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The determinants of dust mite allergen and its relationship to the prevalence of symptoms of asthma in the Asia-Pacific region

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The role that house dust mites play in the primary causation of asthma is controversial. Approximately thirty-six 10-yr-old children in each of 10 centres in the Asia-Pacific region participated. Researchers collected dust from mattresses and living room floors using standardized procedures. Der p1 and Der f1 were analysed using a double monoclonal antibody enzyme-linked immunosorbent assay. Geometric mean allergen levels were calculated for each centre. An ecological analysis was conducted to show the regression of the geometric mean allergen level, using the highest household level, against asthma symptom and severity prevalence data from the International Study of Asthma and Allergies in Childhood, Phase I. Among children aged 13–14 yr, the change in asthma symptom prevalence was associated with per unit change in Der p 1 μg/g (1.08, 95% CI 0.10–2.06) and Der 1 μg/g (Der p1 + Der f1) (0.64, 95% CI 0.02–1.26). The change in having four or more attacks of asthma in the last 12 months was associated with per unit change in Der p 1 μg/g (0.29, 95% CI −0.02 to 0.60) and Der 1 μg/g (0.20, 95% CI 0.01–0.38). There was no effect for total Der p1 or Der f1 (total or μg/g). Among children aged 6–7 yr, neither allergen was related to symptoms or severity prevalence. While our findings suggest that *Dermatophagoides pteronyssinus* may have a role in the primary causation of asthma, the complexity of this association reinforces the need for prospective studies.

Kristin Wickens[1], Jessie de Bruyne[2], Mario Calvo[3], Sam Choon-Kook[4], G. Jayaraj[5], Christopher K. W. Lai[6], Juliette Lane[1], Rameshchandra Maheshwari[7], Javier Mallol[8], Sankei Nishima[9], Gordon Purdie[10], Rob Siebers[1], Thevaruparambil Sukumaran[11], Muthita Trakultivakorn[12] and Julian Crane[1]

[1]Department of Medicine, Wellington Asthma Research Group, Wellington School of Medicine and Health Sciences, University of Otago, New Zealand, [2]Department of Pediatrics, Faculty of Medicine, University of Malaya, Kuala Lumpur, Malaysia, [3]Pediatrics Department, Medicine Faculty, Austral University, Valdivia, Chile, [4]Genetics Division, Institute of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia, [5]Neyveli Lignite Corporation Hospital, Neyveli, India, [6]Department of Medicine and Therapeutics, The Chinese University of Hong Kong, Hong Kong, [7]Asthma Research Clinic, Pune, India, [8]Department of Pediatric Respiratory Medicine, University of Santiago de Chile, Santiago, Chile, [9]The National Minami-Fukuoka Chest Hospital, Fukuoka, Japan, [10]Department of Public Health, Wellington School of Medicine and Health Sciences, University of Otago, New Zealand, [11]Institute of Child Health, Kerala, India, [12]Chiang Mai University, Chiang Mai, Thailand

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Kristin Wickens, Wellington Asthma Research Group, Wellington School of Medicine and Health Sciences, PO Box 7343, Wellington South, New Zealand
Tel.: 064 4 3855999
Fax: 064 4 3895427
E-mail: kwickens@wnmeds.ac.nz

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Previous studies have shown dose–response relationships between dust mite allergen level and the prevalence of sensitization (1, 2), and between increasing sensitization and the risk of asthma (3, 4). In those with established disease, the level of allergen exposure has been shown to predict the severity of symptoms (5, 6). However, the nature of the direct association between dust mite allergen exposure and the development of asthma has been more elusive with the findings from prospective studies of high-risk infants in conflict (7–9). Comparisons between centres within countries have also failed to show associations of dust mite allergen level with asthma prevalence despite showing associations with specific dust mite sensitization (2, 10, 11). We only identified one

across-country comparison and this showed that Hong Kong and Guangzhou, China had similar allergen levels but variable asthma prevalence (12).

We undertook this cross-sectional study of a number of geographically and culturally distinct centres in a range of countries in the Asia-Pacific region. The primary aim of the study was to show whether the exposure of different populations to mite allergen was associated with the prevalence of allergic disease previously reported in the International Study of Asthma and Allergies (ISAAC) Phase I (13). A secondary aim was to determine risk factors for mite allergen level in this population where the prevalence of such factors vary widely.

