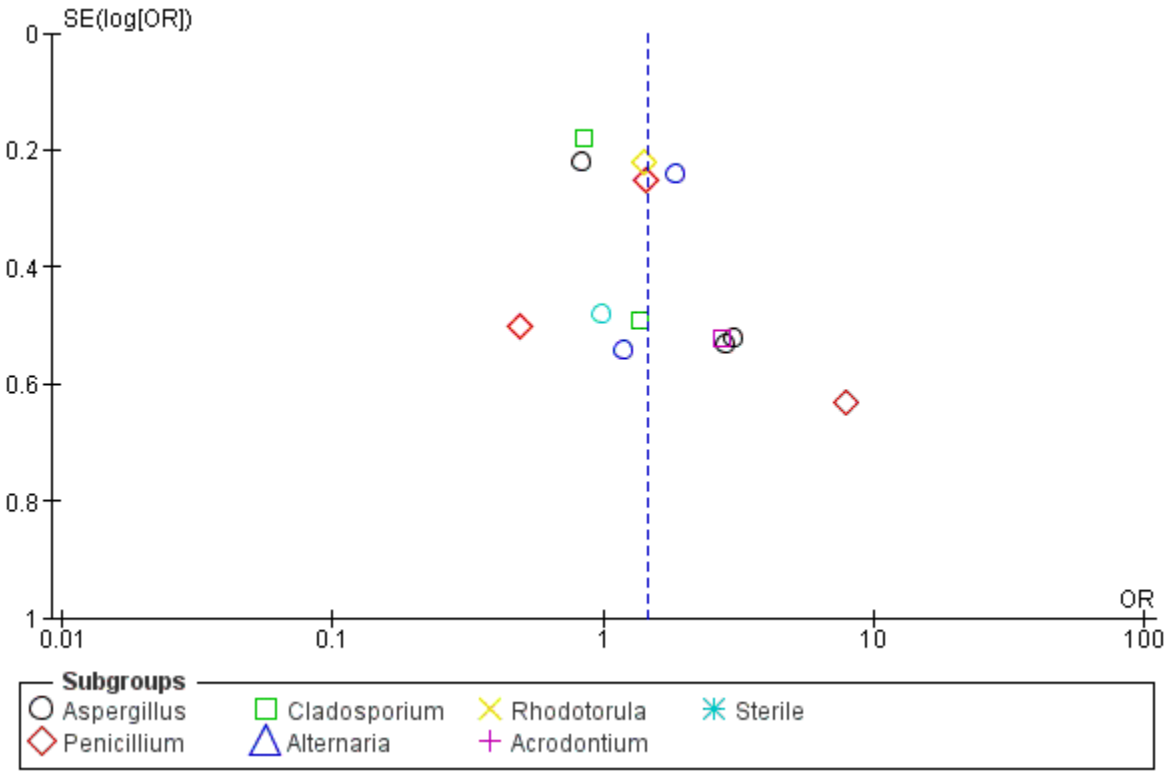
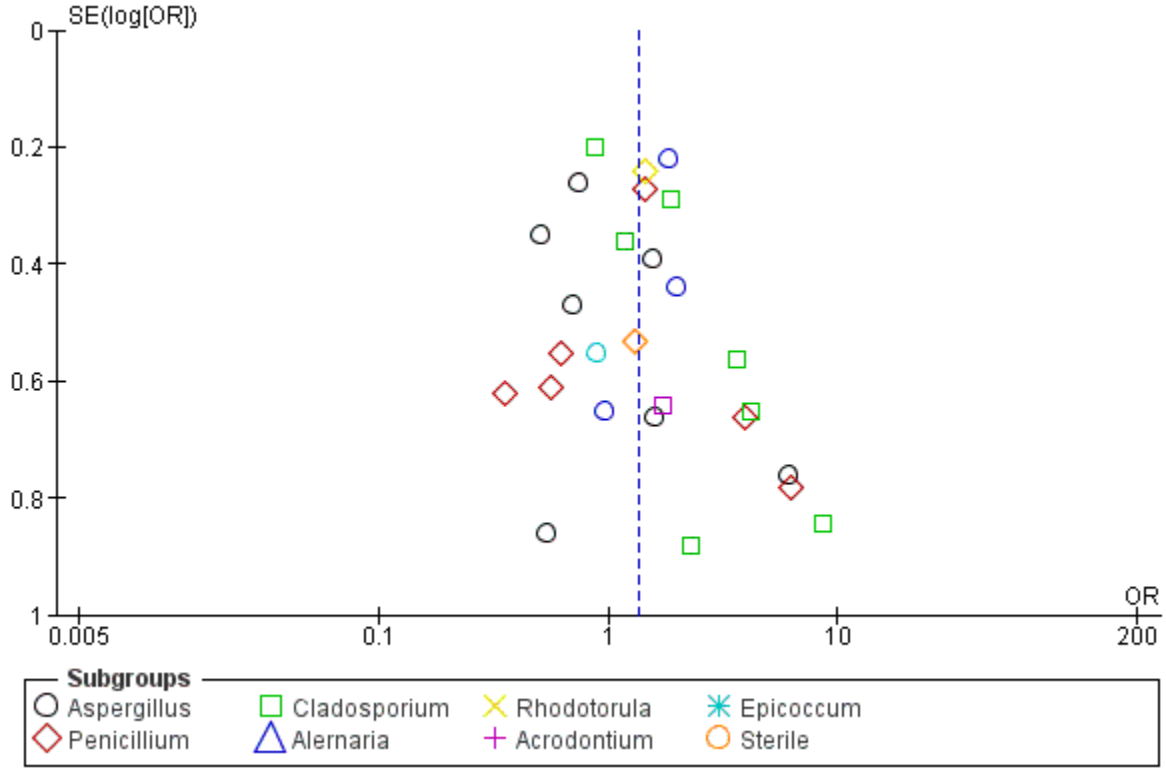


