ORIGINAL ARTICLE Epidemiology of Allergic Disease

Dampness and moulds in relation to respiratory and allergic symptoms in children: results from Phase Two of the International Study of Asthma and Allergies in Childhood (ISAAC Phase Two)

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Clinical & Experimental Allergy

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Cite this as: G. Weinmayr, U. Gehring, J. Genuneit, G. Büchele, A. Kleiner, R. Siebers, K. Wickens, J. Crane, B. Brunekreef, D. P. Strachan and the ISAAC Phase Two Study Group, *Clinical & Experimental Allergy*, 2013 (43) 762–774.

Summary

Background Many studies report that damp housing conditions are associated with respiratory symptoms. Less is known about mechanisms and possible effect modifiers. Studies of dampness in relation to allergic sensitization and eczema are scarce.

Objective We study the influence of damp housing conditions world-wide on symptoms and objective outcomes.

Methods Cross-sectional studies of 8–12-year-old children in 20 countries used standardized methodology from Phase Two of the International Study of Asthma and Allergies in Childhood (ISAAC). Symptoms of asthma, rhinitis and eczema, plus residential exposure to dampness and moulds, were ascertained by parental questionnaires ($n = 46\ 051$). Skin examination, skin prick tests ($n = 26\ 967$) and hypertonic saline bronchial challenge ($n = 5713$) were performed. In subsamples stratified by wheeze ($n = 1175$), dust was sampled and analysed for house dust mite (HDM) allergens and endotoxin.

Results Current exposure to dampness was more common for wheezy children (pooled odds ratio 1.58, 95% CI 1.40–1.79) and was associated with greater symptom severity among wheezers, irrespective of atopy. A significant ($P < 0.01$) adverse effect of dampness was also seen for cough and phlegm, rhinitis and reported eczema, but not for examined eczema, nor bronchial hyperresponsiveness. HDM sensitization was more common in damp homes (OR 1.16, 1.03–1.32). HDM-allergen levels were higher in damp homes and were positively associated with HDM-sensitization, but not wheeze.

Conclusion A consistent association of dampness with respiratory and other symptoms was found in both affluent and non-affluent countries, among both atopic and non-atopic children. HDM exposure and sensitization may contribute, but the link seems to be related principally to non-atopic mechanisms.

Keywords asthma, atopy, dampness, house dust mite, ISAAC, moulds, respiratory and allergic symptoms, wheeze

Submitted 26 September 2012; revised 21 January 2013; accepted 24 January 2013

Introduction

Dampness and mould growth in the home have been shown to be associated with wheeze and asthma in many geographical settings [1]. Results from non-affluent countries are scarce and results are inconsistent.

Positive associations have been found in Nigeria [2] and Kenya [3], but there was no association in a South African study [4]. The most recent comprehensive review [1] rated the evidence as 'sufficient for association and strongly suggestive of causality' only for asthma exacerbation, and as 'sufficient for association' for numerous other outcomes, including wheeze, cough and allergic rhinitis.

*The Phase Two Study Group is listed in Appendix.

The principal mechanisms by which damp housing could cause or exacerbate asthma have usually been considered to relate to indoor moulds and house dust mites. Many species of indoor moulds, including their fragments and spores, possess allergenic proteins (e.g. [5–7]), and a positive association of sensitization to moulds with both building dampness and current asthma was reported from Sweden [8]. A Dutch study found that the observed effect of dampness on respiratory symptoms was mainly mediated by sensitization to house dust mites (HDM), noting that sensitization to HDM was much more common than sensitization to moulds [9]. A study from Germany found an increase in BHR persistence with increasing HDM-allergen levels and a significant correlation between HDM allergens and dampness [10].

Alternatively, non-allergic mechanisms related to dampness and/or moulds include inflammatory reactions to volatile organic compounds [11, 12] or to cell wall components such as $1,3-\beta-D$-glucan [13–15]. Few studies have specifically compared the effects of dampness between atopic and non-atopic individuals, but reviews have concluded that the association of dampness with respiratory symptoms can be observed in non-atopic as well as atopic individuals [1, 16]. Even fewer studies have looked at potential other effect modifiers.

In this article, we report on the association with damp housing conditions and/or visible moulds in an international study investigating the symptom prevalence of asthma, rhinitis and eczema based on the results from 28 centres world-wide differentiating also between atopic and non-atopic individuals. In addition, the sample size in this international study is large enough to investigate in detail potential effect modification by family history of allergic disease symptoms and living conditions such as parental smoking, presence of carpets, type of bedding. Dust samples collected in a subsample allowed us to specifically investigate whether house dust mite allergen levels were higher in damp homes, and whether HDM levels were related to a higher occurrence of wheeze.

Methods

Study populations and field work

The methods of ISAAC Phase Two have been described in detail elsewhere [17, 18]. Briefly, random samples of at least 10 schools from defined geographical areas were chosen and children ($n > 1,000$ per centre) attending classes with a majority of 9–11 year olds were invited to participate. Standardized parental questionnaires were used. In two countries (Brazil and India), the questions were posed by trained interviewers because illiteracy was common.

The ISAAC Phase Two methodology allowed objective measurements to be performed either in the full sample (option A) or in stratified random subsamples of children (option B) [17]. Most centres invited all children to participate in the skin prick testing, while bronchial hyperresponsiveness tests and house dust sampling were carried out mostly in stratified random subsamples of children with and without reports of wheeze in the past year (targeting 100 per centre in each stratum).

