Received: 18 November 2016 | Accepted: 30 July 2017

DOI: 10.1111/ina.12413

ORIGINAL ARTICLE

WILEY

Indoor visible mold and mold odor are associated with new-onset childhood wheeze in a dose-dependent manner

Caroline Shorter[1] | Julian Crane[1] | Nevil Pierse[2] | Phillipa Barnes[1] | Janice Kang[1] | Kristin Wickens[1] | Jeroen Douwes[3] | Thorsten Stanley[4] | Martin Täubel[5] | Anne Hyvärinen[5] | Philippa Howden-Chapman[2] | the Wellington Region General Practitioner Research Network

[1]Wellington Asthma Research Group, Department of Medicine, University of Otago, Wellington, New Zealand

[2]He Kainga Oranga/Housing and Health Research Group, Department of Public Health, University of Otago, Wellington, New Zealand

[3]Centre for Public Health Research, Massey University, Wellington, New Zealand

[4]Department of Paediatrics and Child Health, University of Otago, Wellington, New Zealand

[5]Department of Health Protection, National Institute for Health and Welfare (THL), Kuopio, Finland

Correspondence
Caroline Shorter, Department of Medicine, University of Otago Wellington, Wellington South, New Zealand.
Email: caroline.shorter@otago.ac.nz

Funding information
Health Research Council of New Zealand, Grant/Award Number: HRC 09/071B; Suomen Akatemia, Grant/Award Number: 296587; Asthma and Respiratory Foundation of New Zealand

Abstract

Evidence is accumulating that indoor dampness and mold are associated with the development of asthma. The underlying mechanisms remain unknown. New Zealand has high rates of both asthma and indoor mold and is ideally placed to investigate this. We conducted an incident case-control study involving 150 children with new-onset wheeze, aged between 1 and 7 years, each matched to two control children with no history of wheezing. Each participant's home was assessed for moisture damage, condensation, and mold growth by researchers, an independent building assessor and parents. Repeated measures of temperature and humidity were made, and electrostatic dust cloths were used to collect airborne microbes. Cloths were analyzed using qPCR. Children were skin prick tested for aeroallergens to establish atopy. Strong positive associations were found between observations of visible mold and new-onset wheezing in children (adjusted odds ratios ranged between 1.30 and 3.56; $P \leq .05$). Visible mold and mold odor were consistently associated with new-onset wheezing in a dose-dependent manner. Measurements of qPCR microbial levels, temperature, and humidity were not associated with new-onset wheezing. The association between mold and new-onset wheeze was not modified by atopic status, suggesting a non-allergic association.

KEYWORDS
asthma, children, dampness, housing, leaks, mold

1 | INTRODUCTION

Asthma is a common chronic non-infectious disease, which affects 300 million people worldwide and is responsible for 250 000 deaths each year.[1] Observations of indoor dampness and visible mold have been found to be strongly associated with poor respiratory health, including asthma exacerbations, in several international reports and meta-analyses.[2–5]

Evidence is also accumulating that indoor dampness and mold may be associated with the *development* of asthma[5] as shown in several birth cohort studies using self-reported dampness and mold. An incident case-control study from Finland involving independent home inspections provides the strongest evidence to date; however, this study did not include objective measures of mold or record indoor temperature and humidity.[6]

The underlying mechanisms explaining the associations between dampness and mold and adverse health effects remain unknown, but

See Appendix 1 for the Wellington Region General Practitioner Research Network.

a causal role for fungal fragments, spores, cell wall components, volatile organic compounds, and secondary microbial metabolites such as mycotoxins has been suggested.[7] There are many methods for measuring mold, each with advantages and limitations, but currently, there is no established standard method of quantifying exposure to mold suitable for use in epidemiological studies.[8] Most studies therefore rely on self-reports of mold by questionnaire, which may be subject to bias.[9,10] Advances in molecular technology have allowed the analysis of measures of fungal DNA, and there have been calls for increased use of fungi as a marker for indoor dampness using these methods.[11]

New Zealand is ideally placed to study the associations between wheezy illness and the domestic environment, with high rates of childhood asthma (25%), and poor-quality housing[12] comprising low rates of insulation and high rates of reported mold.[13]

In this study, we investigate the relationships between parental, researcher and independent building assessor reports of dampness, mold and water damage, temperature and humidity measures, qPCR levels of fungi and bacteria collected on electrostatic dust cloths (EDCs), and new-onset wheezing in children.

Practical Implications

Observed dampness and mold were the best indicators of an increased likelihood of new-onset wheezing, whereas measuring temperature, humidity, and levels of mold in collected static dust were not good indicators. While observations of mold could be used to identify areas where remediation of dampness and mold is needed, it is important that researchers continue to seek the "missing link" between such observations and health effects, to better target remediation efforts, and to better understand the potential mechanisms behind these observed adverse health effects. Improving housing, with particular attention to mold removal, may lead to significant improvements in respiratory health for young children.

2 | METHODS

2.1 | Study design

A matched case-control study was conducted involving 150 case children with new-onset wheezing and 300 control children and a range of indoor dampness measures. The study protocol was approved by the central health and disabilities ethics committee (HDEC CEN-09-06-039).

