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

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Background: Indoor dampness increases the risk of indoor fungal growth. A complex interaction between occupant behaviors and the built environment are thought to affect indoor fungal concentrations and species diversity, which are believed to increase the risk of having asthma, exacerbation of asthma symptoms, or both. To date, no systematic review has investigated this relationship.

Objective: This review aims to assess the relationship between exposure to indoor fungi identified to the genera or species level on asthma outcomes in children and adults.

Methods: Ten databases were systematically searched on April 18, 2013, and limited to articles published since 1990. Reference lists were independently screened by 2 reviewers, and authors were contacted to identify relevant articles. Data were extracted from included studies meeting our eligibility criteria by 2 reviewers and quality assessed by using the Newcastle-Ottawa scale designed for assessment of case-control and cohort studies.

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

Conclusion: Longitudinal studies assessing increased exposure to indoor fungi before the development of asthma symptoms suggests that *Penicillium*, *Aspergillus*, and *Cladosporium* species pose a respiratory health risk in susceptible populations. Increased exacerbation of current asthma symptoms in children and adults were associated with increased levels of *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria* species, although further work should consider the role of fungal diversity and increased exposure to other fungal species. (J Allergy Clin Immunol 2014;■■■:■■■-■■■.)

Key words: *Systematic review, damp, indoor fungi and allergic asthma*

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 can be compounded by efforts to reduce the carbon footprint of domestic domiciles[4,5] and the adoption of increased household energy efficiency measures to reduce the domestic carbon footprint, protect against temperature-related morbidity and mortality, and alleviate fuel poverty.[6] Efforts to prevent heat loss by reducing ventilation lead to undesired consequences for IAQ,[7] increasing indoor dampness and the risk of fungal contamination,[8] which currently affects around 16% of European dwellings.[9] Dampness and fungal contamination has been consistently shown to increase the risk of asthma[10] and asthma exacerbation.[11,12] Fisk et al,[11] Mendell,[12] and Quansah et al[10] review the role of fungal contamination, as defined by the presence of visible fungi, a moldy musty odor, or both. We contribute to existing knowledge by investigating the role of individual fungal genera/species (as opposed to the presence of any fungi) on asthma outcomes, which has yet to be explored.

Human behaviors, socioeconomic factors, and the built environment have been shown to increase the fungal load found in house dust.[13] Old terraced houses (≥90 years old) are at increased risk of higher concentrations of *Penicillium* and *Aspergillus* species propagules, exceeding outdoor spores per cubic meter of air per day in homes with no suspected damp or fungal contamination.[14] These fungi are also more frequently cultured from damp indoor home environments[15] and are of interest because they have been shown to increase the risk of asthma development in children.[16] Changes in composition of indoor air spores are complicated by the interaction with outdoor ambient levels. The indoor fungal profile is regulated by the dispersal of outdoor sources of fungi,[17] such as *Cladosporium* and *Alternaria* species.[18] The indoor fungal profile varies by geographic location,[19] season,[20] temperature, humidity, and air

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Abbreviations used
CFU: Colony-forming unit
ERMI: Environmental Relative Moldiness Index
IAQ: Indoor air quality
MSqPCR: Mold-specific quantitative PCR
NOS: Newcastle-Ottawa Scale
OR: Odds ratio
RR: Relative risk

exchange rates,[21] which are modified by ventilation and occupant behaviors. Variations in concentrations and diversity of fungal propagules (hyphae and spores) regulate the risk of asthma.[22] In a public health context we were interested in the development of new asthma-like symptoms over time and the exacerbation of symptoms. For the purpose of this review, we define asthma development as the initiation and progression of the disease to a point at which the reversible airflow obstruction and bronchospasm become common. We use the risk of asthma to describe either the development of new asthma-like symptoms, having "asthma," or the exacerbation of current asthma symptoms, and it is defined either by wheezing episodes (eg, International Study of Asthma and Allergies in Childhood[22]), a doctor's diagnosis, or medical examination using protocols, such as the European Respiratory Society and American Thoracic Society.[23]

The adoption of molecular techniques is advancing our ability to identify and quantify indoor exposures. To our knowledge, there has been no systematic review exploring the role of fungal diversity identified to the genus or species level 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 3 phyla: Ascomycota (including species of *Aspergillus*, *Penicillium*, *Alternaria*, and *Cladosporium* species), Basidiomycota, and Zygomycota.[18] Fungal components have been cultured from sputum samples taken from asthmatic and nonasthmatic subjects and are associated with an impaired postbronchodilator FEV_1.[24] There are several mechanisms that operate together in the pathogenesis of hypersensitivity reactions to fungi. Fungi are potent sources of allergenic molecules, including enzymes, toxins, cell-wall components, and highly conserved cross-reactive proteins.[25] Also, the inhalation of serine proteases of *Alternaria* species can also cause inflammation through stimulation of protease-activated receptor 2 of airway epithelium, which might be implicated in the development and exacerbation of airway allergic diseases.[26] Systematically reviewing studies concerning the diversity and concentrations of indoor fungi and the risk of having asthma, exacerbation of symptoms, or both provide an opportunity to assess associations and improve future health intervention work.

This review aims to assess the role of indoor fungal diversity being identified and quantified to the genus or species level on asthma symptoms in infants, children, and adults.

METHODS

Search strategy

Electronic searches were conducted on April 18, 2013, and limited to studies published after 1990 in accordance with our protocol (PROSPERO reference: CRD42013004333). In addition to electronic searches, author contacts and references of included studies were conducted in August 2013. The full search strategy was used on all 10 databases (listed in Appendix E1 in this article's Online Repository at www.jacionline.org) to identify eligible articles. The screening process was managed in Endnote version X5.0 (Thomas Reuters, New York, NY)[27] and recorded by using the PRISMA guidelines.[28] Articles were independently screened by 2 team members (R.A.S. and N.B.), and where there was disagreement, a third reviewer (N.J.O.) 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 (Fig 1). Forward and backward citation chasing was performed on all included studies, and authors were contacted for additional relevant articles.

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

1. original peer-reviewed articles publishing original data;
2. cohort, case-control, and nonrandomized and randomized controlled trials (including cluster-randomized and crossover trials);
3. studies published in 1990 or later;
4. investigations of the indoor home environment;
5. assessments of indoor fungi identified to the genus or species level;
6. studies with outcomes of asthma ever and/or asthma symptoms in the last 12 months, including wheeze, whistling in the chest, or a dry cough; doctor's diagnosis or skin prick test, peak flow, or spirometric results; and asthma development requiring newly diagnosed new cases of asthma by a physician or doctor; and
7. those that provided a measure of risk for asthma, including the relative risk (RR) or odds ratio (OR) and CI.