Methods

The study design was based on the ISAAC Europe Biomed Project. We approached 44 centres in the Asia-Pacific region that had participated in ISAAC Phase I (13). Ten of these centres within seven countries (India, Hong Kong, Malaysia, Thailand, Japan, Chile and New Zealand) agreed to participate in the present study. Within each centre, six randomly selected schools with 10-yr-old pupils were approached and within each school, six randomly selected children were invited to participate.

Dust samples were collected from the tropical and sub-tropical countries lying north of the equator between November and March (winter), from Japan in October (autumn) and from the temperate countries in the southern hemisphere in summer (November to February) (Fig. 1). For the dust collection, an ALK collection device (ALK Allergologisk Laboratorium A/S, Denmark, was attached to a vacuum cleaner of at least 800 W.

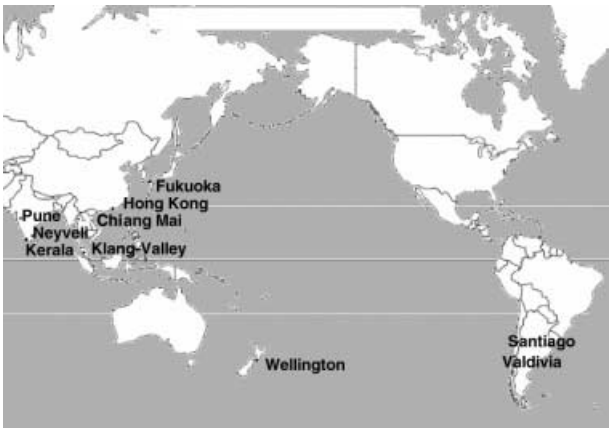

Fig. 1. Map showing the distribution of participating centres.

The investigators sampled the entire upper surface of the child's mattress (for 2 min) and the living room floor at a rate of 1 min/m^2 (2 m^2, if carpeted, or the entire floor, if uncarpeted). After sampling, the dust and filter paper were emptied into tinfoil and placed in a labelled self-sealing plastic bag for storage at −20° before transit to Wellington, New Zealand. Dust was removed from the filter and sieved (425 μm) with the fine dust extracted with phosphate-buffered saline at room temperature, and Der p1 and Der f1 levels determined in the centrifuged supernatants by double monoclonal antibody enzyme-linked immunosorbent assay (14) (Indoor Biotechnologies, Cardiff, UK).

A short questionnaire on housing and bedding characteristics was completed by a parent. This was conducted in Spanish in Validivia and Santiago in Chile, in Thai in Thailand and in English in all other centres.

Ethical approval for the study was granted by the Wellington Ethics Committee.

Statistical analysis

Statistical analysis was conducted using SAS version 8 (SAS Institute, Cary, NC, USA). Der p1, Der f1 and dust weights were log-normally distributed, so analysis was based on log-transformed data of the highest floor or mattress level, in conformity with previous analyses of the association between dust mite allergen and asthma (7, 8). The results of the two dust mite assays (Der p1 and Der f1) were added and expressed in terms of total group 1 (Der 1) allergen. Geometric mean Der p1, Der f1 and Der 1 were calculated for both the concentration (μg/g) and total content in collected dust. For samples where allergen levels were undetectably low, values of 0.01 were assigned for both the concentration and total content. An ecological analysis was conducted using regression analysis to model the change in the population symptom and severity [from ISAAC(13)] prevalence against geometric mean allergen levels for each centre, weighted by the inverse of the variance of the proportion in each centre. This analysis was repeated with adjustment for gross national income (GNI) per capita, using World Bank figures (15). The distribution of allergen levels against selected symptom and severity prevalence data were plotted by centre and presented graphically showing Pearson's correlation coefficients. Finally a regression analysis of the factors that separately determine bed and floor Der p1 and Der f1 levels was conducted using the mixed procedure of the SAS package to control for the fixed effects of possible confounding by

individual home and bed characteristics, with random terms for country, centre within country and school within centre. A similar analysis was conducted to correlate log total dust weight with Der p1 and Der f1 concentrations.