2.2 | Study population

Children with new-onset wheezing were identified by general practitioners (GPs), parental referral from posters in medical facilities, or by electronically searching GP records in Wellington, New Zealand, between June 2010 and July 2012. The inclusion criteria for cases were children aged between 12 and 84 months, who had wheezed for the first time in the last 12 months, were prescribed inhaled bronchodilators by a doctor for wheezing for the first time in the last 12 months, and had taken this medication on at least one occasion. Matched control children were also identified from GP electronic records and selected on the basis of having had no previous medical history, or parental reports, of any wheezing or asthma and having never been prescribed bronchodilators.

Each eligible wheezing child was matched with two eligible control children of similar age (±6 months) to the wheezing child and of same gender and area of residence (to nearest city council). Children were required to have lived in their current home for at least 6 months prior to the onset of wheezing, or for control children, at least 6 months prior to study enrollment. For children who lived in more than one household, the house in which they spent the majority of their time was assessed.

One-third of people approached agreed to take part in the study (Figure 1). Of 311 potential cases identified either by GP record search or self-referral, 155 met our inclusion criteria and were enrolled, with 150 cases completing the study. There were 391 potential control children identified from GP records; of these, 305 were eligible and enrolled, and 300 completed the study—overall a 98% retention rate (Figure 1). Parents were informed that the study was to assess housing conditions in relation to new-onset wheezing, but were not specifically told it was a study investigating mold and dampness.

2.3 | Researcher assessments

Each home was visited by researchers between autumn and spring, with control children visited within ±2 weeks of the matched case child to minimize seasonal differences. A researcher trained in mycology assessed condensation, visible water damage/leaks, mold odor, and visible mold in seven locations in the child's bedroom: bedroom walls, ceilings, floors, windows, curtains, bedding, and wardrobe areas. A scale for mold extent (0-3) was developed, which categorized visible mold for each area: none, small, moderate, or large/extensive using a showcard (Figure 2). Mold odor in bedrooms was also categorized on a 0–3 scale of none, mild, moderate, and severe. To reduce subjectivity in mold odor detection, both researchers and building assessors were trained to identify mold odors using fungal air samples collected onto agar plates, including predominately *Cladosporium* and *Penicillium*, but also containing species of *Alternaria*, *Aspergillus*, and *Penicillium*. These mixed plates were incubated inside a sealed plastic bag for 30 days, and the presence of a damp musty odor was agreed upon by the researchers who assessed the homes. In the homes, researchers were asked to subjectively assess whether any mold odor identified was mild, moderate or severe in intensity.

2.4 | Parental assessments

The mold severity scale was also used for parents (Figure 2) to assess presence/absence and size of mold in children's bedrooms, and

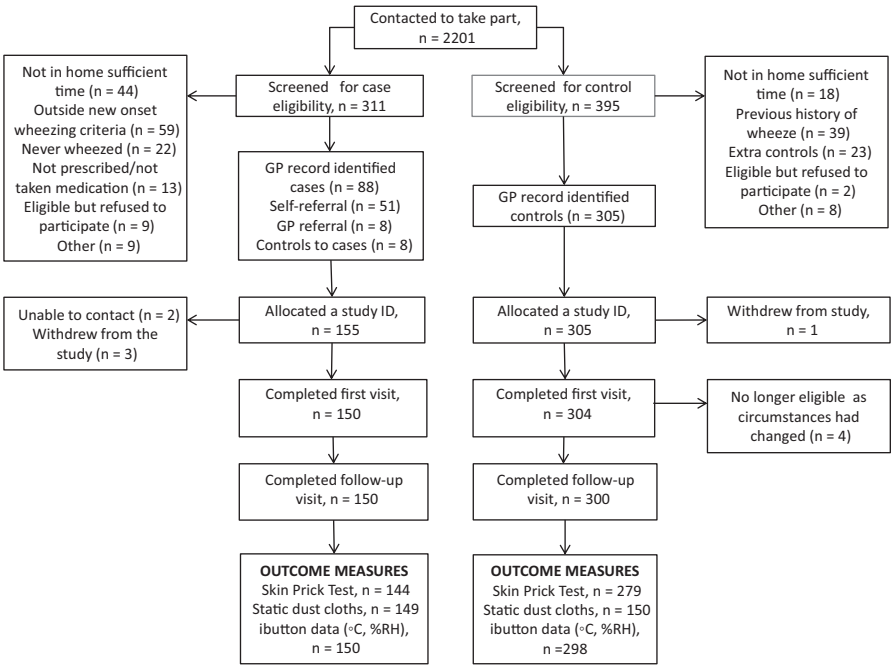

FIGURE 1 Flowchart of HOME study participant recruitment and retention

also mold in other rooms in the home. Parents/caregivers were asked about the child's health and household characteristics including moisture damage/leaks, condensation, mold odor, household smoking, heating, and ventilation practices. Additional questions were asked on potential confounding factors: age, family history of allergy and asthma, gestation, ethnicity, bed sharing, and income.

2.5 | Mold score

Researcher and parental mold observations and severity of mold in the children's bedroom were separately totaled across the seven locations in each child's bedroom to give a researcher and a parental mold score, with a minimum score of 0 (no mold) and a maximum of 21 (extensive areas of mold in all seven locations) possible.