Data extraction

Relevant participant and study characteristics were recorded with a standardized data extraction template (see Appendix E2 in this article's Online Repository at www.jacionline.org), which was subsequently used to populate data synthesis tables.

Quality assessment

Two team members (R.A.S. and N.B.) assessed the quality of each study by using the Newcastle-Ottawa Scale (NOS)[29] modified to reflect fungal exposure (see the case-control form, Exposure point 1, Appendix E3 in this article's Online Repository at www.jacionline.org). 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 (R.A.S. and N.B.) independently scored included articles and a final score was obtained by consensus. Journal article authors were contacted if data were missing.

RESULTS

Synthesis

We provide an overarching narrative synthesis of included studies and a meta-analysis of studies of similar design and those reporting ORs and CIs. We included 7 studies in a meta-analysis of Salo et al,[30] Araki et al,[31] Dales et al,[32] Jones et al,[33] Li and Hsu,[34] Rosenbaum et al,[35] and Dharmage et al[36] because these met our inclusion criteria for conducting a meta-analysis; the other 10 studies were too heterogeneous to be included. We had planned to prioritize studies rated more highly on the NOS rating scale; however, the evidence located was all of a midrange quality, and therefore we did not weight studies in the analysis.

Studies were grouped in our narrative synthesis according to those reporting risk of increased fungal concentrations in homes of asthmatic patients (analysis of indoor fungi in homes being

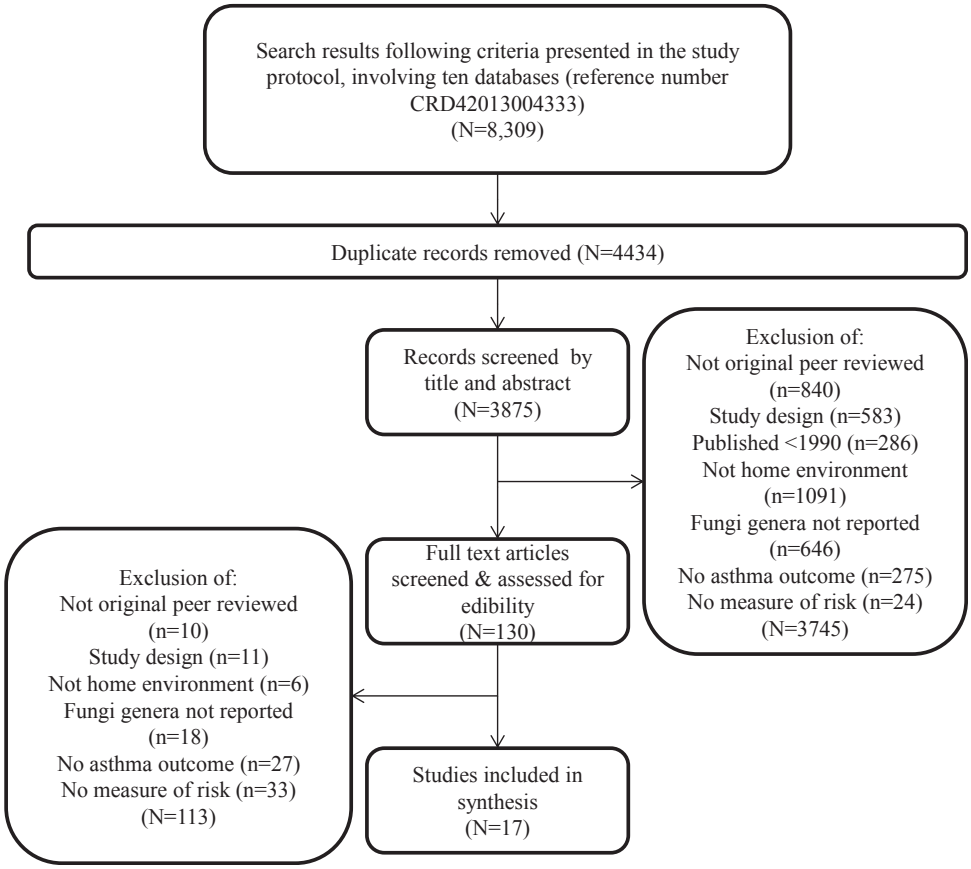

FIG 1. Diagram of the systematic search and included studies.

occupied with ≥1 asthmatic patients). We then assessed fungal genera, total fungi, and risk of asthma in our meta-analyses. Meta-analyses were undertaken to explore the relationship between exposure to individual groups of fungi and current asthma by using the generic inverse variance method[37] to conduct random-effects meta-analysis[38] in RevMan 5 (version 5.2.6; Cochrane Collaboration, Copenhagen, Denmark). Logistic regression was used to calculate ORs and CIs for adjusted and unadjusted data because of the inconsistency of reporting unadjusted data. We were unable to stratify by age, study design, or outcome because of the limited number of studies and inconsistent reporting.

Heterogeneity was assessed by using the I^2 statistic, where an I^2 value of 0% to 40% was considered low heterogeneity and an I^2 value of 75% or greater represented considerable heterogeneity.[37] No further analyses were conducted because of sample size limitations.

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 I).[16,30-36,39-47] One author[31] provided additional analyses to be included in our results synthesis. Eight studies were based on populations living in the United States, and the remaining were from the United Kingdom, Sweden, Taiwan, Colombia, Australia, Canada, and China. Not all studies clearly reported whether they had investigated urban or rural environments. Based on the summary results (Table I), 9 studies assessed indoor fungal concentrations in homes located in predominantly urban areas, with only 1 study specifically investigating homes situated in an agricultural setting.

Thirteen included studies involved children (aged <18 years), 2 included adult populations, and the remaining 2 included all age groups. Demographic variables (ie, variations in the built environment and occupant behaviors) potentially modifying the risk of fungi, asthma, or both were not consistently reported. Reported asthma outcome measures also varied (Table I), and only 2 studies, those of Reponen et al[16] and Matheson et al,[39] examined the development of new asthma symptoms.

Study design characteristics of included studies

We included 4 cohort studies with follow-up periods of 1, 2, and 7 years. Thirteen studies were cross-sectional, which included 9 case-control studies. Funding, recruitment, and statistical analyses varied between studies (see Table E2 in this article's Online Repository at www.jacionline.org). The heterogeneity between study designs and the defined exposure and outcomes prevented the inclusion of all studies in our meta-analysis. The following presents results separately for both our narrative synthesis and meta-analysis.