All centres obtained approval by local ethics committees and investigators were trained in one location to assure comparable data quality [17]. Fuller details of the skin examination, bronchial responsiveness and skin prick tests to six aeroallergens (*Dermatophagoides pteronyssinus*, *D. farinae*, cat dander, *Alternaria tenuis*, mixed tree pollen and mixed grass pollen) have been published elsewhere [19–21] and can be found at http://isaac.auckland.ac.nz/phases/phasetwo/phasetwo.html and in [22].

Questionnaire data

Standardized parental questionnaires including detailed questions on the occurrence and severity of symptoms of asthma (wheeze), rhinitis (with and without conjunctivitis) and flexural eczema were administered. These were identical to those used in ISAAC Phase One for parents of children aged 6–7 years [17, 23]. In addition, in many (but not all) centres, questions about cough and phlegm were asked (http://isaac.auckland.ac.nz/phases/phasetwo/phasetwo.html, and Online Repository).

Exposure to dampness and moulds was assessed by the following questions: 'Does or did the child's home have damp spots on the walls or ceiling? At present? During the child's first year of life?' and 'Does or did the child's home have visible moulds or fungus on the walls or ceiling? At present? During the child's first year of life?' For most analyses presented here, the child was considered 'exposed' if damp spots and/or moulds were reported. In a few centres, there were minor deviations in the exact wording, see the Online Repository.

Dust sampling and laboratory analysis

Mattress dust samples were collected in eight centres in six countries and analysed for house dust mite allergen. These centres are as follows: Tirana (Albania), Dresden and Munich (Germany), Rome (Italy), Hawke's Bay (New Zealand), Linköping and Östersund (Sweden), and West Sussex (UK). In addition, in all but the German centres, living room floor dust samples were collected and analysed for endotoxin. In all centres, one child per household was included. As a result of insufficient numbers of children with a history of wheeze in some centres and as a result of non-response, in most centres

the aim of including 100 wheezers was not achieved. The actual number of participants for which house dust samples were analysed varied from 49 (Rome) to 231 (Hawke's Bay).

Dust samples were collected on filters according to a standardized protocol as described earlier [24, 25]. Laboratory analysis of dust samples from European centres took place at the Institute for Risk Assessment Sciences (Utrecht University, Utrecht, the Netherlands). Dust samples from New Zealand were analysed at the laboratory of the Wellington Asthma Research Group (Wellington School of Medicine and Health Sciences, Wellington, New Zealand).

After weighing, the whole dust sample including filter was extracted using Tween-20 and water and then analysed for house dust mite allergens *Dermatophagoides pteronyssinus* (*Der p 1*) and *Dermatophagoides farinae* (*Der f 1*) with enzyme immunoassays as described earlier [17]. Endotoxin concentrations were determined with a kinetic chromogenic Limulus amoebocyte lysate (LAL) test as described previously [26, 27].

Allergen and endotoxin levels were expressed per gram of dust. Samples with non-detectable amounts of house dust mite allergen or endotoxin were assigned a value of two thirds of the lowest overall observed detectable value.

Statistical analysis

Prevalences and odds ratios (ORs) for health outcomes were calculated with the SURVEY-procedures of SAS (V9.2) using, where necessary, the appropriate weighting and variance estimation to account for stratified subsampling [28, 29]. The association of allergen and endotoxin levels with the mould exposure was estimated with linear regression based on log-transformed data (base10) and results are presented as the ratio of the geometric mean concentration in exposed children to that in non-exposed children. When modelling dichotomous outcomes in relation to exposure, separate logistic regression models were fitted for each centre using PROC SURVEYLOGISTIC and combined estimates of the odds ratio were derived using random effects meta-analysis [30]. For the analyses involving allergen and endotoxin levels, data for all respective centres were pooled, because of the lower number of children and centres for these analyses, which would make a random effects meta-analysis less reliable. To take account of the centre, adjustment terms for the individual centres were added.

Potential confounders were tested by including them one by one in the models fitted by centre and only those that resulted in a notable (10% or greater) change of the combined estimate were retained (Table S2 in the Online Repository). The potential confounders included sex, reported parental allergic disease, pets, use of any combustion fuels for heating or cooking, maternal smoking in pregnancy, anybody smoking in the child's home, older siblings, maternal education and bedroom sharing. Based on the change-in-parameter criterion, only parental allergic disease was retained in the fully adjusted model.

The influence of potential effect modifiers was investigated by performing stratified centre-specific analyses, calculating the combined effect for each stratum and evaluating the difference between strata-specific estimates. Due to small cell counts in some centres in specific strata, the number of centres contributing to the stratum-specific estimates may differ from the number of centres in the corresponding unstratified analyses.

Centres classified by the World Bank as 'high income countries' (i.e. GNI *per capita* per year in 2001 $\geq$ 9200 US \$) were combined in a group called 'affluent countries' and the remaining centres in a group called 'non-affluent countries' [31, 32].

Results

Table 1 presents, for each study centre, the prevalence of the principal exposure variable (current dampness and/or mould in the child's home) and the potential effect modifiers. An expanded version including health-related outcomes is included in the Online Repository (Table S1). The prevalence of homes with reported current damp spots and/or moulds varied widely, from 1.5% in Östersund to 48% in Tallinn. About half of the children who were currently exposed were reported also to have been exposed during the first year of life, but again, this proportion varied greatly between centres.