Figure E1b Adjusted Model for Fungi and Asthma



Online Repository Supporting Tables

Table E1 Participant characteristics of included studies

Author & year	% female	% in poverty / low SES	% ETS exposure	% of damp homes	% homes with visible fungi	% asthma prevalence
Vesper et al. (2006a), USA	-	30 <$20,000	-	-	-	75
Strachan et al. (1990), UK	-	-	-	-	26.3 cases & 12.5 controls	38.6
Holme et al. (2010), Sweden	-	-	-	2.1 visible damp, 18.6 condensation	22.6 mild & 16.3 severe	36.1
Vesper et al. (2008), USA	44	-	-	-	-	-
Su et al. (2001), Taiwan	-	-	-	-	-	-
Meng et al. (2012), USA	51.4 & 52.8	-	-	-	-	72
Gent et al. (2002), USA	50.3	14.2 mothers education <12 years	-	-	21.3	27.5 >30 wheeze days
Herrera et al. (2011), Columbia	45.8	1.2 unemployed	11.4	-	-	8 asthma & 23 wheeze
Reponen et al. (2012a), USA	-	<$20,000; 30 cases, 14 control	-	22	53	24
Matheson et al. (2005), Australia	51.8 & 52.0 in follow up	-	current 17.5 & 16.9	-	-	26.2
Salo et al. (2006), USA	51.8	16.5 in poverty	46	-	-	11.2 Dr diagnosed
Araki et al. (2012), Japan	51.4	-	22.3	68.8	80.7	4.8
Dales et al. (1999), Canada	51	50 <$50,000 & 87 completed 2nd school	47	-	-	19
Jones et al. (2011), USA	-	-	-	69.4	49.5	67
Li and Hsu (1997), China	38.3 asthma, 30.0 atopic & 46.2 control	Education >high school, Father 80.8-95.7 & Mother 75.0-89.3	53.2 25 44	73-85	44-75	-
Rosenbaum et al. (2010), USA	55	46% of mothers <high school educated	50	71	25	38
Dharmage et al. (2001), Australia	53	51 Occupational class 1, 6.5 unemployed	51	-	-	23

Table E2 Study characteristics of included studies

Author & year	Study, Region & country	Funder	Recruitment	Analysis
Vesper et al. (2006a), USA	Cleveland, USA	US Dept. of Housing and Urban Development	Recruitment from the Cleveland asthma study	Wilcoxon statistic
Strachan et al. (1990), UK	Department of Epidemiology and Population Sciences	Wellcome fellowship, Asthma Research Council & BRE	Original questionnaire survey conducted by DPS in 1986-7	Student t-test 88 degrees of freedom
Holme et al. (2010), Sweden	Dampness in Buildings and Health (DBH) phase II	Not reported	First phase of the DBH cross-sectional questionnaire	Pearson chi-squared test
Vesper et al. (2008), USA	SE Michigan, USA	US Environmental Protection Agency's (NHEERL)	Enrolled in a non-profit managed care organization in SE Michigan	Wilcoxon Rank-sum test p-values
Su et al. (2001), Taiwan	Southern Taiwan	Taiwan National Science Council	Citywide random survey	Mann-Whitney test
Meng et al. (2012), USA	Mid-West, USA	Clorox Corporation and Physician's Award at CMH	From allergy clinic visits at the Children's Mercy Hospital	Chi-square test, Fisher exact test & logistic regression
Gent et al. (2002), USA	Connecticut / Western Massachusetts, USA	National Institute of Environmental Health Sciences	New-borns recruited from hospital	Rate ratio
Herrera et al. (2011), Columbia	Bucaramanga, Columbia	Research Vice Presidency University Extension Industrial Santander	Children participating in the previous project.	Prevalence ratio
Reponen et al. (2012a), USA	European Community Respiratory Health Survey (ECRHS), Australia	The Victorian Health Promotion Foundation and Victorian Department of Human Services	Participants in ECRHS (European Community Respiratory Health Survey)	Holm method & Rate Ratio
Matheson et al. (2005), Australia	Cincinnati cohort	US Department of Housing and Urban Development (NIEHS)	Full-term infants born in Cincinnati, Ohio, and N. Kentucky	Logistic regression
Salo et al. (2006), USA	NSLAH study, USA	Intramural Research Program of the National Institutes of Health	NSLAH study participants	Logistic regression
Araki et al. (2012), Japan	Nationwide epidemiological study on SBS, Japan	Japan's MoH, Labor and Welfare, Health and Labor Sciences	Single family home - 2nd partial follow up from prospective study	Logistic regression
Dales et al. (1999), Canada	Wallace burg Ontario, Canada	Panel for Energy Research & Dev.	Families of elementary schools	Logistic regression
Jones et al. (2011), USA	Buffalo, New York	Not reported	Children <17 years of age living in Buffalo, New York	Logistic regression
Li and Hsu (1997), China	Taiwan, China	The Taiwan National Science Council	National Taiwan University Hospital	Logistic regression
Rosenbaum et al. (2010), USA	The Assessment of urban dwellings for indoor toxins	Environmental Protection Agency	Mothers with asthma were recruited in 2001 & 2002	Logistic regression
Dharmage et al. (2001), Australia	European Community Respiratory Health Survey (ECRHS), Australia	The Victorian Health Promotion Foundation and Victorian Department of Human Services	Participants in ECRHS (European Community Respiratory Health Survey)	Logistic regression

Table E3a Results Synthesis - Risk of Fungi Measured as Cell Equivalents per gram

		Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes								
Study		*Aspergillus* spp. *flavus* *fumigatus* *niger* *ochraceus* *penicillioides* *restrictus* *sclerotiorum* *sydowii* *unguis* versicolor ustus			*Penicillium* spp. *brevicompactum* *corylophilum* *penicillium* group 2 *purpurogenum* *spinulosum* *variabile* *chrsogenum*			*Cladosporium* spp. sphaerospemum cladosporioides 1 cladosporioides 2 herbarum		
	Outcome	Case	Control	P value	Case	Control	P value	Case	Control	P value
Vesper et al. (2006a)	GM CE/g	NR 493.98 NR 1895.46 103285.40 227.79 474.12 NR 3831.60 4261.87 1039.10	NR 733.76 NR 2117.95 72823.67 298.52 429.75 NR 1881.66 1948.05 1794.22	NR 0.411 NR 0.794 0.863 0.740 0.812 NR 0.316 0.402 0.219	3652.60 2317.31 2604.09 478.79 710.90 1050.69 11362.78	2353.54 1328.69 654.48 474.68 3600.06 1033.93 11222.07	0.629 0.437 **0.078** 0.959 **0.012** 0.920 0.830	4714.39 177704.30 16155.37 33532.34	8172.98 544160.00 50671.42 48206.32	**0.028** **<0.001** **0.012** 0.344
Vesper et al. (2008)	Median CE/mg	** 1 67 40 52 ** 2 17 3 12 5	1 2 24 24 52 ** 2 6 2 14 3	0.848 0.386 **0.007** 0.092 0.507 ** 0.281 0.242 **0.024** 0.372 0.094	14 3 16 ** ** 27 6	17 2 11 2 ** 14 8	0.725 0.547 0.495 0.783 ** 0.389 0.752	16 325 7 135	9 370 10 160	0.102 0.588 0.703 0.780
Reponen et al. (2012a)	GM	2.3 6.5 13.7 6.8 25.6 1.7 2.4 2.0 2.6 5.5 5.2	1.4 4.3 5.7 2.0 19.5 1.2 1.6 0.9 1.0 1.8 2.5	NS NS **<0.05** **<0.05** NS NS NS NS **<0.05** NS NS	20.6 1.0 - 0.8 1.1 12.6 51.1	14.6 0.7 - 0.6 0.9 4.0 31.2	NS NS NS NS NS **<0.05** NS	137.2 2099.3 28.1 232.0	70.5 1349.2 27.7 186.9	NS NS NS NS

NR not reported

NS not significant

Table E3b Results Synthesis - Risk of Fungi Measured as Cell Equivalents per gram

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes									
Study		*Aureobasidium pullulans*			*Epicoccum nigrum*			*Scopulariopsis brevicaulis*		
	Outcome	Case	Control	P value	Case	Control	P value	Case	Control	P value
Vesper et al. (2006a)	GM CE/g	417991.00	727917.30	**0.02**	407868.70	920578.10	**0.002**	1179.00	480.64	**0.035**
Vesper et al. (2008)	Median CE/mg	5400	5700	0.374	275	300	0.534	3	2	0.461
Reponen 2012	GM	4599.4	3891.3	NS	315.9	245.2	NS	3.7	1.8	NS

Table E3c Results Synthesis - Risk of Fungi Measured as Cell Equivalents per gram

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes									
Study		*Trichoderma viride*			*Alternaria alternata*			*Wallemia sebi*		
	Outcome	Case	Control	P value	Case	Control	P value	Case	Control	P value
Vesper et al. (2006a)	GM CE/g	1602.96	284.82	**0.009**	16452.45	55594.45	**0.001**	18954.01	8442.97	**0.051**
Vesper et al. (2008)	Median CE/mg	2	2	0.771	42	46	0.596	70	96	0.471
Reponen et al. (2012a)	GM	14.3	9.3	NS	262.3	216.6	NS	85.2	43.2	NS

- Vesper et al. (2006a) & Vesper et al. (2008) 36 Group 1 & 2 species reported as part of ERMI. Only selected fungi of interest or showing a significant association between cases and controls have been reported. Vesper 2008 also reports percentage of occurrence between homes. Vesper 2008 Medians and Wilcon tests for fungi species with fewer than 20% detections (**) were not calculated

Table E4a Results Synthesis - Risk of Fungi Measured as Colony Forming Units per meter cubed

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes															
Study	Outcome	*Aspergillus* spp.			*Penicillium* spp.			*Cladosporium* spp.			*Alternaria* spp.			*Epicoccum* spp.		
		Case	Control	P value	Case	Control	P value	Case	Control	P value	Case	Control	P value	Case	Control	P value
Strachan et al. (1990)	GM CFU/m^3	NR			39	55	-0.78	16	12	+0.46	NR			NR		
Holme et al. (2010) On DG-18 On MEA	Mean CFU/m^3	 113 229	 128 57	 0.602 0.147	 104 95	 119 106	 0.298 0.699	 92 70	 125 100	 0.130 0.762	NR			NR		
Su et al. (2001) Spring Summer Fall Winter	Total CFU/m^3	 306.7 738.0 303.1 451.2	 226.9 427.0 269.8 165.0	 NS NS NS NS	 839.6 568.4 454.0 496.8	 608.3 260.7 479.3 276.3	 NS NS NS **<0.05**	 4972.9 2085.0 6469.51 17696.0	 3906.1 2303.9 6726.1 16999.3	 NS NS NS NS	 3039.1 47.4 87.9 251.0	 4098.6 4.5 178.8 336.53	 NS NS NS NS	NR		
Meng et al. (2012)	Mean CFU/m^3	3.62	3.33	0.24	4.12	3.72	0.09	5.18	4.43	**<0.0001**	3.99	3.60	0.07	3.63	3.62	0.98

Table E4b Results Synthesis - Risk of Fungi Measured as Colony Forming Units meters cubed