2.6 | Home inspections

An independent building assessment was made by a trained building inspector, who was blinded to case status, within a month of the researcher's second visit. This involved a version of the healthy housing index survey,[14] expanded to include an examination of the rooms in the house for evidence of water damage, presence and size of visible mold (in square meters), and roof rafter moisture levels.

2.7 | Temperature and humidity assessment

Temperature and humidity were recorded every 10 minutes over a 4-week period using an i-button data logger (DS1923-F5; Maxim Integrated™, San Jose, CA, USA). Where possible, the building inspector also took repeated moisture measurements from two roof rafters using a protimeter mini (GE Sensing, EMEA, Billerica, MA, USA).

2.8 | qPCR and static cloth assessments of mold

Electrostatic dust cloths (Pledge Dust & Allergen Grab Its, 29.8 cm × 15 cm; SC Johnson, Racine, WI, USA) were placed in the bedrooms of 300 children enrolled in the study (including every case and one randomly matched control subject), using a shelf attached to the bedroom wall (1.5 m height ±0.2 m), and left in place for 4 weeks. The cloths (n = 299, 1 damaged and unanalyzed) were stored frozen until analysis and then extracted as described previously.[15] The total fungal DNA, *Penicillium* spp. + *Aspergillus* spp. + *Paecilomyces variotii*, *Cladosporium cladosporioides*, *Aspergillus versicolor*, and Gram-positive and Gram-negative bacteria levels were measured. Determination of Gram-negative bacteria was hampered by background DNA contamination in the magnetic beads of the DNA extraction kit, and so results are not presented.

2.9 | Atopic sensitization

Atopic sensitization was assessed using skin prick tests against a panel of aeroallergens using a standard protocol.[16] The panel included three common environmental allergens: house dust-mite (*Dermatophagoides pteronyssinus*), cat (Fel d I), and grass pollen mix; and four fungal allergens (*Alternaria alternata*, *Aspergillus* mix, *Penicillium* mix, *Cladosporium* mix) (Allergologisk Laboratorium A/S, Horsholm, Denmark).

2.10 | Statistical analysis

The four roof rafter moisture measurements taken by the building inspectors were averaged. Mean values of temperature (°C) and relative humidity (%) were calculated for each bedroom. Associations between mold scores and mean temperature, humidity, dew point, and absolute humidity measurements were assessed using generalized

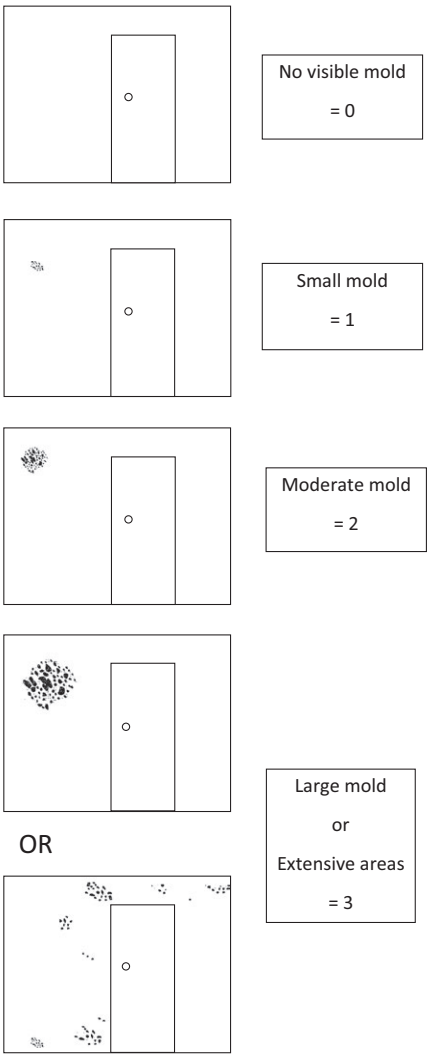

FIGURE 2 Mold severity scale—used by researchers and parents to identify and quantify mold in children's bedrooms

linear quasi-Poisson models to give ratios of means. The log-transformed qPCR microbial static cloth data were used to assess the association with mold score using Pearson's correlation test, and for the presence/absence of moisture damage using Wilcoxon signed ranked tests. The presence of moisture damage observed by inspectors was calculated for areas that the child spent most time in or traversed regularly: child's bedroom, family bathroom, kitchen, living room, and hallways. Other areas such as spare bedrooms, parents' bedroom, and laundry rooms were excluded from the primary analysis.

The associations between wheezing and mold and dampness observations were analyzed using conditional logistic regression models and expressed as odds ratios and 95% confidence intervals. Results for two models are given: model 1, the unadjusted model, and model 2, adjusted for potential confounders, including the matched variables of age and gender, were either significantly related to case status ($P \leq .05$) or changed the odds ratio for researcher mold score by 10% or more. Statistical analyses were performed using R 3.2.2 (www.R-project.org).[17]

3 | RESULTS

Wheezing children were more likely to have a family history of asthma and allergic disease, be of Māori ethnicity, and have a lower gestational age than control children (Table 1). Higher rates of maternal smoking were observed for wheezing children than non-wheezing children; however, this did not reach significance.

Visible mold and dampness were reported frequently in homes and bedrooms of children with new-onset wheezing (Table 2). Significantly elevated odds ratios were found for visible mold, mold odor, condensation, and leaks/water damage (Table 2, Model 1 unadjusted). Adjusting for potential confounders (Table 2, Model 2) increased the strength of most associations.