In the centres that had collected dust samples, the concentration of house dust mite allergens in sampled dust was lowest in Sweden (geometric mean of 46.1 and 61.5 ng/g) and highest in New Zealand (22582.5 ng/g). Endotoxin levels in sampled dust were more similar among centres with a minimum again in Sweden (geometric mean of 7032.6 endotoxin units (EU)/g in Linköping) and highest in Albania (32673.2 EU/g).

Association of dampness with wheeze prevalence and severity

The associations calculated for wheeze in the past year were similar in magnitude for current exposures to dampness alone, moulds alone, and both, and also for exposures in the first year and at present (Fig. 1). Therefore, to maximize the power of the analyses, we analysed the combination damp spots and/or moulds as the principal exposure, allowing the maximum number

Table 1. Characteristics of the study populations (prevalence (%) at present if not otherwise indicated)

Centres	N children[†]	Damp* or mould present	Modules available	Damp* or mould 1st year	Damp* or mould present AND damp* or mould present 1st year	HDM-sensitization	Geometric mean HDM allergens (ng/g dust)	Geometric mean endotoxin (EU/g dust)	Feather pillow	Feather bedding	Carpet	Only double sealed window	Air conditioning	ETS
Albania, Tirana	975	18.3	WH, RH, EC, ED, RF, SP, BR, SD	18	11.8	11.1	2629.4	32673.2	22	12.6	17.2	5	6.6	47.7
Brazil, Uruguaiana	1966	47.1	WH, RH, EC, RF, CP, SP	36.7	34.7	11.8	–		1.3	1.3	10	40.7	12.4	51.5
China, Beijing	4136	7.9[‡]	WH, RH, EC, ED, RF, SP	12.9	3.7	9	–	–	24.8	36.6	9.5	14.2	33.1	62.3
China, Guangzhou	3481	9.1[‡]	WH, RH, EC, ED, RF, SP	11.9	4.4	25.3			27.1	34.7	4.7	8.7	68.6	54.8
China, Hong Kong	2969	17.6[‡]	WH, RH, EC, ED, RF, SP	8.3	4.7	41.9			8.3	22.9	7.1	17.6	95.4	33.6
Estonia, Tallinn[‖]	290	47.6[¶]	WH, RH, EC, ED, RF, SP	–	–	5.1			–	–	–	–	–	–
France, Creteil	1310	17.1[¶]	WH, RH, EC, ED, RF	19.5	6.7	–			20.8	14.8	–	–	6.5	–
Georgia, Tbilisi	938	15.6	WH, RH, EC, ED, RF, CP, SP, BR	9.5	5.7	17.7			91.9	12.1	5.5	3.2	26.4	58.7
Germany, Dresden	2893	13.6[¶]	WH, RH, EC, ED, RF, SP, BR, SD	25.4	5.7	11.1	2777.6		57.4	43	81.7	55.7	–	32.7
Germany, Munich	3074	8.6[¶]	WH, RH, EC, ED, RF, SP, BR, SD	13.6	3.5	8.4	4556.6		58.1	41.6	73.2	52.1	–	42.2
Greece, Athens	977	11.3	WH, RH, EC, ED, RF, CP, SP, BR	13.9	5.2	3.8			29.6	14.6	51.9	41.4	43.8	58.5
Greece, Thessaloniki	1013	9.5	WH, RH, EC, ED, RF, CP, SP, BR	9	4.3	14.4			29.1	8.3	27.6	40.4	37.8	55.7
Iceland, Reykjavik	908	6.6	WH, RH, EC, ED, RF, CP, SP	7.9	1.8	3.2			40.9	39.8	7.9	85.6	–	30
India, Mumbai	1646	22.9	WH, RH, EC, ED, RF, CP, SP	22.6	21.7	4.2			3.5	2.2	4	4	1.2	19.8
Italy, Rome	1314	16.2	WH, RH, EC, ED, RF, CP, SP, BR, SD	15.2	7.9	17.5	2267.3	26282.3	15.4	61.7	1.9	47.9	8.3	49.1
Latvia, Riga	889	17.4	WH, RH, EC, ED, RF, CP, SP, BR	17.5	7	12.9			82.8	20.6	22	7.6	4.9	39.2
Netherlands, Utrecht	3480	24.5[¶]	WH, RH, EC, ED, RF, CP, SP, BR	–	–	21.9			–	–	54.2	–	–	56.3

(continued)

Table 1 (continued)