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes															
Study	Outcome	*Acremonium*			*Ulocladium*			White rot basidiomycetes			*Mycelia sterilia*			Total Fungi		
		Case	Control	P value	Case	Control	P value	Case	Control	P value	Case	Control	P value	Case	Control	P value
Strachan et al. (1990)	GM CFU/m^3	NR			NR			2.5	1.3	+1.45	2.1	0.7	**+2.84**	NR		
Holme et al. (2010) On DG-18 On MEA	Mean CFU/m^3	NR			NR			NR			NR			 212 168	 199 188	 0.994 0.306
Su et al. (2001) Spring Summer Fall Winter	Total CFU/m^3	NR			NR			NR			NR			 11233.0 7288.9 10727.3 20676.1	 10834.4 5857.5 11765.2 20313.3	 NS NS NS NS
Meng et al. (2012)	Mean CFU/m^3	3.32	0	**<0.02**	3.06	0	**<0.001**	NR			NR			5.92	5.19	<0.0001

- Meng et al. (2012) provides several analyses between cases and controls. Only the viable fungal colony level have been provided in this synthesis with unadjusted P Values
- Strachan et al. (1990) Geometric Mean (GM) airborne fungal counts (CFU/m^3), all visits combined by history of wheeze in last 12 months. Student t-test with 88 degrees of freedom

Table E5a Results Synthesis – Fungal Exposure and Risk of Asthma or Wheeze

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes											
Study	Analysis	*A ochraceus*, *A uniguis* & *Penicillium variabile*		*Penicillium* spp.		*Cladosporium* spp.		*Acremonium* spp.		Other Fungi	
		un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted
Gent et al. (2002)	Rate Ratio CFU/m^3 0 1–499 500–999 ≥ 1,000	-	-	 1.0 1.06 (0.82–1.36) 1.10 (0.51–2.34) **2.46 (1.63–3.70)**	 1.0 1.11 (0.87–1.42) 1.29 (0.65–1.48) **2.15 (1.34–3.46)**	 1.0 1.12 (0.87–1.45) 1.07 (0.71–1.61) 0.83 (0.50–1.40)	 1.0 0.92 (0.69–1.22) 0.95 (0.61–1.49) 0.91 (0.53–1.56)	-	-	 1.0 1.31 (1.00-1.63) 1.13 (0.63-2.03) 0.88 (0.39-1.98)	 1.0 0.97 (0.75-1.26) 0.91 (0.49-1.68) 1.02 (0.49-2.11)
Herrera et al. (2011)	Prevalence Ratios	-	-	-	-	-	-	NR	**6.2 (3.8-10.0)**	-	-
Reponen et al. (2012a)	Rate Ratio	**1.8 (1.3-2.4)**	**2.2 (1.8-2.7)**	-	-	-	-	-	-	-	-

- Herrera et al. (2011) analyses of >50% probability of Respiratory symptoms indicative of bronchial asthma reported no significant associations with exposure to *Cladosporium, Fusarium, Scopulariopsis, Aspergillus, Penicillium, Absidia, Mucor, Curvularia , Curvularia and Alternaria*

Table E5b Results Synthesis – Risk of Asthma or Wheeze Associated with other Reported Factors

	Demographic and Housing characteristic risk factors for outcome: asthma					
Factor	Gent et al. (2002) Rate Ratio		Herrera et al. (2011) Prevalence Ratios		Reponen et al. (2012a) Rate Ratio	
	un adjusted	adjusted	un adjusted	adjusted	Model 1	Model 2
Reported fungi	1.23 (0.94-1.61)					
Positive SPT response to any aeroallergen					**1.5 (1.2-2.0)**	**1.7 (1.3-2.1)**
Upper respiratory tract symptoms					**2.2 (1.6-3.1)**	**2.5 (1.7-3.7)**
Season of sampling: Summer Fall Winter Spring		1.0 1.00 (0.73-1.38) 0.87 (0.59-1.29) 0.81 (0.57-1.15)				
Water Leaks		1.18 (0.90-1.55)				
Humidifier use		**1.41 (1.11-1.79)**				
Dehumidifier use		0.83 (0.61-1.13)				
Parent /s with asthma		**1.40 (1.10-1.78)**		**2.6 (1.4-5)**	**1.7 (1.3-2.1)**	**1.4 (1.1-1.8)**
Low education level <12 years (Gent 2012)		**1.87 (1.25-2.80)**				
Income: >$40,000 <$20,000 $20,000-$40,000						1.0 **1.4 (1.02-1.8)** **1.4 (1.1-1.8)**
Smoking in the home		0.88 (0.62-1.25)				
Heating system Forced air Steam/hot water Electric		1.0 0.89 (0.68-1.15) 1.30 (0.93-1.82) 0.43 (0.15-1.19)				
Male vs female		**1.60 (1.26-2.02)**			1.1 (0.9-1.4)	1.1 (0.8-1.4)
Multifamily home		**1.50 (1.10-2.02)**				
House dust mites				1.7 (1.0-3)		
Pet ownership Cat allergen				**0.4 (0.2-0.9)**	**0.5 (0.3-0.7)**	**0.6 (0.4-0.9)**

- Gent et al. (2002). Adjusted for socioeconomic factors and housing characteristics. Other fungi defined as total spore counts minus counts for *Penicillium, Cladosporium* and Yeasts
- Herrera et al. (2011). Adjustment not reported or not translated
- Reponen et al. (2012a). Initial models included ERMI value, race, sex, parental asthma, income, cigarette smoking, central air-conditioning, endotoxin, cat allergen, and SPT. Only the adjusted model for 3 species associated with asthma are summarized, refer to article for comparisons between different models for predicting asthma based on ERMI and variations in Group 1 and 2 fungi.