Results of studies included in our narrative synthesis

We provide a narrative synthesis of 10 studies[16,39-47] that were not included in the meta-analysis because of heterogeneity

TABLE I. Summary of participant characteristics of included studies

Reference	Country	Study population	Urban/rural, region	Study design	Study size
Rosenbaum et al[35]	United States	Infants aged <1 y	Urban; Syracuse, NY	Birth cohort	39 cases, 64 control subjects
Matheson et al[39]	Australia	Adults aged 20-45 y	Urban, Melbourne	Longitudinal	360
Reponen et al[16]	United States	Children aged 7 y	Cincinnati, Ohio, and northern Kentucky	Birth cohort	69 cases, 220 control subjects
Gent et al[46]	United States	Infants aged <1 y	Connecticut/western Massachusetts	Cohort, longitudinal	819
Jones R et al[33]	United States	Children aged 3-17 y	Buffalo, NY	Nested case-control	50 cases, 59 control subjects
Araki et al[31]	Japan	All ages	Not specified; study conducted across 6 regions	Case-control	609
Holme et al[42]	Sweden	Children aged 1-6 y	Mixed, Värmland	Nested case-control	198 cases, 202 control subjects
Vesper et al[43]	United States	Children aged 9-12 y	Urban, Detroit, Mich	Case-control	28 cases, 83 control subjects
Su et al[44]	Taiwan	Children aged 10-12 y	Urban, Taiwan	Case-control	23 cases, 12 control subjects
Strachan et al[41]	United Kingdom	Children aged 6-7 y	Scotland	Case-control	34 cases, 54 control subjects
Li and Hsu[34]	China	Children aged 7-15 y	Urban, Taiwan	Case-control	46 cases, 26 control subjects
Vesper et al[40]	United States	Children; mean age, 6.8 y	Cleveland, Ohio	Case-control	60 cases, 22 control subjects
Meng et al[45]	United States	Children aged 2-18 y	Rural, agricultural area in the Midwest	Case-control	88 cases, 85 control subjects
Salo et al[30]	United States	All ages	Metropolitan areas, nationwide	Cross-sectional	2456
Dharmage et al[36]	Australia	Adults aged 20-44 y	Urban, Melbourne	Cross-sectional	485
Dales et al[32]	Canada	Children aged 10 y	Ontario	Cross-sectional	400
Herrera et al[47]	Colombia	Children aged 7 y	Urban, Bucaramanga	Cross-sectional	678

ATS, American Thoracic Society; *CE*, cell equivalents; *EISL*, International Study of Wheezing in Infants; *GINA*, Global Initiative for Asthma; *ISAAC*, International Study of Asthma and Allergies in Childhood; *NA*, not applicable; *NP*, nurse practitioner.

between study designs, statistical analyses, and outcomes. The first part of the narrative synthesis includes 2 main elements because 7 studies[16,40-45] used 2 different sampling and analysis techniques to quantify fungal concentrations in homes of asthmatic patients (Tables II and III).[16,40-45] These are defined by those reporting fungal species as cell equivalents per gram of house dust and fungal genera as colony-forming units (CFU) per cubic meter of air. This is followed by a review of the associated risk of asthma exacerbation assessed as rate or prevalence ratios (see Table E4, A, in this article's Online Repository at www.jacionline.org) and then ORs (Table IV[30-36,39] and see Table E5 in this article's Online Repository at www.jacionline.org), which were subsequently included in the meta-analysis.

Three studies from the United States assessed the risk of increased fungal concentrations in the homes of asthmatic patients[16,43,48] by using house dust samples and mold-specific quantitative PCR (MSqPCR) to quantify fungal concentrations (Table II). This method has been developed to assess 36 fungi of the Environmental Relative Moldiness Index (ERMI) developed to quantify the indoor fungal load.[49] These studies quantify 9 fungal genera at the species level that were found to be present in higher concentrations in homes of asthmatic patients, although these were not consistent, and concentrations varied considerably (see Table E3 in this article's Online Repository at www.jacionline.org). The other studies[41,42,44,45] used air sampling and microscopy to quantify indoor fungus to the genus level as CFU per cubic meter of air (Table III), as opposed to MSqPCR. Studies using microscopy to define CFU per cubic meter of fungi present indoors showed that levels of *Penicillium* species (496.8 vs 276.3 total CFU/m^3),[44] *Cladosporium* species (5.18 vs 4.43 mean CFU/m^3), *Ulocladium* and *Acremonium* species (3.32 vs 0 mean CFU/m^3), and total fungi (5.92 vs 5.19 mean CFU/m^3)[45] were higher in homes of asthmatic patients, although other studies did not report this relationship. Studies with molecular techniques to quantify indoor fungal concentrations reported higher concentrations of *Aspergillus*, *Penicillium*, *Cladosporium*, *Ulocladium*, *Acremonium*, *Aureobasidium*, *Epicoccum*, *Scopulariopsis*, *Trichoderma*, *Alternaria*, and *Wallemia* species in house dust. Not all studies found this relationship, and only higher concentrations of *Aspergillus* and *Penicillium* species were reported by 2 of the studies.

TABLE I. *(Continued)*

Follow-up y	Exposure measurement	Definition of asthma	Final quality score
2	Air sampling (CFU/m^3)	Diagnosis of wheeze during the first year of life was defined as (1) primary care provider documenting wheezing, reactive airway disease, asthma, or bronchiolitis; (2) wheeze heard on physical examination by the NP; or (3) prescription for bronchodilator, inhaled steroid, or steroid pulse prescription documented in medical records.	7/13
2	Air sampling (CFU/m^3)	Wheeze <12 mo, spirometry plus bronchial hyperreactivity to methacholine and clinical activity	7/13
1 and 7	House dust sampling (ERMI)	Parental self-reports and then diagnosis of asthma based on asthma symptoms and objective measures of lung function and airway hyperresponsiveness; all children completed spirometric testing (Koko; nSpire Health, Longmont, Colo) according to ATS criteria.	6/13
3 in 1 y	Air sampling (CFU/m^3)	Respiratory symptoms of wheeze and persistent cough defined by yearly symptom counts	5/13
NA	Air sampling (CFU/m^3)	Self-reported questionnaire and clinical interview to assess medication use and asthma symptoms, including lung function and skin prick testing for allergens	8/10
NA	Air sampling (CFU/m^3)	Self-reported questionnaire for receiving medical treatment for bronchial asthma	7/10
NA	Air sampling (CFU/m^3)	Doctor-diagnosed asthma defined by medical examination	6/10
NA	House dust by vacuum (CE/mg dust [ERMI])	Parental self-reported use of asthma medication using the GINA classification system	6/10
NA	Air sampling (CFU/m^3)	Adult self-reported child receiving a diagnosis from a physician and re-examined by a pediatrician at the National Cheng Kung University Hospital before they were included into the year-long study	6/10
NA	Air sampling (CFU/m^3)	Examination followed the ATS protocol: wheeze in <12 mo and bronchial lability >10%. Wheeze and bronchial lability were defined as the difference between the postexercise and pre-exercise FEV divided by the pre-exercise FEV.	5/10
NA	Air sampling (CFU/m^3)	Asthma status defined by ATS criteria	5/10
NA	Air and dust sampling (mg/g [ERMI])	Homes with an asthmatic child	4/10
NA	Air sampling (CFU/m^3)	Persistent asthma defined by National Heart, Lung, and Blood Institute	4/10
NA	Dust sampling (mg/g)	Doctor-diagnosed asthma and allergy defined at interview to obtain information on doctor-diagnosed asthma and allergies, asthma symptoms in the past year, and current asthma medication use	7/10
NA	Air sampling (CFU/m^3)	Wheeze <12 mo, spirometry plus bronchial hyperreactivity to methacholine and clinical activity	6/10
NA	Self-reported and house dust samples collected	Self-reported questionnaire of current and diagnosed asthma; cough or wheeze during the night and within the last 12 mo; asthma defined by a doctor confirming the child has asthma or regularly takes asthma medication	5/10
NA	Air sampling (CFU/m^3)	Self-reported via questionnaire, respiratory symptoms suggestive of asthma, which is measured by questionnaires from EISL and the ISAAC	4/10