The strongest increases in the likelihood of new-onset wheezing were associated with the highest levels of the severity scales used to assess visible mold and mold odor in the children's bedroom. A one-unit increase in researcher reported mold score equated to a 1.46 fold increased odds of wheezing. Therefore, a child in a bedroom with a mold score of 7 had 14.1 times odds of wheezing compared to a child with a mold score of 0 (Figure 3). Researcher-assessed mold odor severity showed a similar dose-dependent response for new-onset wheezing with an OR 2.35 for every unit increase in the severity of perceived odor (Table 2, Model 2), with the highest severity of odor associated with 13 times increased the likelihood of new-onset wheeze. For the dichotomous variables, parent identified mold or condensation in the living areas of the home was associated with the greatest likelihood of new-onset wheezing (Table 2, Model 2, with OR of 5.0-5.5).

The majority of visible mold in children's bedrooms was detected on or around windows (present in 42% of bedrooms reported by parents and in 57% of bedrooms reported by researchers), followed by curtains (16%, 23%), walls (10%, 5%), ceilings (4%, 5%), with less observed in bedding, wardrobes, and other areas (occurring in less than 5% of bedrooms).

Differences between observers were found when reporting dampness and mold; visible mold was more frequently detected by parents and researchers than by building inspectors (Table 2). Researcher and parental mold scores for the children's bedrooms were moderately correlated, $r = .48$, as were researchers' and building inspector's observations, $r = .43$. However, building inspector reports of bedroom mold and parental mold score were only weakly correlated, $r = .29$, perhaps due to differences in observation timeframes or potential over-reporting by parents of wheezy children. Building inspector reports of mold in the child's bedroom were not significantly associated with risk of new-onset wheezing. However, when inspector observations of mold were combined between the child's bedroom, living room, and bathroom, to give a dichotomous variable of inspector identified visible mold presence in the house, this was associated with a risk of new-onset wheezing (Table 2, Model 2, OR 1.73). This relationship disappeared when the analysis was expanded to include observed mold in other less frequented areas of the home, such as the laundry and spare bedrooms. This was also the case for the relationship between building inspector identified leaks/water damage.

TABLE 1 Characteristics of study participants, their households, and homes

Variable	Case (wheezing), n = 150	Control (non-wheezing), n = 300	Odds ratios and 95% confidence intervals
Median age at first visit	32 mo	33 mo	0.92 (0.86-0.99)*
Gender	55% male	55% male	
Ethnicity NZ European	67.8%	77.2%	1.00
Ethnicity Māori	16.7%	9.4%	2.13 (1.16-3.91)
Ethnicity Pacific	4.0%	2.0%	2.57 (0.76-8.77)
Ethnicity other	12.0%	11.4%	1.18 (0.63-2.20)
Home ownership	81.2%	85.7%	1.06 (0.59-1.90)
Home occupancy (average people)	4.2	4.1	1.08 (0.89-1.30)
Bedroom sharing	37%	32%	1.32 (0.84-2.07)
Bed sharing	18.2%	19.5%	0.80 (0.47-1.37)
Maternal smoking in pregnancy	7.4%	3.4%	2.09 (0.84-5.12)
Household income before tax (NZ$ median)	$80 000-100 000	$80 000-100 000	1.01 (0.90-1.18)
House condition—self-reported			
Excellent	12.1%	18.9%	1.09 (0.81-1.48)[a]
Good	47.5%	43.4%	
Average	36.2%	34.9%	
Poor	3.5%	2.1%	
Very poor	0.7%	0.7%	
Roof insulation (average thickness x cover)	96.6 mm	97.5 mm	1.00 (0.99-1.01)
Gestation (wk)	39.0	39.6	0.88 (0.79-0.97)*
Family history of asthma, eczema, or hay fever	96.7%	84.7%	6.97 (2.56-19)*
Atopy (skin prick test positive)	44%	21.4%	3.25 (2.03-5.22)*

*$P \leq .05$.
[a]Per category change.

qPCR levels of mold and bacteria determined from the static dust cloths were not related to new-onset wheezing (Table 2), although all markers, except *Cladosporium cladosporioides*, were significantly positively correlated with building inspector measurements of mold area in square meters and researcher and parental mold scores, with correlations strongest for researcher mold score as we have previously shown.[15]

No difference in mean bedroom temperature, relative humidity, or roof rafter moisture levels was observed between wheezing children's and non-wheezing children's homes (Table 2). No significant effect was observed between dew point or absolute humidity and new-onset wheezing status (data not shown). Several of the observations of dampness and mold correlated with measured mean temperature, mean relative humidity, mean absolute humidity, and mean dew point (Table 3). The most significant and strongest correlations were for mean relative humidity, with absolute humidity and dew point showing weaker correlations, except for the correlations with mold odor and condensation (Table 3).

Wheezing children were significantly more likely to be atopic than control children (Table 1), with 44% of the wheezing children and 21.4% of the control children tested reacting to one or more of the environmental allergens; however, only 2 children were sensitized to the fungal allergens tested. Atopy was not associated with any of the dampness and mold observations ($P > .05$), either in wheezing children, non-wheezing children or when both groups were combined. When the association between researcher mold score and new-onset wheeze was stratified by atopy, the association was largely unchanged at 1.33 (1.08-1.64) amongst atopics, and 1.23 (1.07-1.42) amongst non-atopics.