Centres	N children†	Damp* or mould present	Modules available	Damp* or mould 1st year	Damp* or mould present AND damp* or mould present 1st year	HDM-sensitization	Geometric mean HDM allergens (ng/g dust)	Geometric mean endotoxin (EU/g dust)	Feather pillow	Feather bedding	Carpet	Only double sealed window	Air conditioning	ETS
New Zealand, Hawkes Bay	1314	19.7	WH, RH, EC, ED, RF, SP, BR, SD	24.2	11.2	24.6	22582.5	15185.1	10.8	25.8	83.9	–	–	27.9
Norway, Tromso	3362	3.3	WH, RH, EC, ED, RF, CP, SP, BR	6.5	1	5	–	–	19.3	19.3	13.6	60.8	34.5	37.6
Palestine, Ramallah‖	270	21.6	WH, RH, EC, ED, RF, SP, BR	27.8	16.7	6.6	–	–	10.1	2.8	16.7	11.5	10.5	64.2
Spain, Almeria	1090	5.2	WH, RH, EC, ED, RF, CP, SP, BR	10.9	2.3	37.8	–	–	4.4	11.3	1.2	14.6	30.3	60.4
Spain, Cartagena	1376	8.8	WH, RH, EC, ED, RF, CP, SP, BR	14.8	4.2	19.2	–	–	4.7	10.7	1.9	14.3	20.5	58
Spain, Madrid	919	10	WH, RH, EC, ED, RF, CP, SP, BR	12.5	3.2	9.8	–	–	8.6	14.9	4.6	16.9	17.3	57
Spain, Valencia	1322	3.5	WH, RH, EC, ED, RF, CP, SP, BR	6.8	1.3	10.7	–	–	5.6	14.5	2.1	16.6	36.2	56.3
Sweden, Linkoping‖	193	5.8§	WH, RH, EC, ED, RF, CP, SP, BR, SD	–	–	1.8	61.5	7032.6	–	–	–	–	–	–
Sweden, Oestersund‖	267	1.5§	WH, RH, EC, ED, RF, CP, SP, BR, SD	–	–	1	46.1	12735.8	–	–	–	–	–	–
Turkey, Ankara	2868	7.7	WH, RH, EC, ED, RF, CP, SP, BR	14.9	4.5	7.9	–	–	9.5	1.2	13.1	6.2	4.7	63.1
UK, West Sussex	811	8.8	WH, RH, EC, ED, RF, SP, BR, SD	10.7	3.8	8.9	2611.2	17544.7	24.2	11.7	96.7	63.8	2.1	29

*Damp is defined as presence of damp spots and/or visible moulds in the child's home.
†Number of children with valid answers to the wheeze questionnaire and the questions on exposure to damp and mould.
‡Damp or mould variable only contains damp spots.
§Damp or mould variable only contains visible moulds.
¶Not the exact ISAAC wording was used; however, questions were equivalent (see online supplement for exact wordings).
‖Stratified subsamples.
WH, Questionnaire on wheezing; RH, Questionnaire on rhinitis; EC, Questionnaire on eczema; ED, Examination for flexural dermatitis; EU, Endotoxin Units; RF, Risk factor questionnaire; CP, Additional respiratory questionnaire: cough and phlegm; SP, Skin prick tests for atopy; BR, Bronchial responsiveness to hypertonic saline; SD, Dust sampling; HDM, House dust mite; ETS, Environmental tobacco smoke.
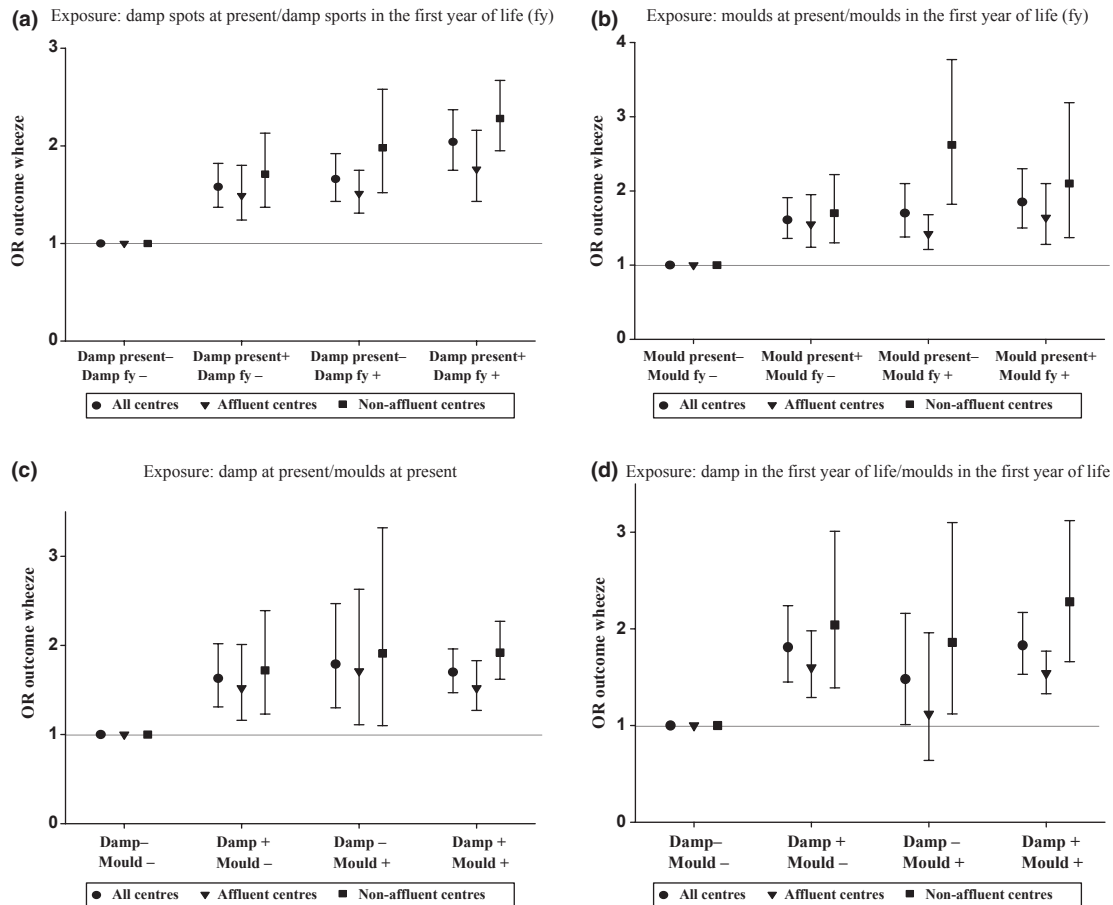
Fig. 1. (a–d) Association between wheeze and parent-reported indicators of damp housing conditions (combined estimates from random effects meta-analysis on all centres). (a) Exposure: damp spots at present/damp spots in the first year of life (fy). (b) Exposure: moulds at present/moulds in the first year of life (fy). (c) Exposure: damp at present/moulds at present. (d) Exposure: damp in the first year of life/moulds in the first year of life.

of children to be retained in the analysis. In this article, unless otherwise stated, we show results for current exposure, based on data for 46 051 children from 28 centres.