The evidence reviewed here is weak and requires further investigation into variations in species diversity and the interaction between the indoor and outdoor environments.

In an attempt to examine the role of fungi in asthma beyond exacerbation, 2 longitudinal studies have enabled the investigators to assess the effect of fungal diversity before the development of asthma symptoms. Birth cohorts at risk of atopy showed a 2-fold increased risk of higher rates of infant wheeze[46] and the onset of childhood asthma[16] associated with exposure to species of *Penicillium* and *Aspergillus*. *Cladosporium* species increased the risk of a new asthma attack in the last 12 months by 50% in adults.[39] We were unable to include these studies[16,39,46] in a separate meta-analysis because of the limited number of studies (with varying study designs) assessing fungal concentrations and the risk of having asthma symptoms in longitudinal analyses. The longitudinal study designs also prevented them from being included in our meta-analysis of the cross-sectional and case-control studies assessing the risk of asthma exacerbation.

Investigations into specific groups of fungi and associated risk of asthma exacerbation were not consistent and limited our syntheses, particularly with respect to variations in asthma outcome definitions (Table I). Three studies[16,46,47] assessed the potential risk of asthma by calculating prevalence or rate ratios and were omitted from our meta-analysis because of heterogeneity of study designs. In summary, Herrera et al[47] reported an increased probability (>50%) of respiratory symptoms (indicative of bronchial asthma) being associated with *Acremonium* species (prevalence ratio, 6.2; 95% CI, 3.8-10.0). Gent et al[46] reported that the highest level of *Penicillium* species (≥1000 CFU/m^3) was associated with higher rates of wheeze (adjusted RR, 2.2; 95% CI, 1.3-3.5) in the first year of life, although it is impossible to assess whether reported wheeze developed into asthma later in childhood. Finally, the summation of *Aspergillus ochraceus*, *Aspergillus unguis*, and *Penicillium variabile* were associated with the development of asthma in children aged 7 years (adjusted RR, 2.2; 95% CI, 1.8-2.7).[16]

Results of studies included in the meta-analysis

We identified 7 studies[30-36] that met our criteria for conducting a meta-analysis to assess fungal (identified to the genus

TABLE II. Results synthesis: risk of fungi identified to the species level (CE) in homes of asthmatic patients

		Fungi measured as CE/g of house dust								
		Aspergillus niger			*Penicillium* species group 2			*Cladosporium sphaerospermum*		
		Aspergillus ochraceus			*Penicillium spinulosum*			*Cladosporium cladosporioides* 1		
		Aspergillus unguis			*Penicillium variabile*			*Cladosporium cladosporioides* 2		
Study	**Fungal analysis**	**Case**	**Control subject**	***P* value**	**Case**	**Control subject**	***P* value**	**Case**	**Control subject**	***P* value**
Vesper et al[40]	GM CE/g	NR	NR	NR	2,604.09	654.48	.08	4,714.39	8,172.98	.03
		1,895.46	2,117.95	.79	710.90	3,600.06	.01	177,704.3	544,160.00	.00
		3,831.60	1,881.66	.32	1,050.69	1,033.93	.92	16,155.37	50,671.42	.01
Vesper et al[43]	Median CE/mg	67	24	.01	16	11	.49	16	9	.10
		40	24	.09	*	*	*	325	370	.59
		3	2	.02	27	14	.39	7	10	.70
Reponen et al[16]	GM CE/g	13.7	5.7	<.05	–	–	NS	137.2	70.5	NS
		6.8	2.0	<.05	1.1	0.9	NS	2,099.3	1,349.2	NS
		2.6	1.0	<.05	12.6	4.0	<.05	28.1	27.7	NS
		Aureobasidium pullulans			*Epicoccum nigrum*			*Scopulariopsis brevicaulis*		
Vesper et al[40]	GM CE/g	417,991.00	727,917.30	.02	407,868.70	920,578.1	.00	1,179.00	480.64	.04
		Trichoderma viride			*Alternaria alternata*			*Wallemia sebi*		
Vesper et al[40]	GM CE/g	1,602.96	284.82	.01	16,452.45	55,594.45	0	18,954.01	8,442.97	.05

CE, Cell equivalents; *GM*, geometric mean; *NR*, not reported; *NS*, not significant.
*Missing data.

TABLE III. Results synthesis: risk of fungi identified to the genus level (CFU) in homes of asthmatic patients

		***Aspergillus* species**			***Penicillium* species**			***Cladosporium* species**			***Alternaria* species**		
Study	**Fungal analysis**	**Case**	**Control subject**	***P* value**	**Case**	**Control subject**	***P* value**	**Case**	**Control subject**	***P* value**	**Case**	**Control subject**	***P* value**
Strachan et al[41]	GM CFU/m^3	NR			39	55	−.78	16	12	.46	NR		
Holme et al[42]	Mean CFU/m^3										NR		
On DG-18		113	128	.602	104	119	.298	92	125	.130			
On MEA		229	57	.147	95	106	.699	70	100	.762			
Su et al[44]	Total CFU/m^3												
Spring		306.7	226.9	NS	839.6	608.3	NS	4,972.9	3,906.10	NS	3,039.1	4,098.6	NS
Summer		738.0	427.0	NS	568.4	260.7	NS	2,085.0	2,303.90	NS	47.4	4.5	NS
Fall		303.1	269.8	NS	454.0	479.3	NS	6,469.51	6,726.10	NS	87.9	178.8	NS
Winter		451.2	165.0	NS	496.8	276.3	<.05	17,696.0	16,999.3	NS	251.0	336.53	NS
Meng et al[45]	Mean CFU/m^3	3.62	3.33	.24	4.12	3.72	.09	5.18	4.43	<.0001	3.99	3.60	.07
		Acremonium species			*Ulocladium* species			*Epicoccum* species					
		3.32	0	<.02	3.06	0	<.001	3.63	3.62	.98			