4 | DISCUSSION

Strong associations were found between observations of visible mold, mold odor and leaks, and new-onset wheezing in children. Increased

TABLE 2 Prevalence and unadjusted/adjusted odds ratios for new-onset wheezing

Mold/dampness factor Identified in study child's bedroom or house	% Prevalence or mean		Model 1 OR	*P* value	Model 2 OR	*P* value
	Case	Control				
Visible mold present (bedroom)						
Researcher identified	71%	58%	1.73 (1.14-2.63)	<.01	2.24 (1.4-3.60)	<.001
Parent identified	66%	43%	1.80 (1.44-2.25)	<.001	1.88 (1.46-2.43)	<.001
Inspector identified	19%	16%	1.29 (0.77-2.16)	.328	1.60 (0.89-2.87)	.113
Visible mold present (house)						
Parent identified	96%	82%	4.89 (2.15-11.09)	<.001	5.52 (2.22-13.74)	<.001
Inspector identified	47%	37%	1.57 (1.03-2.38)	.036	1.73 (1.09-2.75)	.02
Visible mold score (mean score) (bedroom)						
Researcher identified	2.21	1.20	1.33 (1.18-1.50)	<.001	1.46 (1.26-1.69)	<.001
Parent identified	2.00	0.85	1.30 (1.16-1.46)	<.001	1.30 (1.15-1.48)	<.001
Visible mold, inspector (mean total area per house, m^2)	0.42	0.32	1.07 (0.92-1.26)	.375	1.12 (0.94-1.33)	.221
Mold odor						
Research identified (bedroom) severity scale 0–3: mean)	0.31	0.11	1.97 (1.34-2.91)	<.001	2.35 (1.46-3.76)	<.001
Parent identified (bedroom)	25%	10%	3.08 (1.77-5.38)	<.001	2.97 (1.63-5.42)	<.001
Parent identified (house)	54%	36%	2.25 (1.47-3.46)	<.001	2.36 (1.49-3.74)	<.001
Inspector identified (house)	22%	15%	1.61 (0.97-2.66)	.066	1.64 (0.94-2.86)	.083
Condensation						
Researcher identified (bedroom)	65%	49%	2.04 (1.33-3.12)	<.01	2.00 (1.26-3.15)	<.01
Parent identified (bedroom)	81%	67%	1.94 (1.20-3.14)	<.01	1.77 (1.07-2.96)	.026
Parent identified (house)	98%	88%	6.65 (2.01-22.04)	<.01	5.01 (1.5-17.27)	<.01
Leaks/water damage						
Researcher identified (bedroom, includes stains)	13%	7%	2.26 (1.14-4.47)	.02	2.33 (1.08-5.01)	.031
Parent identified (house, 12 mo)	49%	36%	1.82 (1.2-2.73)	<.01	1.81 (1.16-2.82)	<.01
Inspector identified (bedroom, living, bathroom, halls)	9%	3%	3.56 (1.46-8.67)	<.01	3.07 (1.18-7.97)	.021
qPCR Electrostatic dust cloth (median)						
Cladosporim cladosporioides	294	304	1.00 (0.81-1.23)	.987	1.01 (0.79-1.31)	.914
Penicillium/Aspergillus	31 360	28 150	0.99 (0.82-1.12)	.901	1.06 (0.85-1.31)	.606
Total fungi	51 470	48 820	0.96 (0.76-1.20)	.691	1.01 (0. 76-1.34)	.950
Gram-positive bacteria	1 968 000	2 393 000	0.96 (0.80-1.15)	.667	0.97 (0.76-1.24)	.821
Aspergillus versicolor (% prevalence)	8.7%	6%	1.50 (0.61-3.67)	.374	1.66 (0.58-4.78)	.349
Bedroom temperature (mean)	18.2°C	18.1°C	1.02 (0.92-1.15)	.695	1.03 (0.91-1.16)	.700
Bedroom relative humidity (mean)	65%	64.6%	1.01 (0.98-1.04)	.552	1.01 (0.98-1.04)	.628
Roof rafter moisture level (mean)	8%	7.7%	1.04 (0.90-1.21)	.579	1.08 (0.92-1.27)	.365

Model 1 – unadjusted, Model 2 – adjusted for age, family history of allergic disease and gestation.

levels of dampness and mold observed by researchers, parents, and an independent building assessor were associated with an elevated risk of new-onset wheezing. Researcher and parental-reported mold scores were associated with new-onset wheezing in a dose-dependent manner. We also observed a significant positive dose-response effect of researcher-assessed mold odor severity in the children's bedrooms, but the positive associations with mold odor of the whole house assessed by building inspector reports did not reach significance. While we attempted to train the building inspector and researchers in mold odor perception, we believe this is still widely open to subjectivity, and individual differences in odor perceptions have previously been documented.[10] However, given the strong association we found, further research to develop objective measurements of odor would be warranted. No associations were found between indoor