The combined odds ratio for wheeze in the past year in relation to dampness was 1.58 (95%-CI:1.40;1.79) and there was a significantly (*P* < 0.01) stronger association in non-affluent centres (Table 2). Association results for each centre individually are shown in a forest plot in the Online Repository (Fig. S1). There was no indication of effect modification by reported parental allergic disease, nor by skin prick test sensitization to any allergen, nor by sensitization to HDM. None of the indoor living conditions investigated seemed to influence markedly the observed association between current dampness and recent wheeze. This association was also robust to adjustment for potential confounding factors (Table 2).

Among wheezers, exposure to dampness increased the occurrence of severe wheeze with odds ratios of 1.16 (0.96;1.41) for having four or more wheezing attacks per week, 1.33 (1.08;1.63) for speech limiting

wheeze and 1.60 (1.13;2.25) for wheeze disturbing sleep. This held true for children that were exposed only at present [ORs of 1.17 (0.86;1.60), 2.21 (1.28;3.80) and 1.46 (1.03;2.07)], as well as for those only exposed in the first year of life [ORs of 1.36 (1.05;1.76), 1.49 (1.01;2.19) and 1.51 (1.12;2.04)].

Dust concentrations of mite allergen and endotoxin

Analysis of the house dust mite allergen and endotoxin levels for eight centres showed that the concentrations of house dust mite allergen in mattresses were higher in damp homes, whereas endotoxin concentrations in floor dust were not (Fig. 2). However, in this subsample, log_{10}-transformed HDM-allergen levels were not related to wheeze in the past year [OR 0.91 (0.75;1.10) per tenfold increase in allergen level], although there was a significant association between sensitization to house dust mites and log_{10}-transformed HDM-allergen levels [OR 1.54, (1.04;2.29) per tenfold increase in allergen level].The association of wheeze with dampness in this

Table 2. Association of dampness* with wheeze and effect modification: adjusted (for parental allergies) OR with 95%-CI unless otherwise indicated

	OR (95%-CI)	N (children)	N (centres)	P for difference btw strata
All children				
Crude OR	1.58 (1.40–1.79)	46 051	28	
Adjusted OR	1.54 (1.39–1.72)	45 761	27	
Crude in reduced adjustment data set	1.62 (1.44–1.82)	45 761	27	
Children in the following strata				
Affluent centres	1.39 (1.24–1.56)	28 592	18	
Non-affluent centres	1.80 (1.57–2.06)	17 169	9	0.005
Atopics	1.69 (1.42–2.02)	6633	25	
Non-atopics	1.54 (1.33–1.77)	19 786	26	0.403
HDM sensitized	1.68 (1.35–2.08)	3356	21	
Not HDM sensitized	1.47 (1.28–1.68)	22 974	26	0.303
Parental allergies[†]	1.51 (1.31–1.74)	15 956	27	
No parental allergies[†]	1.63 (1.41–1.89)	29 805	27	0.452
Parental asthma[†]	1.48 (1.13–1.93)	4205	25	
No parental asthma[†]	1.63 (1.46–1.82)	40 884	27	0.508
Feather bedding	2.17 (1.56–3.02)	4034	17	
No feather bedding	1.57 (1.37–1.80)	23 323	24	0.077
Feather pillow	1.82 (1.38–2.40)	5296	20	
No feather pillow	1.58 (1.37–1.82)	22 652	24	0.365
Carpet	1.41 (1.18–1.68)	11 141	17	
No carpet	1.62 (1.45–1.81)	31 274	23	0.187
Only double glazing windows	1.63 (1.30–2.05)	10 667	18	
Also other types of windows	1.72 (1.50–1.97)	27 077	21	0.694
Air conditioning	1.63 (1.29–2.06)	10 312	18	
No air conditioning	1.65 (1.46–1.87)	21 784	19	0.908
Environmental Tobacco Smoke (ETS)	1.49 (1.31–1.69)	20 552	24	
No ETS	1.65 (1.37–1.98)	22 588	24	0.377
Bedroom sharing	1.57 (1.40–1.77)	28 404	24	
No bedroom sharing	1.60 (1.35–1.90)	13 939	24	0.891

*Dampness defined as presence of damp spots and/or visible moulds in the child's home.
[†]Crude odds ratio.
HDM, House dust mite (*Dermatophagoides pteronyssinus* or *D. farinae*).

subsample with dust measurements was slightly weaker [OR 1.44 (0.94;2.21)] than in the full analysis with all centres, although this difference between the dust-centres and other centres was not statistically significant.

Association of dampness with other health-related outcomes

As there was no substantial confounding we report here crude odds ratios, which retain the maximum number of children in the analysis. Dampness was not associated with bronchial hyperresponsiveness, but was significantly associated with rhinitis symptoms and even more strongly with reported coughed up phlegm (Table 3).