Table E6a Indoor Fungal Exposure & Risk of Asthma

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes								
Study	Analysis	*Aspergillus* spp.		*Penicillium* spp.		*Cladosporium* spp.		*Alternaria alternata*	
		un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted
Salo et al. (2006) 2 fold increase in concentration	<3.90 3.90-6.27 ≥6.28 µg/g All ages Children <18 Adults >18							1.0 1.60 (0.90-2.77) **1.84 (1.21-2.93)** NR NR NR	1.0 1.52 (0.90-2.55) **1.84 (1.18-2.85)** **1.31 (1.05-1.64)** 1.47 (0.83-2.62) 1.25 (0.99-1.58)
Araki et al. (2012)	>GM CFU/m^3	0.83 (0.53-1.29)	0.73 (0.45-1.21)	1.44 (0.89-2.33)	1.43 (0.84-2.42)	0.84 (0.59-1.20)	0.87 (0.59-1.28)		
Dales et al. (1999), Night cough/wheeze Asthma	Detectable limits CFU/g		0.92 (0.35–2.44) 0.50 (0.25-1.00)				0.46 (0.18–1.21) 0.69 (0.33-1.41)		1.90 (0.55–6.59) 2.00 (0.85-4.74)
Jones et al. (2011) Viable counts	≥85th percentile CFU/m^3 Spores/m^3	2.81 (1.00-7.90)	**6.11 (1.37-27.19)**[1] 0.54 (0.10-2.92)[2]	0.49 (0.19-1.31) 0.70 (0.27-1.82)[3]	0.35 (0.11-1.17) 0.94 (0.31-	1.37 (0.52-3.56) 1.93 (0.73-5.14)	1.19 (0.39-3.60) 2.37 (0.77-7.26)		
Li 1997 Summer Winter			1.55 (0.71-3.36) 0.69 (0.28-1.73)		0.61 (0.21-1.81) 0.56 (0.17-1.84)		**1.88 (1.07-3.30)** **4.14 (1.17-14.67)**		
Rosenbaum 2010	Not detected v high CFU/m3	**3.00 (1.07-8.39)**	1.58 (0.43-5.79)	**7.88 (2.30-26.99)**	**6.18 (1.34-28.46)**	2.74 (0.98-7.66)	2.28 (0.41-12.67)	1.18 (0.41-3.41)	0.96 (0.27-3.45)
Dharmage et al. (2001)	Highest quartile for BHR only				**3.9 (1.1-14.3)**		**8.5 (1.6–44.3)**		
Matheson et al. (2005)	Doubling exposure CFU/m3						0.96 (0.80-1.16)[4] 1.11 (0.91-1.37)[5] **1.52 (1.08-2.13)**[6]		

Table E6b Indoor Fungal Exposure & Risk of Asthma

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes								
Study	Analysis	*Rhodotorula*		*Epicoccum*		*Acrodontium*		Yeast	
		un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted
Araki et al. (2012)	>GM CFU/m^3	1.40 (0.91-2.14)	1.44 (0.91-2.30)						
Dales et al. (1999), Night cough/wheeze Asthma	Detectable limits CFU/g				0.88 (0.30–2.57) 0.88 (0.30-2.57)				1.06 (0.51-2.18) 2.16 (0.73-6.39)
Jones et al. (2011) Viable counts	≥85th percentile CFU/m^3 Spores/m^3							1.93 (0.72-5.17)	1.37 (0.45-4.15)
Li and Hsu (1997)	Summer Winter								1.30 (0.63-2.68) 3.26 (0.83-12.81)
Rosenbaum et al. (2010)	Not detected v high CFU/m3					2.75 (0.99-7.61)	1.72 (0.49-6.03)	0.98 (0.36-2.68)	0.76 (0.23-2.27)

Table E6c Indoor Fungal Exposure & Risk of Asthma

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes								
Study	Analysis	Sterile		Ascospores		Basidiospores		Total Fungi	
		un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted
Araki et al. (2012)	>GM CFU/m^3							0.62 (0.29-1.29)	0.59 (0.26-1.35)
Jones et al. (2011) Viable counts Total counts	≥85th percentile CFU/m^3 Spores/m^3	0.98 (0.38-2.52)	1.30 (0.46-3.64)	 0.70 (0.27-1.82)	 1.15 (0.38-3.84)	 0.70 (0.27-1.82)	 0.94 (0.31-2.83)	1.37 (0.52-3.56) 0.49 (0.19-1.31)	1.59 (0.54-4.72) 0.59 (0.19-1.84)
Rosenbaum et al. (2010)	Not detected v high CFU/m3							1.61 (0.50-5.22)	0.96 (0.19-4.84)
Dharmage et al. (2001)	Highest quartile BHR Current asthma Wheeze								NS graph representation, no data provided
Matheson et al. (2005)	Doubling exposure CFU/m3								1.53 (0.93-2.53)[4] 1.24 (0.83-1.84)[5] 1.54 (0.98-2.43)[6]

Table E6d Indoor Fungal Exposure & Risk of Asthma

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes								
Study	Analysis	Basidiomycetes		Hyaline unknown		Ergosterol		Dark unknown (Rosenbaum 2010) or Other (Matheson 2005)	
		un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted
Rosenbaum et al. (2010)	Not detected v high CFU/m3	0.77 (0.27-2.19)	0.77 (0.24-2.49)	1.00 (0.33-3.06)	0.71 (0.20-2.52)			1.62 (0.60-4.42)	1.01 (0.27-3.74)
Matheson et al. (2005)	Doubling exposure CFU/m3						1.06 (0.67-1.69)[4] 1.08 (0.67-1.75)[5] 0.92 (0.59-1.44)[6]		1.06 (0.85-1.33)[4] 0.89 (0.72-1.09)[5] 1.23 (0.92-1.66)[6]