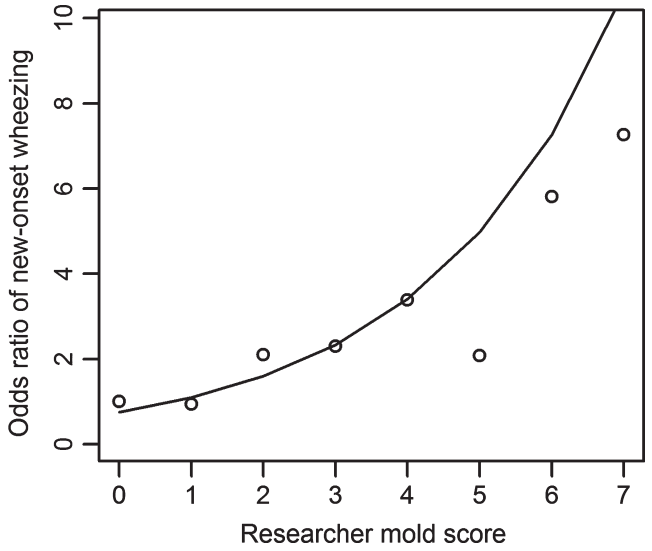

FIGURE 3 Plot of researcher mold score (adjusted) and odds ratio of new-onset wheezing. Line indicates modeled relationship (Model 2)

climate (average temperature, relative humidity, absolute humidity, dew point, or building moisture content) and new-onset wheezing in children, suggesting the mold itself may be an important mediator of new-onset wheezing.

Our results are consistent with the findings from one other incident case-control study[6] that mold and water damage are associated with new-onset asthma, as well as meta-analyses of associations of indoor dampness and mold with asthma, with similar odds ratios.[4,18] This raises the issue as to what extent early childhood wheezing might be explained by housing conditions and therefore how much could be prevented by remediation. We also confirmed previous findings[6] that the location of moisture damage and visible mold in the home is important, with the spaces most occupied by the child in a home associated with a greater risk of wheezing in children.

Worldwide prevalence of indoor mold reportedly occurs in 5%-10% of homes in cold climates, and in 10%-30% of homes in temperate or warm climates,[18] although a prevalence of up to 47% has been reported for U.S. homes.[19] The prevalence of visible mold in the current study was high, with the lowest reports provided by the building assessor in 37% and 47% of control and case homes, respectively, and the highest reports provided by parents of wheezing children, in 96% of homes. Interestingly, researcher and parental observations of mold were more frequent in this study than in previous NZ surveys; almost twice that reported (35.1%) by occupants in a telephone survey.[13] It may be that the mold severity visual scale (Figure 2) helped to jog the memory of participants, or a telephone interview may have elicited different responses than a face-to-face interview in the home, as performed in this study.

While researcher and parental prevalence of observed mold were similar in this study, the building inspector-observed prevalence was lower. This may reflect not using the same visual showcard for the building inspector assessments. The visual scale applied as a tool by the parents and researchers required every minute amount of mold to be recorded, whereas it is possible that there was some threshold below which the building inspector did not measure or identify visible mold, when measuring in square meters. It is also possible that parental and researcher observations might have positively biased findings if mold were more likely to be reported in homes of wheezy children.

The majority of mold observed by researchers and parents was located on and around windows, which could be due to the high levels of single glazing found in New Zealand homes, and our high winter indoor humidity, which increase levels of condensation.[20,21] Parental reports of condensation were particularly high for both wheezing and control children, with around 90% of all homes experiencing some condensation. This essentially delivers a daily supply of water to fungi on building surfaces throughout many months of the year and could explain why high levels of window mold were observed. If conditions are damp around windows, then curtains, which are highly permeable, will absorb moisture which creates another substrate on which fungi are readily able to grow, particularly if there is a lack of solar radiation present to dry the windows or curtains through the daytime. Less mold was observed on curtains than windows in this study, which would indicate that a certain threshold level of dampness or condensation on the windows may need to occur before conditions are favorable for mold growth on curtains.

There were no associations of visible mold or water leaks with atopy. Only two children tested positive to mold allergens, suggesting that mold allergy does not explain the association between mold and wheezing. Alternatively, the mold allergens chosen in our study might not reflect the specific allergen exposure experienced by the study population.

While atopic sensitization to aeroallergens is well recognized as a risk factor for the subsequent development of recurrent wheeze (and asthma),[22] mold in the home appears to be associated with the development of wheeze in children independent of their atopic status.

A question raised by the current study is whether visible mold observations alone are sufficient to measure fungal exposure in homes. Many of the mold observations predicted wheezing status. While correlated with several mold and moisture damage observations objective fungal and bacterial measurements using qPCR were not found to be related to wheezing. In our study, only a limited number of fungal and bacterial species or groups were targeted using qPCR, and it is possible that one or more health relevant mold taxa were missed. This is an area that requires further research, as studies do not routinely collect objective measurements of fungi alongside reports of observations. Moreover, while most current DNA targets in qPCR are based on knowledge from cultivation studies, future work using next generation sequencing of fungal ITS and bacterial 16S amplicons will allow the identification of relevant targets based on DNA signatures in sample materials. Such efforts are currently under way in the herein described study population. As in our study, other researchers have found markers such as mold odor to be related to other independent fungal markers; Roussel et al[23] found increased airborne levels of viable *Cladosporium*, *Penicillium*, and *Aspergillus* associated with mold odor; Reponen et al[24] found that odor was associated with increased levels