Due to the reduced number of centres contributing data on cough and phlegm (18 centres, see Table 1), we report for comparison here the association of dampness with recent wheeze in these centres: the OR was 1.71 (1.47;1.99) in the children who had valid data for

'Coughed up phlegm without a cold' and 1.91 (1.51;2.41) in children with valid data for 'Coughed up phlegm frequently'. Thus, the association of dampness with respiratory symptoms in general was stronger in these centres.

The association of dampness with rhinitis symptoms was stronger for non-affluent countries (Table 3), particularly with rhinoconjunctivitis [OR 2.17 (1.91;2.48) in non-affluent countries and 1.37 (1.23;1.51) in affluent countries]. The association with rhinoconjunctivitis was stronger in children without reported parental allergic disease [OR 1.73 (1.49;2.00) vs. 1.40 (1.22;1.60)] or without positive skin prick results, especially among those not sensitized to pollen [1.91 (1.64;2.22) vs. 1.11 (0.83;1.49)]. There was no effect modification by sensitization to house dust mite (data not shown).

Although there was a significant positive association with reported eczema, there was no relation with examined eczema. This was not related to the selection of
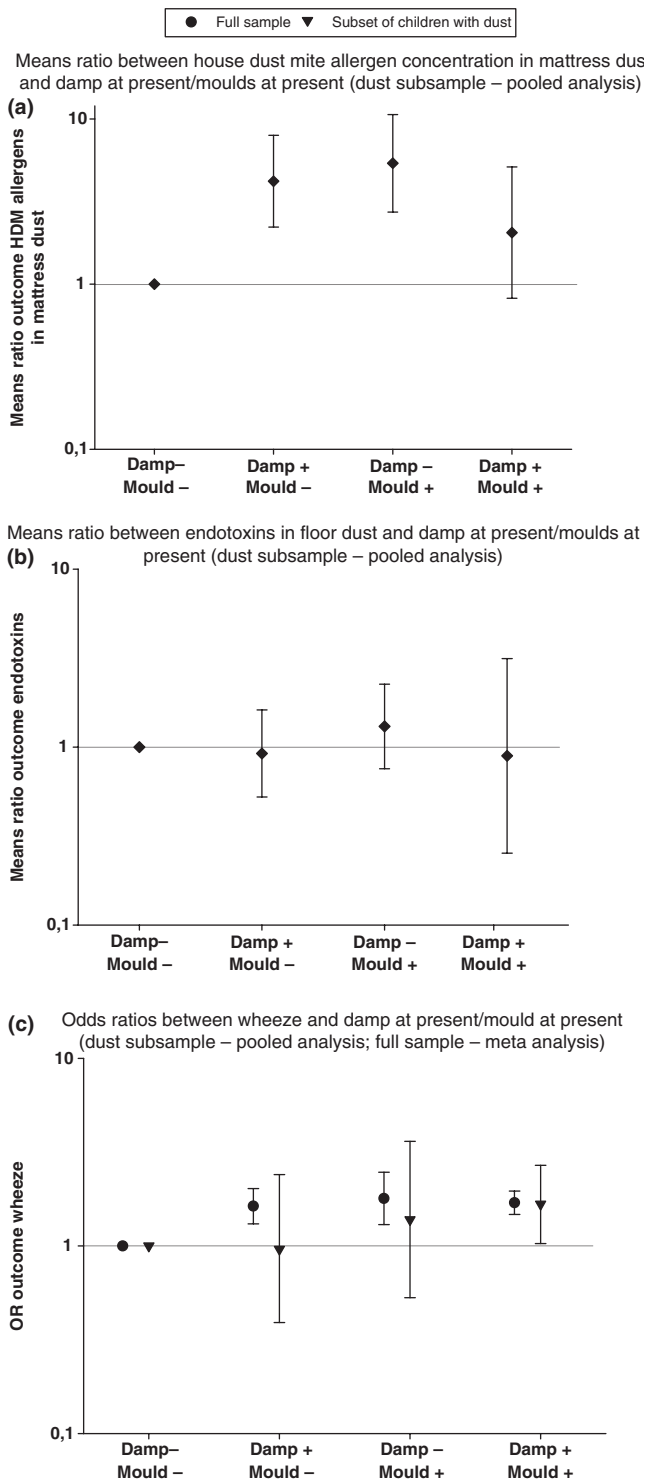
Fig. 2. (a–c) Associations of parent-reported indicators of damp housing conditions at present with house dust mite allergen concentration (a), endotoxin levels (b) and wheeze in the past year (c). (a) Means ratio between house dust mite allergen concentration in mattress dust and damp at present/moulds at present (dust subsample – pooled analysis). (b) Means ratio between endotoxins in floor dust and damp at present/moulds at present (dust subsample – pooled analysis). (c) Odds ratios between wheeze and damp at present/mould at present (dust subsample – pooled analysis; full sample – meta-analysis).

children with a skin exam, because the OR for reported eczema when limited to those that had a skin examination [1.50 (1.26;1.78)] was almost identical to the estimate based on the larger sample of children with data for reported eczema.

Although there was no association with overall sensitization, dampness was positively associated with sensitization to house dust mite, but only in affluent centres (Table 3). When children, whose parents reported having made changes in bedding and carpets due to allergies or asthma were omitted, the results did not change (data not shown).