- [1] without family history of asthma ; [2] with family history of asthma ; [3] model for *Aspergillus* and *Penicillium* combined ; [4] effect of doubling allergen or fungal exposure on the risk of developing current asthma ; [5] Effect of doubling exposure to allergens or fungi on the remission of current asthma ; [6] effect of doubling allergen or fungal exposure on the risk of developing attack of asthma in last 12 months
- Salo et al. (2006), adjusted model for age, sex, race, education, smoking, and sampling season. NB other adjusted models provided and all showing positive associations in the 3rd quartile. Analysis for 2 fold increase (children <18 years) has fewer observations because of missing values.
- Araki et al. (2012), adjusted for gender, age, tobacco smoking exposure, renovation history, wall-to wall carpeting, dampness index, and hay-fever
- Dales et al. (1999), adjusted for child's age, parental illness, passive smoking, and dust mites
- Jones et al. (2011), adjusted for age and one or more family members with asthma. There was a strong interaction between an elevated level of Aspergillus and one or more family members with asthma. Therefore, separate models were generated for individuals with and without a family member with asthma.
- Li and Hsu (1997), adjusted for age, parental education, number of household smokers, and use of gas stove for cooking
- Rosenbaum et al. (2010), adjusted for season of visit, maternal smoking during pregnancy, any smoker in the home, day care center or non-relative care, endotoxin
- Dharmage et al. (2001), adjusted for potential confounders – Socio-demographic factors, current smoking, parental asthma/allergy, medication use, and the season during which the participant was investigated were considered as possible confounders
- Matheson et al. (2005), adjusted for season of sampling and smoking status. Analysis provided for asthma attack in the last 12 months, atopy and Doctor diagnosed asthma

Table E6e Results Synthesis – Risk of Asthma or Wheeze Associated with other Reported Demographic Factors

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes									
Factor	Salo et al. (2006) Odds Ratio		Araki et al. (2012)		Li and Hsu (1997)		Rosenbaum et al. (2010)		Matheson et al. (2005)	
	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted
Season of birth; winter Spring Summer fall							1.00 1.67 (0.50-6.61) **4.52 (1.44-14.20)** 1.40 (0.35-5.55)			
Race White Black/other							 1.00 1.56 (0.69-3.50)			
Diagnosed allergies		**1.28 (1.04-1.57)**								
Low education level Mothers ≤ high school							**3.47 (1.18-10.19)**			
Not married							1.64 (0.64-4.21)			
Ever breast feeding							0.46 (0.20-1.03)			
Day care / non-relative care							0.57 (0.24-1.35)			
Insurance Private vs Medicaid							**6.69 (1.45-30.82)**			
Smoking in the home Maternal smoking,							1.63 (0.67-3.93) 1.47 (0.66-3.27)			
Male vs female							2.16 (0.96-4.85)			

- Salo et al. (2006), adjusted model for 2 fold increase has fewer observations because of missing values. Current asthma in relation to two fold increase in average *Alternaria* stratified by diagnosed allergies

Table E6f Results Synthesis – Risk of Asthma or Wheeze Associated with other Reported Residential Factors

	Outcome of interest is risk of fungi in asthmatic and non-asthmatic homes									
Factor	Strachan et al. (1990) Odds Ratio		Araki et al. (2012)		Li and Hsu (1997)		Rosenbaum et al. (2010)		Matheson et al. (2005)	
	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted	un adjusted	adjusted
Visible fungi Moldy odor Self-reported Surveyed	 3.70 (2.22-6.15) 3.25 (1.60-6.60)					1.02 (0.39-2.69) **3.19 (1.08-9.42)**	0.90 (0.35-2.29) 1.32 (0.58-3.02)			
Season of sampling: Winter Spring							1.00 0.86 (0.30-2.46) 1.49 (0.51-4.42) 3.76 (1.02-13.92)			
Self-dampness Water damage Flooding						1.46 (0.55-3.85) 0.70 (0.27-1.86) 1.18 (0.27-5.17)	1.32 (0.54-3.22)			
Humidifier use							1.30 (0.47-3.61)			
House dust mites Der P 1 floor Der p 1 bed			0.95 (0.60-1.49)	1.07 (0.64-1.81)						1.24 (0.88-1.73) [4] 0.93 (0.70-1.25) [5] 0.81 (0.52-1.27) [6] 0.85 (0.57-1.27) [4] 0.84 (0.58-1.20) [5] 0.74 (0.51-1.06) [6]
Living room carpet / rug							**0.38 (0.16-0.88)**			
Fel d1 Cat Dog Cockroaches							 0.77 (0.30-2.03) 1.55 (0.66-3.65) 1.93 (0.76-4.86)			0.65 (0.40-1.08) [4] 0.89 (0.57-1.39) [5] 0.81 (0.52-1.27) [6]
MVOCs (consolidation			0.86 (0.16-4.64)	1.19 (0.19-7.36)						
Endotoxin >100 EU/mg dust							**2.62 (1.12-6.13)**			
Bacterial							0.58 (0.18-1.92)	0.6 (0.16-2.20)		

Table E7a Synthesis 1 Strengths and Weaknesses

Author & year	Limitations of Study Identified by Authors	Limitations of Study Identified by Reviewers	NOS
Rosenbaum et al. (2010)	No cause effect relationship, small sample size, not all molds tested	Study includes children at risk of asthma: Eligibility for the study required that at least 1 parent was atopic	12
Vesper et al. (2006a)	Only some mold PCR-able. Other factors that weren't recorded might impact asthma	Don't really talk about housing conditions or SES status	6
Vesper et al. (2008)	Asthma definition using the GINA guidelines for treatment of "persistent asthma" and by definition, the "persistent asthma" group would be consistent with our "severe" asthmatic classification. It is this severe or "persistent asthma" group that had higher ERMI values in their homes	Does not report demographics	9