The outcome of interest is risk of fungi in homes of asthmatic and nonasthmatic subjects.
GM, Geometric mean; *NR*, not reported; *NS*, not significant.

level) exposure and risk of asthma exacerbation. Included studies were of case-control or cross-sectional study designs and consistently assessed increased exposure to concentrations of *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* species and risk of asthma exacerbation. Included studies also assessed increased exposures to other fungi, such as *Rhodotorula*, *Epicoccum*, and *Acrodontium* species, as well as total fungi, ergosterol, and yeasts, although these were not consistently investigated by all studies. Reported health outcomes were defined as doctor-diagnosed asthma, self-reported use of asthma medication, wheeze plus bronchial hyperreactivity to methacholine, and medical examination according to the American Thoracic Society criteria (Table I). In some cases studies did not report unadjusted data (see Table E5), which prevented the inclusion of raw data into our meta-analysis. This meant that we used unadjusted data (when reported) and data from the adjusted models reported by each study. Included studies did not consistently adjust for potential covariates (Table III), although a number of built environment and demographic risk factors were assessed (see Tables E5 and E6 in this article's Online Repository at www.jacionline.org).

We were unable to assess the risk associated with fungal species because identification was only made to the genus level (eg, for *Aspergillus*, *Penicillium*, *Cladosporium*, and *Alternaria* species), with the exception of 1 study.[30] Increased exposure to these fungi was associated with an increased risk of asthma in child and adult populations (Table III), although this relationship was not consistently reported. Other fungi investigated included *Rhodotorula*, *Epicoccum*, and *Acrodontium* species and sterile fungi (those lacking asexual or sexual spore production), which were not associated with increased risk of residents having asthma (see Table E5). 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* species and risk of asthma exacerbation (Table V). We excluded data concerning the associated

TABLE IV. Summary table of studies reporting ORs and CIs

Study	Fungal analysis	*Aspergillus* species		*Penicillium* species		*Cladosporium* species		*Alternaria* species	
		Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted	Unadjusted	Adjusted
Salo et al[30]	<3.90	Not reported		Not reported		Not reported		1.0	1.0
	3.90-6.27							1.60 (0.90-2.77)	1.52 (0.90-2.55)
2 fold increase in concentration	≥6.28 μg/g							1.84 (1.21-2.93)	1.84 (1.18-2.85)
	All ages							Not reported	1.31 (1.05-1.64)
	Children <18 y							Not reported	1.47 (0.83-2.62)
	Adults >18 y							Not reported	1.25 (0.99-1.58)
Araki et al[31]	>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[32]	Detectable limits CFU/g		0.92 (0.35-2.44)	Not reported			0.46 (0.18-1.21)		1.90 (0.55-6.59)
Jones R et al[33]	≥85th percentile							Not reported	
Viable counts	CFU/m^3	2.81 (1.00-7.90)	6.1 (1.37-27.19)*	0.49 (0.19-1.31)	0.35 (0.11-1.17)	1.37 (0.52-3.56)	1.19 (0.39-3.60)		
Total counts	Spores/m^3		0.54 (0.10-2.92)†	0.70 (0.27-1.82)‡	0.94 (0.31-2.83)‡	1.93 (0.73-5.14)	2.37 (0.77-7.26)		
Li and Hsu[34]	Summer		1.55 (0.71-3.36)		0.61 (0.21-1.81)		1.88 (1.07-3.30)		
	Winter		0.69 (0.28-1.73)		0.56 (0.17-1.84)		4.14 (1.17-14.67)		
Rosenbaum et al[35]	Not detected v high CFU/m^3	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[36]	Highest quartile	Not reported			3.9 (1.1-14.3)		8.5 (1.6-44.3)	Not reported	
Matheson et al[39]	CFU/m^3	Not reported		Not reported			0.96 (0.80-1.16)§	Not reported	
							1.11 (0.91-1.37)‖		
							1.52 (1.08-2.13)¶		

The outcome of interest is risk of fungi in homes of asthmatic and nonasthmatic subjects.
GM, Geometric mean.
Individual analyses in studies: *without family history of asthma; †with family history of asthma; ‡model for *Aspergillus* and *Penicillium* combined (Jones et al[33]); §effect of doubling allergen or fungal exposure on the risk of current asthma; ‖effect of doubling exposure to allergens or fungi on the remission of current asthma; and ¶effect of doubling allergen or fungal exposure on the risk of asthma attack in the last 12 months (Matheson et al[39]).
Adjusted models in each study:

- Salo et al[30] adjusted for age, sex, race, education, smoking, and sampling season. Please note other adjusted models were provided, with all showing positive associations in the third quartile. Analysis for a 2-fold increase (children <18 years) has fewer observations because of missing values.
- Araki et al[31] adjusted for sex, age, tobacco smoke exposure, renovation history, wall-to-wall carpeting, dampness index, and hay fever.
- Dales et al[32] adjusted for child's age, parental illness, passive smoking, and dust mites.
- Jones et al[33] adjusted for age and 1 or more family members with asthma. There was a strong interaction between an increased level of *Aspergillus* species and 1 or more family members with asthma. Therefore separate models were generated for subjects with and without a family member with asthma.
- Li and Hsu[34] adjusted for age, parental education, number of household smokers, and use of a gas stove for cooking.
- Rosenbaum et al[35] adjusted for season of visit, maternal smoking during pregnancy, any smoker in the home, day care center or nonrelative care, and endotoxin.
- Dharmage et al[36] adjusted for potential confounders: sociodemographic factors, current smoking, parental asthma/allergy, medication use, and season during which the participant was investigated.
- Matheson et al[39] adjusted for season of sampling and smoking status. Analysis provided for asthma attack in the last 12 months, atopy, and doctor-diagnosed asthma.

risk of asthma resulting from models investigating the associated level of risk with doubling fungal exposures[30,39] because the methodology differed from that for other included data.