Discussion

Our results confirm that dampness is a potentially modifiable risk factor for wheeze world-wide. In particular, our study that used the same methodology in all centres showed that the effect is even stronger in non-affluent than in affluent countries. There was an equally strong association with rhinitis symptoms and an even stronger one for cough with phlegm, whereas there was no association with bronchial hyperreactivity and only a weak association with house dust mite sensitization. Concentrations of house dust mite allergens were higher in damp homes and were associated with HDM-sensitization, but this did not account for the observed association between dampness and wheeze, which was found among both atopic and non-atopic children. Our results stress the importance of non-atopic processes, which is of considerable importance given that only relatively small fractions of asthma, rhinitis and eczema symptoms are attributable to atopy in non-affluent centres and even in affluent countries non-atopic symptoms are frequent [18, 20, 33]. Our estimate of the odds ratio linking current dampness with recent wheeze (1.6) is very similar to that cited in reviews by Antova (2008) [34] [1.43 (1.36 to 1.49)] and Fisk (2007) [35] [1.53 (1.39–1.68)]. The fact that our result comes from a multi-centre study and is not subject to publication bias strengthens the evidence from previous meta-analyses based mainly on literature reviews [1, 34, 35].

Our odds ratio estimates for cough with phlegm (1.9) are higher than those reported for children in Fisk (2007) [35] and Antova (2008) [34] for cough (OR from 1.3 to 1.5). However, these meta-analyses included different definitions of cough and may therefore not be directly comparable with ours. Our estimates for rhinitis with and without conjunctivitis (1.3 to 1.6) are comparable to these two reports covering upper respiratory symptoms with odds ratios in the range from 1.3 to 1.7 [1, 34, 35]. Our OR for reported eczema of 1.5 is very similar to that from a previous British study in school-children that reported an OR of 1.4 [36].

Table 3. Association of dampness* with respiratory and allergic outcomes: crude OR with 95%-CI

	All centres			Affluent			Non-affluent		
	OR (95%-CI)	N	N (centres)	OR (95%-CI)	N	N (centres)	OR (95%-CI)	N	N (centres)
HDM-sensitization	1.16 (1.03–1.32)	26 560	25	1.25 (1.06–1.48)	17 367	15	1.01 (0.84–1.23)	9193	10
Positive skin prick test	0.99 (0.91–1.08)	26 967	27	0.99 (0.89–1.10)	17 774	17	0.99 (0.85–1.16)	9193	10
Bronchial hyperresponsiveness	0.90 (0.69–1.17)	5713	21	0.85 (0.61–1.18)	4582	15	0.95 (0.58–1.58)	1131	6
Rhinitis	1.51 (1.37–1.66)	45 774	28	1.38 (1.24–1.55)	28 466	18	1.71 (1.48–1.98)	17 308	10
Rhinoconjunctivitis	1.61 (1.42–1.83)	45 651	28	1.37 (1.23–1.51)	28 378	18	2.17 (1.91–2.48)	17 273	10
Rhinitis without conjunctivitis	1.27 (1.16–1.39)	45 378	27	1.30 (1.12–1.51)	28 102	17	1.25 (1.11–1.41)	17 276	10
Coughed up phlegm without a cold	1.9 (1.59–2.26)	24 573	18	1.97 (1.52–2.56)	16 121	12	1.98 (1.63–2.40)	8452	6
Coughed up phlegm frequently	2.71 (2.15–3.41)	14 972	15	2.91 (1.89–4.49)	9524	9	2.64 (1.99–3.49)	5448	6
Flexural eczema symptoms past year	1.52 (1.34–1.73)	45 856	28	1.34 (1.18–1.51)	28 544	18	1.96 (1.62–2.37)	17 312	10
Flexural eczema on skin examination	0.95 (0.78–1.16)	25 966	23	0.92 (0.74–1.14)	18 879	16	1.10 (0.70–1.72)	7087	7

N, Number of children; HDM, House dust mite (*Dermatophagoides pteronyssinus* or *D. farinae*).

We did not find an association of dampness with bronchial hyperresponsiveness, in contrast to other studies in adults and among asthmatic children [37, 38] as well as children from a Swedish population. However, no relationship was found between reported dampness and exercise-induced bronchospasm among Scottish children [39].

A special strength of the ISAAC collaborative framework is the diversity of study centres. Most previous studies of dampness and health have been carried out in industrialized Western countries, and only a small number of previous studies in non-affluent countries such as Kenya, Nigeria and Sri Lanka [2, 3, 40] have suggested that dampness might be a risk factor world-wide.

Despite the consistent association of dampness with symptoms of wheeze, rhinoconjunctivitis and eczema the underlying mechanisms remain more speculative. House dust mite exposure has been proposed as a mediating factor [9, 41] relating to the fact that house dust mites thrive well in humid environments (e.g. [42]) and higher levels of the house dust mite allergen *Der p1* were found in mattresses in bedrooms with higher measured humidity [43–45].

In our study, as in others [43] mite allergen levels were indeed higher in mattress dust samples from homes where the parents reported dampness. Furthermore, sensitization to house dust mite was also positively related to dampness and to HDM-allergen levels in the smaller subsample. However, in this subsample wheeze was not related to allergen levels and similar results have been found in several cohort studies published in the last decade [46, 47]. Also a study in Sweden, where mite levels are low, reported that there was no relation between HDM-sensitization and either current asthma or damp buildings [8].

In our study, the effect of dampness was very similar in subjects with and without house dust mite sensitization, which suggests that house dust mite allergy is not the only or even the main pathway underlying the observed association between dampness and wheeze. However, it has recently been suggested that the effect of house dust mite (allergens) may not always be IgE-mediated, but could also involve airway remodelling in asthmatics through other mechanisms [48].