Table E7b Synthesis 2 Strengths and Weaknesses

Author & year	Limitations of Study Identified by Authors	Limitations of Study Identified by Reviewers	NOS
Gent et al. (2002)	Limitations primarily from fungal sampling methodology due to a single air borne sample being taken during the first year of life that were not taken during the same time of year. Air sampling and agar may also omit some species, particularly rare fungi and fungi favoring different growth mediums	Potential for selection bias, participants had at least one sibling with asthma. Response rate of 80% due to non-response/follow up of the initial 1,002 infants enrolled	10
Herrera et al. (2011)	Did not use clinical diagnosis of asthma as outcome. Measurements biological time were dry and not covered different climatic seasons to establish seasonal changes	Article written in Spanish and translated by Google translate	8
Rosenbaum et al. (2010)	No cause effect relationship, small sample size, not all molds tested	Study includes children at risk of asthma: Eligibility for the study required that at least 1 parent was atopic	12
Strachan et al. (1990)	The viable mold counts obtained from three minute air samples may not adequately reflect peaks and troughs of exposure. Volumetric sampling may underestimate the true exposure of mobile people to fungal spores. Potential for reporting bias	Doesn't look at other housing conditions (heating, temp etc.) and reporting bias / potential for chance findings in table 4 due to multiple comparisons. Limited by the methodological difficulties of quantifying fungi in indoor air and by the relatively small number of homes studied	11
Holme et al. (2010)	Short air sampling time of 1 minute that may not accurately reflect exposure. CFU analysis can overlook fungal species that are not easily culturable and may represent faster growing species. Potential for selection bias - Factors associated with participating were more health problem in the case families, more health-related lifestyle factors such as non-smoking parents, and a higher socio economic status of the family	Does not report demographics or funder	12
Su et al. (2001)	Short term study	Does not report demographics	9
Meng et al. (2012)	Difficult to conclude whether environmental exposure can be linked to causes of asthma incidence or exacerbation because population derived from cleaning product research project and some homes with grossly contaminated fungi and unsound and unsafe houses were excluded	Eligibility criteria only required families to have lived in property >2 months and potential for selection bias. Homes located in the amid agricultural and grassland areas expected that many yeasts and other fungal species may have been overlooked	9

Table E7c Synthesis 3 Strengths and Weaknesses

Author & year	Limitations of Study Identified by Authors	Limitations of Study Identified by Reviewers	NOS
Salo et al. (2006)	No measure of sensitivity of patients to *Alternaria*. Only self-reported asthma (bias)	Little info on physical house structure	14
Araki et al. (2012)	Possible misclassifications in questionnaire response, no lab tests for allergy, cross-sectional study design	No older homes (>8 years)	13
Dales et al. (1999)	Discrepancies between findings based on self-reports and those based on objective health measures	self-reported exposure and outcomes	11
Jones et al. (2011)	Small sample sizes for the analysis because of the large number of fungi, likely that children who live in homes with fungi are also exposed to other indoor environmental risk factors. Fungi allergen sensitization and cross-reactivity were not evaluated for these analyses, which could serve to modify asthma risk following fungi exposure. Nature of sampling activity inclusion of outdoor fungi not accurately accounted for due to cross sectional study design. Case sampling method used for this study is subject to potential selection bias, although analyses confirmed that the case–control population was representative	Does not report demographics or funder. Unable to assess whether concurrent exposure to multiple species of other important allergenic fungi (e.g., *Cladosporium* or *Penicillium*) demonstrated similar associations with asthma risk, because isolates of these genera were not speciated. Similarly, the lack of any significant associations with total spore counts may be due in part to the lack of precise species identification in relevant total count samples	14
Li and Hsu (1997)	Possible reporting bias for atopic children. Air cleaner use is something that is not common in other studies	Only urban environment and only concerns middle income families	11
Rosenbaum et al. (2010)	-	Recruitment of children with lower SES and potentially at greater risk of poorer housing conditions and increased fungi and/or asthma. Also parental asthma and may not represent normal population. High percentage of prenatal smoking	13
Dharmage et al. (2001)	Potential for selection bias and weighting undertaken to represent original cohort, but no significant different and un-weighted data used in analysis. Fungal analysis restricted given that *Aspergillus, Epicoccum* and *Alternaria* were presented at too lower level to include in analysis. Outcomes also potentially influenced by fungal avoidance being undertaken by allergic subjects. Cross sectional design unable to adjust for seasonal fungal changes	Adjusted models does not adjust for age / sex or season given the cross sectional nature of the project	11
Matheson et al. (2005)	May be a threshold effect for ergosterol which isn't investigated. Few other studies. Varied relationship between asthma & allergy. Issues of systematic error, the authors tried modelling the data. Follow up incomplete. Air sampling may not be a reliable measuring method. Sampling occurred at different times of the year. Exposure measurements such as the dust and air sampling methods performed in this study are likely to be subject to random measurement error	-	16

Online Repository: Appendix E1-E3

Appendix E1 Search Strategy

The below search strategy was conducted on the 18th April 2013 and with "title and abstract" searches being conducted with ten databases:

1. Cochrane Library (Wiley),
2. Medline (via the OVID platform)
3. AMED
4. Web of Science
5. Scopus
6. Environment Complete (EBSCO)
7. GreenFile (EBSCO)
8. Embase (via the OVID platform)
9. British Nursing Index (BNI)
10. Applied Social Sciences Index and Abstracts (ASSIA)