Random-effect estimates were calculated in combined models to investigate the role of fungal load and then individual fungal genera to further explore associations between different fungal genera. Effect estimates of each model were calculated with the number of included studies and the I^2 statistic, indicating that included studies were subject to medium-high heterogeneity (Table V). No associations were reported with the total fungal load found indoors (model 1), and model 2 suggests a 29% to 40% risk. In accordance with our protocol, we omitted exposures not defined to the genera or species level, and this suggests a 34% to 51% (model 3) and 34% to 51% (model 4) increased risk of asthma symptoms. The combination of the most consistently reported fungal genera and the 4 prevalent indoor fungi *Cladosporium*, *Alternaria*, *Penicillium*, and *Aspergillus* species (model 5) increased the risk of current asthma by 48% in the unadjusted model and 36% in the adjusted model. Studies were subject to medium heterogeneity, with an I^2 statistic ranging from 61% to 67% (Table V). Because of the heterogeneity, we carried out subgroup analyses of the 4 most commonly reported fungal genera to determine which genus or genera modified our combined effect estimates. This analysis suggests that the association was primarily caused by increased levels of *Cladosporium* and *Alternaria* species (models 6-9), with no significant association with exposure to *Penicillium* and *Aspergillus* species (Figs 2 and 3 and see Appendix E1). Further analyses showed that the findings might be driven by a single study[30] demonstrating a strong association between *Alternaria alternata* and asthma exacerbation. The fungal analysis of this study differed by the use of ELISA techniques to quantify concentrations of *A alternata* antigen in

TABLE V. Summary effect estimates and heterogeneity scores of results synthesis

Model in subgroup 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 value	No. of studies included in analysis	Summary effect estimates for pooled adjusted data (95% CI)	I^2 value
Model 1: Total fungi	3	0.98 (0.53-1.82)	25%	3	0.86 (0.46-1.59)	1%
Model 2: Identified and unidentified fungi (*Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhodotorula*, *Acrodontium*, and *Epicoccum** species; sterile; Basidiomycetes; hyaline unknown and dark unknown)	4	1.40 (1.07-1.82)	54%	7	1.29 (1.02-1.62)	50%
Model 3: Fungi, including nonsporulating (*Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhodotorula*, *Acrodontium*, and *Epicoccum** species; sterile)	4	1.47 (1.09-1.97)	61%	7	1.34 (1.05-1.71)	54%
Model 4: Fungi, excluding nonsporulating (*Aspergillus*, *Penicillium*, *Cladosporium*, *Alternaria*, *Rhodotorula*, *Acrodontium*, and *Epicoccum** species)	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*, and *Alternaria* species)	4	1.48 (1.03-2.14)	67%	7	1.36 (1.02-1.82)	61%
Model 6: *Aspergillus* species	3	1.74 (0.66-4.60)	76%	5	0.98 (0.59-1.63)	54%
Model 7: *Penicillium* species	3	1.66 (0.48-5.70)	83%	5	1.19 (0.56-2.54)	67%
Model 8: *Cladosporium* species	3	1.29 (0.64-2.59)	61%	6	1.96 (1.13-3.41)	66%
Model 9: *Alternaria* species	2	1.71 (1.11-2.63)	0%	3	1.77 (1.22-2.56)	0%

*Only unadjusted data are available.

house dust. Analyses in these models excluded *Rhodotorula*, *Acrodontium*, and *Epicoccum* because data concerning these fungi were not consistently reported.

Risk of bias of individual studies

The NOS for included items (Table I) indicated the studies were of medium quality, suggesting the potential inclusion of bias. There is also the potential for the inclusion of reporting bias resulting from inclusion of unadjusted and adjusted data into the random-effects models. Funnel plots present the variability between individual fungal groups (see Fig E1 in this article's Online Repository at www.jacionline.org) and the I^2 statistic (Table V) suggests that there is medium-to-considerable heterogeneity, further suggesting conservative effect estimates, with the exclusion of combined models for total fungi and *Alternaria* species (I^2 value ranging from 0 to <25).

DISCUSSION

Our findings suggest that exposure to *Aspergillus*, *Penicillium*, *Cladosporium*, *Ulocladium*, *Acremonium*, *Aureobasidium*, *Epicoccum*, *Scopulariopsis*, *Trichoderma*, *Alternaria*, and *Wallemia* species might represent a respiratory health risk to asthmatic patients living in homes with increased fungal concentrations. These analyses do not provide sufficient detail to assess whether these fungi exacerbated asthma symptoms or potential health outcomes resulting from increased exposure to known allergenic fungal species (ie, fungi only identified to the genus level) present in higher concentrations at the time of sampling. Development of the ERMI and use of MSqPCR[49] enables us to more reliably quantify fungal species present indoors.[50] *Aspergillus niger*, *A unguis*, *Cladosporium cladosporioides*, *Aureobasidium pullulans*, *Epicoccum nigrum*, and *A alternata* were found in higher concentrations in homes of asthmatic patients in studies using MSqPCR. These fungi are allergenic species that might induce type I hypersensitivity.[18] It is not clear which factors regulate indoor fungal diversity and the risk of asthma at the individual level or how potential covariates might modify the outcome.

We identified a limited number of longitudinal studies to explore the risk of new cases of asthma symptoms in populations exposed to increased concentrations of indoor fungi. Included studies highlight that exposure to species of *Penicillium*, *Aspergillus*, and *Cladosporium* species increases the risk of asthma in children and adults, although these studies do not embrace the full extent of indoor fungal diversity and exposure to other allergenic fungi.[18] Seven of the identified studies reviewed investigate exposure to increased fungal concentrations and increased risk of the exacerbation of asthma symptoms, which were included in our meta-analysis.

Meta-analysis: Indoor fungal contamination and asthma exacerbation of asthma symptoms

Our meta-analysis suggests that a number of fungal genera investigated by the included studies increase the risk of exacerbation of asthma symptoms. The associated level of risk did not appear to be significantly different when grouped by all reported fungi (model 2) versus those identified to the genera level (model 5). These findings could be affected by increased heterogeneity (I^2 statistic) as we refine our meta-analysis model in accordance with our protocol. Our refined model for the 4 most consistently reported fungal genera, *Penicillium* and *Aspergillus* species,[51] *Penicillium* species,[35,36] *Aspergillus* species,[33] *Cladosporium* species,[34,36] and *Alternaria* species,[30] increases the risk of the exacerbation of asthma symptoms by 36% to 48% in our effect estimates. These fungi have also been shown to be associated with an increased risk in longitudinal studies discussed in our narrative analyses[16,39,46] and warrant further investigation in future research. Further analyses suggest that exposure to increased concentrations of *Cladosporium* and *Alternaria* species are primarily associated with increased risk of the exacerbation of asthma symptoms. However, this might be a result of the adopted