Nevertheless, the dampness effect is observed world-wide in very different climatic conditions including areas where house dust mites do not occur frequently (e.g. [49, 50]) suggesting that mite exposure can, at best, explain only a part of the associations between symptoms and dampness in homes [16].

Our study is unusual in reporting results stratified by sensitization. Stratum-specific effects for atopic and non-atopic individuals are reported in few studies with partly contradictory results. A case-control study of English primary schoolchildren suggested that the effect of surface wall moisture on parent-reported wheeze was stronger in atopics though the interaction term was not statistically significant [51]. A case-control study of newly diagnosed childhood asthma in Finland showed a significantly stronger effect in atopic individuals for visible mould, but no significant difference for moisture damage [52]. Similarly, in children 7–8 years of age, Rönmark et al. (1999) [53] found no significant difference in the association of dampness at home with atopic and non-atopic asthma respectively. In our study, the effect of dampness, including also visible moulds, on wheeze was essentially the same in atopic and non-atopic individuals.

The ISAAC data has previously shown [18, 20, 33] that a substantial proportion of wheezing, rhinitis and

eczema symptoms among children, particularly in less affluent countries, is unlikely to have an atopic basis, so the significantly stronger association between dampness and wheeze in centres from less affluent countries provides further argument for the link being through non-atopic mechanisms such as non-allergic inflammatory processes.

One limitation of our study is that the only mould species we have information on sensitization for is *Alternaria alternata*. Only a limited number of children was sensitized to that mould species (1.5%) an even less were sensitized exclusively to *Alternaria* (0.7%) with most being also sensitized to house dust mite. In the one centre that tested for sensitization to *Cladosporium*, a similar pattern emerged (1% and 0.8% respectively). Therefore, we could not perform a meaningful analysis on this data. However, the low prevalence of *Alternaria* and *Cladosporium* sensitization precludes a significant role in the observed association of disease with dampness. Although we cannot make any statements regarding other mould species, they seem unlikely to be the relevant mechanism, given that overall atopy is not associated with dampness.

An explanation of a notable non-allergic effect could relate to inflammatory reactions to cell wall components such as 1,3-β-D-glucan [13–15] or volatile organic compounds emanating from degrading building materials or microbial activity [11, 12]. A recent study in a population-based sample including children and adults has found a relation between MVOCs such as 1-octen-3-ol with rhinitis and conjunctivitis, though not with asthma [54]. Furthermore, mycotoxins have been shown to hinder macrophage functioning *in vitro* [55] and animal models have shown inflammatory, non-allergic respiratory effects with toxic fungal metabolites [56] and a 1,3-β-D-glucan [57]. On the other hand, one study in infants found that 1,3-β-D-glucan was negatively associated with asthma in a longitudinal investigation [58] which however could not be confirmed at age three of the children [59].

One limitation to our study is that we report cross-sectional associations and have no data on wheeze incidence. However, the positive association was also found when investigating early exposure retrospectively. Potential over-reporting due to differential parental recall may have exaggerated this association, although the results were similar for current exposure.

In general, recall bias may have influenced the results as parents of wheezy children may be more likely to notice or report dampness due to perceived health effects. Some previous studies have actually found that the associations were stronger when dampness was assessed by trained personnel or objective measurements [39, 49, 60, 61] probably because this personnel is trained to find moulds also in hidden locations. However, one study which compared parental report with trained personnel reports actually found that parents reported more damp spots, but there was no over-reporting of parents of cases relative to parents of controls [9]. While reporting bias is plausible and reported in the literature from some affluent countries, we found that the association was observed consistently in diverse countries, even where preconceptions regarding health effects are unlikely to occur.

We found no association of dampness with the objective measures of bronchial hyperresponsiveness and skin examination, while the wheezing and eczema symptoms showed a clear association. A third objective measurement (house dust mite sensitization) did show the expected association with dampness and with allergen exposure.

However, BHR is not perfectly correlated with wheeze, either at the individual level or the level of whole populations [19]. This may be, in part, because BHR is a measure at one point in time (point prevalence) and thus fails to capture those with asymptomatic wheeze at the time of the test but who have contributed to the 12-month period prevalence of wheeze, therefore resulting in a lower power. Similar considerations apply to the relationship between examined eczema and reported eczema. In addition, for BHR, the power of our study was reduced because bronchial challenges were performed in stratified subsamples in many centres.

Another limitation is that we are not able to distinguish whether the association with present exposure is mainly due to inducing the development of the disease or to triggering asthma symptoms in the past 12 months. However, the fact that the association holds also true for children for which exposure was reported only for the first year of life, that is, before the survey, suggests that the effect is related to the onset of asthma rather than triggering of asthma attacks.

In conclusion, our study confirms an adverse effect of damp housing conditions on respiratory symptoms based on results from centres world-wide. Exposure to dampness, both at present and earlier in life was associated with wheeze occurrence as well as wheeze severity, mainly, we suggest, through non-atopic mechanisms. Further research is needed to investigate these non-allergic pathways, the lesser contributing role of house dust mite allergens, and the timing of the relevant environmental exposures.

for field work in several low income countries without charge.

Conflict of interests

The authors declare no conflict of interests.

Appendix

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The agencies funding the field work are listed elsewhere (Weiland et al. 2004).

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Odds ratios (OR) (with 95%-CI) for the association between wheeze in the past year and reported damp spots and/or visible moulds at present

Table S1. Frequency of the health-related outcomes in the analysis data set (%).

Table S2. Test of potential confounders of the association between wheeze in the past year and damp spots and/or visible moulds in the child's home at present