TABLE 3 Correlations (Rho ρ values) between observed dampness and mold and measured temperature and humidity

Measure	Mean temperature	Mean relative humidity	Mean absolute humidity	Mean dew point
Visible mold bedroom				
Researcher identified	**−0.08**	**0.14**	0.06	0.06
Parent identified	−0.05	**0.11**	0.06	0.06
Inspector identified	**−0.18**	**0.20**	0.03	0.03
Visible mold house				
Parent identified	−0.06	**0.09**	0.03	0.03
Inspector identified	**−0.11**	**0.16**	0.05	0.05
Visible mold score bedroom				
Researcher identified	**−0.11**	**0.19**	**0.08**	**0.08**
Parent identified	−0.05	**0.12**	0.06	0.06
Visible mold in square meters				
Inspector identified	**−0.11**	**0.16**	0.06	0.06
Mold odor				
Researcher identified (bedroom)	−0.07	**0.16**	**0.08**	**0.08**
Parent identified (bedroom)	**−0.08**	**0.23**	**0.16**	**0.16**
Parent identified (house)	−0.03	**0.11**	**0.09**	**0.09**
Inspector identified (house)	−0.011	**0.15**	**0.16**	**0.16**
Condensation				
Researcher identified bedroom	0.00	**0.10**	**0.10**	**0.10**
Parent identified bedroom	−0.04	**0.15**	**0.11**	**0.12**
Parent identified house	**−0.08**	**0.13**	0.07	0.07
Leaks/water damage				
Researcher identified (current, bedroom)	**−0.16**	**0.14**	0.01	0.01
Parent identified (house, 12 mo)	0.02	−0.01	0.03	0.02
Inspector identified (current, includes bedroom, living room, bathroom, hallways)	−0.03	0.06	0.06	0.06

Significant results in bold ($P \leq .05$).

of endotoxin, beta glucans, and qPCR levels of mold. While visible observations of mold and dampness are valuable and could be used to identify sites as a focus for remediation, we believe it is important researchers continue to seek the "missing link" between such observations and health effects, to better target our remediation efforts, and to better understand the mechanisms behind these apparent health effects. Identifying objective non-visual markers is particularly important for determining exposure to "hidden mold" where visible observations fail to indicate indoor mold and dampness problems such as in building cavities.

There are several limitations to our study. Recall bias may have occurred with parents of children who have recently started wheezing being more likely to report mold. Reporting bias was also possible as parents and researchers were not blinded to the case-control status of the child. To minimize these biases, we looked at data collected from three independent observers (parents, researchers, building assessors), each of whom independently showed positive associations between observed dampness measures and new-onset wheezing. Additionally, the objective measures of mean temperature, mean humidity, building assessments of mold, and qPCR levels of airborne fungi in the children's bedrooms were all found to be significantly correlated with researcher mold scores, suggesting that researcher mold assessments were not particularly biased. Selection bias could have occurred as parents of children with asthma who had poor

housing may have been more likely to agree to take part in the study than parents of children with asthma living in good-quality housing. However, there is little evidence of this bias as there was no difference in measures of socio-economic status or income between cases and controls.

The current study did not attempt to examine children with doctor-diagnosed asthma, but instead focussed on those with new-onset wheezing. As asthma is difficult to diagnose in young children, increasing numbers of GPs are deferring a diagnosis of asthma until children are older. If we had waited for a doctor diagnosis of asthma, we would therefore not have been able to assess the effects of early mold exposures. As a result, this study captured not just those that will go on to develop asthma, but all those who "only" had transient wheezing. A follow-up study may be warranted to determine whether the long-term effects of early mold exposure persist, as some studies have reported.[25,26]

5 | CONCLUSIONS

Strong associations were found between visible mold, mold odor, or leaks in the home and new-onset wheezing in children when observed by parents, researchers, and an independent building inspector. Visible mold and mold odor were associated with new-onset wheezing in a dose-dependent manner, with the strongest mold odor and highest levels of mold associated with 13-14 times increased odds of new-onset wheezing over those with no mold odor or mold. No associations were found between being atopic and having high levels of mold or dampness suggesting the positive relationship between increased mold exposure and wheeze may be due to different mechanisms not operating through an allergic association. Objective measurements of qPCR microbial levels, temperature, and humidity were not associated with new-onset wheezing, so the mechanisms by which dampness and mold conditions are associated with early childhood wheeze remain to be elucidated.

ACKNOWLEDGEMENTS

The authors wish to thank the Health Research Council of New Zealand for funding the research (Grant number HRC 09/071B) and the Asthma and Respiratory Foundation of New Zealand and the Academy of Finland (Grant number 296587) for additional funding support for the qPCR fungal testing. The authors are indebted to the parents and children who participated in the study and the general practitioners who helped identify potential children for the study.