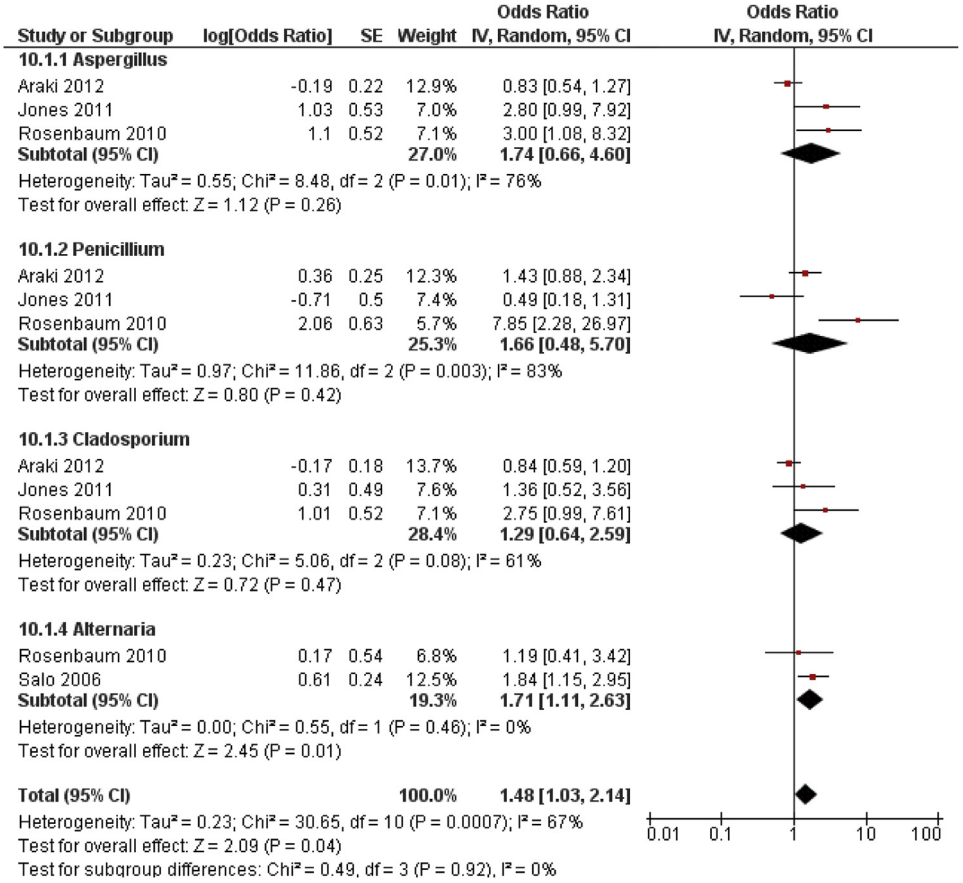

FIG 2. Unadjusted model for indoor fungi and risk of asthma.

study designs and limited sample sizes. For example, the strong association with *Alternaria* species results from the inclusion of one study[30] with a large sample size (n = 2456) compared with other studies and used ELISA to quantify concentrations of *A 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 per cubic meter vs dust CFU per gram sampling) because of their poor correlation in estimating potential exposures[52] and differences in fungal identification techniques.[40,51] Resultant health risks depend on the timing and extent of exposure to other groups of fungi, as well as ambient outdoor/indoor conditions, growth substrates, and levels of dampness,[8] which cannot be ascertained from the included studies. Included studies did not consistently account for potential covariates, and few considered the role of indoor dampness and increased relative humidity, which increase the biological (house dust mite and fungi) and chemical load[53] and should be considered potential covariates. Sensitization to pet allergens increases asthma exacerbation, with cat allergen being the most insidious,[54] although other work suggests that early dog ownership is associated with changes in immune development and reductions in wheezing and atopy.[55]

It is also not clear from the evidence reviewed here how fungal diversity and risk of asthma might be modified by residential characteristics and the influx of outdoor fungal spores, which regulates the indoor fungal profile.[8] *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria* species sporulation rates have considerable daily and seasonal variability and, combined with the adoption of different sampling techniques,[56,57] add another level of complexity. Indoor fungal concentrations used to calculate ERMI values have also been shown to be heterogeneously distributed across the United States.[19] 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 4 fungal groups represents a respiratory risk for asthmatic patients, but the evidence is not conclusive when assessing species diversity and asthma risk. It is yet unknown how exposure to fungi influences the development of new asthma-like symptoms, exacerbation of asthma symptoms, or both.

Synthesis with existing knowledge

Our combined random-effect estimates concerning exposures to individual fungal genera are similar to the meta-analyses of Fisk et al,[11] who reported an approximate 30% to 50% increased risk of asthma outcomes. Two cohort studies have demonstrated that exposure to increased fungal contamination and risk of atopy increase the risk of asthma development in children[58] and adult[59] populations. A recent systematic review reported a significant association with