Context: home* OR hous* OR dwelling* OR residence* OR residential OR indoor* OR domicile* OR "living unit*" OR propert* OR build* OR "built environment*" OR "domestic environment*" OR bedroom* OR "living room" OR wall* OR floor* OR ceiling* OR "construction material*" OR "skirting board*" OR "window sill*" **AND Fungal Exposures:** damp* OR fungi OR mold* OR mould* OR fungal OR fungus* OR microbial OR aspergillus OR penicillium OR cladosporium OR alternaria OR helminthosporium OR epicoccum OR aureobasidium OR acrodontium OR didymella OR phoma OR botrytis OR rhizopus OR speciation **AND Outcomes:** asthma* OR wheez* OR cough* OR dyspnea OR bronchitis

Appendix E2 Data Extraction – Summary Contacting Author Details and Forward/Backward Citation Chasing

Library Reference Number, Author Year:

Study Details	Population	Description / Context	Exposure	Outcome
Name of Study: **Authors:** **Year published:** **Language:** **Title:** **Aims:** **Study Design:** **Statistical Analysis (e.g. OR models):** **Covariates / Confounders:** **Funders:** **Country:** **Region:** **Rural / Urban:**	**Population Included:** **Participant Characteristics:** • Sample size: • Age: • % females: • Ethnicity: • SES: • % smokers: • Mean BMI: • Pets: • Other: **Recruitment:** **Case Group:** **Control Group:**	**Built Environment Characteristics:** • Build age: • Build type: • Materials: • Heating: • Energy Efficiency: • Ventilation: • Other: • Damp prevalence: • Fungal prevalence: **Environmental Monitoring / Averages:** • Ambient temperature: • Relative Humidity: • Due Point temperature: • Vapor Pressure: • Moisture: • Water Activity: • Other: **Intervention Description:** **Follow up Period:**	**Description of Exposure:** **Prevalence of Exposure:** **Sampling Method/s:** **Sampling Location/s:** **Sampling Duration / Season:** **Sample Storage:** **Description of Protocol / Controls:** **Level of Fungal Identification:** **Identification Methods used:** **microscopy**	**Definition of Asthma Symptoms:** **Methods used / adopted to Classify Symptoms:** **Asthma Characteristics:** • Asthma prevalence: • Spirometry: • PEV/FEV: • Peak Flow: • Skin Prick Test: • IgE: • Other: **Other Symptoms Measured:**
Notes				
Limitations of Study Identified by Authors: **Limitations of Study Identified by Reviewers:**				
Results from Crude and Adjusted Models (insert results table from article)				
Self-Reported Health Outcomes: **Doctor Diagnosed Health Outcomes:**				
Newcastle-Ottawa Scale: NOS Score				
RS: Score **NB: Score** **Combined Score**				
Author Contact				
Contact Details: **Number of articles identified:** **Forward citation chasing:** **Backward citation chasing:** **Author contact:** **Number of studies omitted from the original database search:**				

Appendix E3 the Newcastle-Ottawa Scale (NOS) Scoring Template

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
CASE CONTROL STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Exposure categories. A maximum of two stars can be given for Comparability.

Selection

1) Is the case definition adequate?
   a) yes, with independent validation □
   b) yes, e.g. record linkage or based on self-reports
   c) no description

2) Representativeness of the cases
   a) consecutive or obviously representative series of cases □
   b) potential for selection biases or not stated

3) Selection of Controls
   a) community controls □
   b) hospital controls
   c) no description

4) Definition of Controls
   a) no history of disease (endpoint) □
   b) no description of source

Comparability

1) Comparability of cases and controls on the basis of the design or analysis
   a) study controls for _____ (Select the most important factor.) □
   b) study controls for any additional factor □ (This criteria could be modified to indicate specific control for a second important factor.)

Exposure

1) Ascertainment of exposure
   a) Fungal exposure measured quantitatively by molecular techniques e.g. qPCR or rtPCR□
   b) Qualitative description or by mycological examination □
   c) Visible damp and/or fungi assessed by physician
   d) self-reported visible damp and/or fungi
   e) no description

2) Same method of ascertainment for cases and controls
   a) yes □
   b) no

3) Non-Response rate
   a) same rate for both groups □
   b) non respondents described
c) rate different and no designation

NEWCASTLE - OTTAWA QUALITY ASSESSMENT SCALE
COHORT STUDIES

Note: A study can be awarded a maximum of one star for each numbered item within the Selection and Outcome categories. A maximum of two stars can be given for Comparability

Selection

1) Representativeness of the exposed cohort
   a) truly representative of the average _____ (describe) in the community □
   b) somewhat representative of the average _____ in the community □
   c) selected group of users e.g. nurses, volunteers
   d) no description of the derivation of the cohort

2) Selection of the non-exposed cohort
   a) drawn from the same community as the exposed cohort □
   b) drawn from a different source
   c) no description of the derivation of the non-exposed cohort

3) Ascertainment of exposure
   a) secure record (e.g. surgical records) □
   b) structured interview □
   c) written self-report
   d) no description

4) Demonstration that outcome of interest was not present at start of study
   a) yes □
   b) no

Comparability

1) Comparability of cohorts on the basis of the design or analysis
   a) study controls for _____ (select the most important factor) □
   b) study controls for any additional factor □ (This criteria could be modified to indicate specific control for a second important factor.)

Outcome

1) Assessment of outcome
   a) independent blind assessment □
   b) record linkage □
   c) self-report
   d) no description

2) Was follow-up long enough for outcomes to occur
   a) yes (select an adequate follow up period for outcome of interest) □
   b) no

3) Adequacy of follow up of cohorts
   a) complete follow up - all subjects accounted for □
   b) subjects lost to follow up unlikely to introduce bias - small number lost - > ____ % (select an adequate %) follow up, or description provided of those lost) □
   c) follow up rate < ____% (select an adequate %) and no description of those lost
d) no statement