REFERENCES

1. Strina A, Barreto ML, Cooper PJ, et al. Risk factors for non-atopic asthma/wheeze in children and adolescents: a systematic review. *Emerg Themes Epidemiol*. 2014;11:1.
2. Clark NM, Ammann HM, Brunekreef B, et al., eds. *Damp Indoor Spaces and Health*. Washington, DC: The National Academies Press; 2004.
3. Fisk WJ, Eliseeva EA, Mendell MJ. Association of residential dampness and mold with respiratory tract infections and bronchitis: a meta-analysis. *Environ Health*. 2010;9:1.
4. Fisk WJ, Lei-Gomez Q, Mendell MJ. Meta-analyses of the associations of respiratory health effects with dampness and mold in homes. *Indoor Air*. 2007;17:284-296.
5. Mendell MJ, Mirer AG, Cheung K, et al. Respiratory and allergic health effects of dampness, mold, and dampness-related agents: a review of the epidemiologic evidence. *Environ Health Perspect*. 2011;119:748.
6. Pekkanen J, Hyvärinen A, Haverinen-Shaughnessy U, et al. Moisture damage and childhood asthma: a population-based incident case–control study. *Eur Respir J*. 2007;29:509-515.
7. Kirjavainen P, Täubel M, Karvonen A, et al. Microbial secondary metabolites in homes in association with moisture damage and asthma. *Indoor Air*. 2016;26:448-456.
8. Holme J, Hägerhed-Engman L, Mattsson J, et al. Culturable mold in indoor air and its association with moisture-related problems and asthma and allergy among Swedish children. *Indoor Air*. 2010;20:329-340.
9. Strachan D. Damp housing and childhood asthma: validation of reporting of symptoms. *BMJ*. 1988;297:1223-1226.
10. Hägerhed-Engman L, Bornehag C-G, Sundell J. How valid are parents' questionnaire responses regarding building characteristics, mouldy odour, and signs of moisture problems in Swedish homes? *Scand J Public Health*. 2007;35:125-132.
11. Norbäck D, Cai GH. Dampness, indoor mould, fungal DNA and respiratory health–molecular methods in indoor epidemiology. *Clin Exp Allergy*. 2015;45:840-843.
12. Isaacs N, Donn M. Health and housing—seasonality in New Zealand mortality. *Aust J Public Health*. 1993;17:68-70.
13. Howden-Chapman P, Saville-Smith K, Crane J, et al. Risk factors for mold in housing: a national survey. *Indoor Air*. 2005;15:469-476.
14. Keall M, Baker M, Howden-Chapman P, et al. *Healthy Housing Index Pilot Study Final Report*. Wellington: University of Otago, Department of public health; 2007.
15. Shorter C, Täubel M, Pierse N, et al. Objective assessment of domestic mold contamination using quantitative PCR. *J Allergy Clin Immunol*. 2016;137:622-624.
16. Wickens K, Black PN, Stanley TV, et al. A differential effect of 2 probiotics in the prevention of eczema and atopy: a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol*. 2008;122:788-794.
17. Team RC. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing; 2014.
18. Quansah R, Jaakkola MS, Hugg TT, et al. Residential dampness and molds and the risk of developing asthma: a systematic review and meta-analysis. *PLoS One*. 2012;7:e47526.
19. Mudarri D, Fisk WJ. Public health and economic impact of dampness and mold. *Indoor Air*. 2007;17:226-235.
20. Becker R, Jaegermann C. The influence of the permeability of materials and absorption on condensation in dwellings. *Build Environ*. 1982;17:125-134.
21. Mithraratne N, Vale B. Life cycle analysis model for New Zealand houses. *Build Environ*. 2004;39:483-492.
22. Sears M. Risk factors: immunoglobulin E and atopy. *Asthma*. 1997;1:71-79.
23. Roussel S, Reboux G, Bellanger A-P, et al. Characteristics of dwellings contaminated by moulds. *J Environ Monit*. 2008;10:724-729.
24. Reponen T, Singh U, Schaffer C, et al. Visually observed mold and moldy odor versus quantitatively measured microbial exposure in homes. *Sci Total Environ*. 2010;408:5565-5574.
25. Reponen T, Vesper S, Levin L, et al. High environmental relative moldiness index during infancy as a predictor of asthma at 7 years of age. *Ann Allergy Asthma Immunol*. 2011;107:120-126.
26. Karvonen AM, Hyvärinen A, Korppi M, et al. Moisture damage and asthma: a birth cohort study. *Pediatrics*. 2015;135:e598-e606. peds. 2014-1239.

How to cite this article: Shorter C, Crane J, Pierse N, et al. Indoor visible mold and mold odor are associated with new-onset childhood wheeze in a dose-dependent manner. *Indoor Air*. 2017;00:1-10. https://doi.org/10.1111/ina.12413

APPENDIX 1

Wellington Region General Practitioner Research Network, New Zealand

Dr Debbie Chitty; Brooklyn Central Health, Dr Denis Delany; Ngaio Medical Centre, Dr Theodore Dorfling, Dr Rod Ferguson; Linden Surgery, Dr Kevin Fitzsimons; Tawa Medical Centre, Dr Martin Harris; The Terrace Medical Centre, Dr Tony Jackson; Newlands Medical Centre, Dr Tim Jefferies; Onslow Medical Centre, Dr Lise Kljakovic; Upper Hutt Medical Centre, Dr Chris Masters; Ropata Medical Centre, Dr Richard Medlicott; Island Bay Medical Centre, Dr David Robinson; Whitby Doctors, Dr Penny Rowley; Khandallah Medical Centre, Dr Jill Shepherd; Newtown Medical Centre, Dr Tim Smith; Paraparaumu Medical Centre, Dr Philip Wong; Hataitai Medical Centre, Dr Christopher Wright; Naenae Medical Centre.