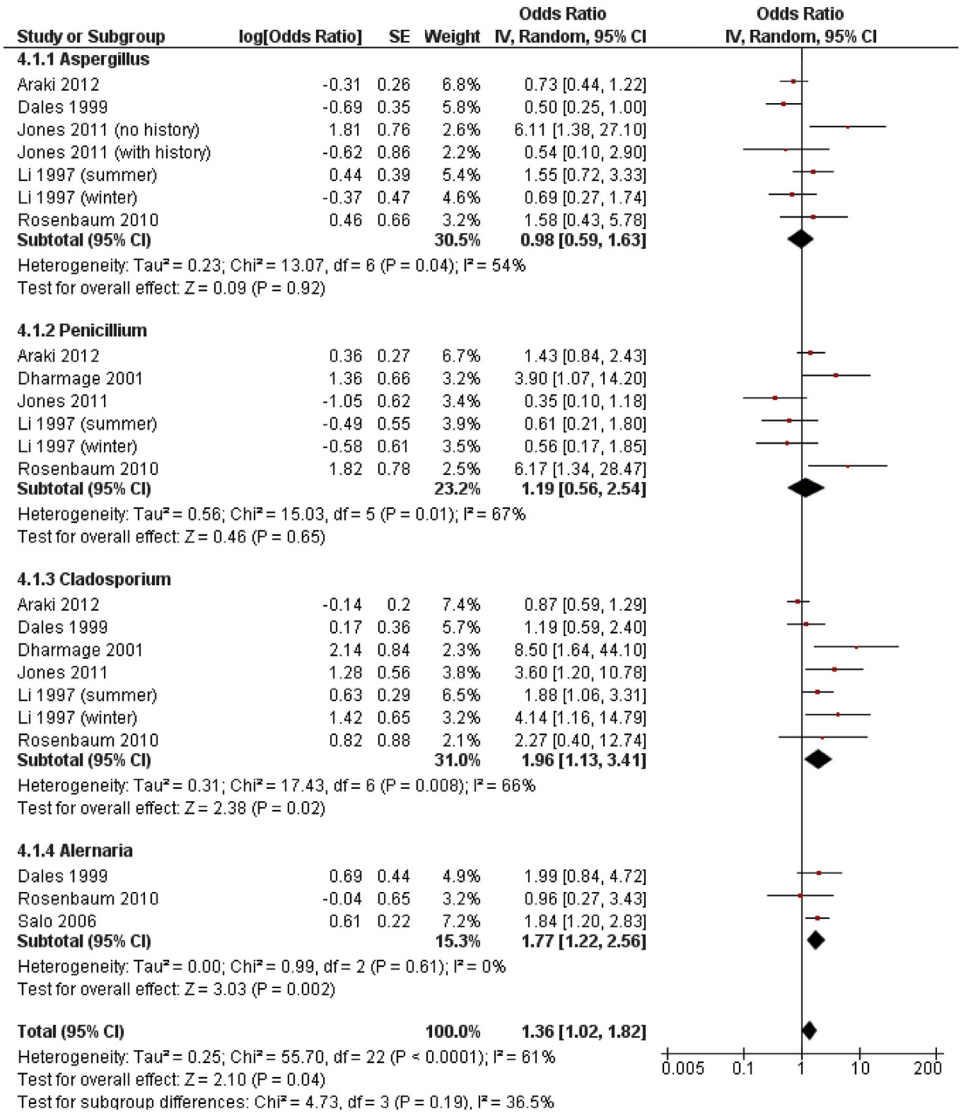

FIG 3. Adjusted model for indoor fungi and risk of asthma.

increased exposure to fungal odor (random-effects model; effect estimate, 1.7; 95% CI, 1.2-2.5) and the development of asthma.[10] Fungal diversity and concentrations of *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria* species vary considerably between different populations.[45,60,61] This is likely to regulate asthma outcomes in different populations given that variations in residential characteristics regulate fungi found in US[13] and United Kingdom[14] homes. Included studies in our meta-analysis used predominantly microscopy to identify and quantify the genus of fungi, which is likely to underestimate microbial exposures compared with molecular techniques.[51]

Exposure to *Cladosporium* and *Alternaria* species increased the risk of asthma in our effect estimates, which might be due to asthma severity being associated with *Cladosporium*[39,62] and *Alternaria* species.[63,64] It is not clear how the risk of asthma and severity of symptoms might be modified in sensitized populations, which is important to consider given that the development of allergic asthma (presence of IgE antibodies) in adults has been associated with *Aspergillus fumigatus* and *Cladosporium* species.[65] *Penicillium* species is frequently cultured from damp indoor home environments and has been associated with asthma severity,[66] peak flow variability,[67] and asthma morbidity[68] when present in low concentrations.[69] The lack of association between exposure to *Penicillium* and *Aspergillus* species and asthma in meta-analyses might be due to the limitations discussed above. These are important fungi to consider in future work because they dominate the damp indoor environment, where propagule concentrations exceed those in their natural outdoor environments,[8] and have been implicated in the development of childhood asthma.[16] Dampness appears to be a high risk for fungal growth present both in the US and European scenarios.

There is insufficient evidence to support targeted interventions to decrease exposures to high-risk fungi in the general public and reduce symptoms or the initiation of disease. It is accepted that fungal sensitization is associated with an increased risk of asthma.[70] Fungal diversity and concentrations of different fungal groups appear to modify asthma outcomes in atopic and nonatopic subjects. However, this might also be the result of inhalation

of different indoor/outdoor fungal propagules. Variations in the composition of ambient fungal spores also influence asthma episodes resulting from increased seasonal sporulation rates or those resulting from extreme weather events, such as thunderstorm-related asthma in *A alternata*–sensitized populations.[71] For example, outdoor fungal exposure is associated with increased asthma symptoms and exacerbation in an-inner city population.[66]

Thermotolerant filamentous fungi, such as *Aspergillus* and *Penicillium* species, can germinate and colonize the bronchial tree and regulate fungal sensitization and asthma severity.[24] Allergenic proteins have been identified in 23 fungal genera, although not all are considered major allergens, such as Alt a 1 of *A alternata*, Cla h 8 of *C herbarum*, and Asp f 1 from *A fumigatus.*[18] Fungal sensitization has been reported in up to 80% of asthmatic patients, although research into fungal allergies has been compounded by high variability between their protein compositions.[18] In terms of asthma severity, it is thought that more than 6.5 million persons have severe asthma with fungal sensitization and up to 50% of adult asthmatic patients attending secondary care have fungal sensitization.[72] This is likely influenced by a high aeroallergen load,[73] which can have opposing health effects.[74] 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,[75] along with clearly defined outcomes for developing asthma (long-term) or exacerbation (short-term) and epidemiologic techniques to investigate the cause 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 in Table E6 in this article's Online Repository at www.jacionline.org. 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 exacerbation. The included studies had relatively small sample sizes, resulting in 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-high heterogeneity and were of medium quality, meaning that our findings might include reporting bias. We were unable to conduct further analyses to explore potential bias associated with the heterogeneity between studies because of the small number of included studies.

The majority of the included studies used cross-sectional or case-control study designs, which reduces our confidence in these results because bias can be introduced as a result of incorrect estimation of exposures and failure to account for confounders, as evidenced by the decrease in the strength of the relationship between moisture-related risk factors and asthma in longitudinal analyses.[76] We identified a limited number of longitudinal study designs, which restricted our meta-analysis to assess the role of fungal exposures taken from a single sample on the exacerbation of asthma symptoms. Few studies quantified indoor fungal contamination defined to the species level by using molecular techniques, which restricted analyses to the fungal genera and potentially underestimate exposures. Potential covariates were not consistently assessed, and studies did not account for the effect of increased dampness and relative humidity on concentrations of house dust mites or volatile organic compounds or the seasonality of outdoor air spore composition. It is also not clear how fungal exposures and risk of asthma exacerbation might be modified by different occupant behaviors, such as heating and ventilation patterns, which have been shown to modify the indoor fungal profile, and this adds another layer of complexity in assessing indoor exposures.

Longitudinal studies assessing increased exposure to indoor fungi before the development of asthma symptoms suggests that species of *Penicillium*, *Aspergillus*, and *Cladosporium* pose a respiratory health risk in susceptible populations. Increased exacerbation of current asthma symptoms in children and adults were associated with increased levels of *Penicillium*, *Aspergillus*, *Cladosporium*, and *Alternaria* species, although further work should consider the role of fungal diversity and increased exposure to other fungal species. Adoption of a holistic approach to the complex disease of asthma in atopic and nonatopic 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 because it will be expensive to achieve at the population level. We recommend that future studies should consider the adoption of a multidisciplinary approach using both molecular and epidemiologic tools to accurately estimate the extent and timing of exposures and reliably assess potential health effects.

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Key message

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

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