Results

The number of samples with detectable allergen, geometric mean concentration (Table 1) and total allergen level (Table 2) of the highest (floor or mattress) Der p1, Der f1 and Der 1 allergens are reported. Low amounts of dust were collected from most centres (Table 2), as is reflected in the low levels of total allergen in these centres. Log total dust weight was correlated with log highest Der p1 concentration ($\beta = 0.55$, $p < 0.0001$) and log highest Der f1 concentration ($\beta = 0.25$, $p = 0.006$). In all centres except Hong Kong and Pune, India Der p1 was the predominant allergen.

Table 3 presents the results of the ecological analysis of the asthma symptom and severity prevalence, as reported in ISAAC Phase I for children aged 6–7 and 13–14 yr (13), with the geometric mean of the highest (floor or mattress) allergen level for each centre, collected as part of the current study. Among children aged 13–14 yr, increasing concentrations of Der p1 and Der 1, were significantly associated with increases in asthma symptom prevalence, measured using the written questionnaire, and non-significantly associated with increases in asthma symptom prevalence, measured using the video questionnaire. The concentrations of Der p1 and Der 1 were also related to the frequency of asthma attacks but not to wheeze limiting speech. Fig. 2a–d illustrate the relationship for Der p1 concentration graphically. There was no association between allergen level and asthma symptom or severity prevalence for children aged 6–7 yr. Total allergen (Der p1 or Der f1) was not a predictor of asthma symptom or severity prevalence.

A separate analysis calculated geometric mean values for each centre restricted to samples with dust weights >0.02 g. Pune was excluded from this analysis as there were only a small number of samples with sufficient dust. At 13–14 yr, the associations for Der p 1 μg/g [0.63 (95% CI 0.22–1.04, $p = 0.008$)] and Der 1 μg/g [0.45 (95% CI 0.10–0.79, $p = 0.02$)] with asthma symptoms and Der p 1 μg/g [0.26 (95% CI 0.09–0.42, $p = 0.008$)] and Der 1 μg/g [0.19 (95% CI 0.08–0.30)] with frequency of attacks increased in significance. The significance of the associations of Der p 1 μg/g [0.26 (95% CI −0.02 to 0.54, $p = 0.06$)] and Der 1 [0.19 (95% CI 0.00–0.37, $p = 0.05$)] concentrations with the frequency of

Table 1. Geometric mean (95% CI) highest (floor or mattress) Der p1, Der f1 and Der 1 concentration by study centre

Centre	n = 353	Der p1 detectable	Der p1 (μg/g)	Der f1 detectable	Der f1 (μg/g)	Der 1 (μg/g)
Pune, India	33	4	0.04 (0.01–0.12)	16	0.69 (0.13–3.50)	0.78 (0.14–4.24)
Kerala, India	36	19	0.28 (0.09–0.90)	4	0.02 (0.01–0.03)	0.28 (0.09–0.91)
Neyveli, India	36	32	4.04 (1.76–9.26)	7	0.03 (0.01–0.06)	4.25 (1.84–9.80)
Hong Kong	36	16	0.16 (0.05–0.47)	23	0.60 (0.19–1.89)	0.98 (0.29–3.25)
Klang Valley, Malaysia	35	22	0.70 (0.22–2.28)	15	0.13 (0.04–0.37)	1.28 (0.39–4.15)
Chiang Mai, Thailand	36	36	9.02 (5.85–13.90)	33	2.50 (1.15–5.45)	15.81 (10.71–23.34)
Fukuoka, Japan	35	31	5.98 (2.34–15.31)	32	3.63 (1.62–8.13)	20.28 (11.13–36.95)
Valdivia, Chile	36	35	9.28 (4.97–17.32)	4	0.01 (0.01–0.02)	9.30 (4.98–17.35)
Santiago, Chile	36	16	0.10 (0.04–0.25)	4	0.01 (0.01–0.02)	0.12 (0.05–0.29)
Wellington, New Zealand	34	30	15.86 (5.60–44.91)	4	0.02 (0.01–0.03)	23.31 (10.19–53.33)

Table 2. Geometric mean (95% CI) highest (floor or mattress) total Der p1, Der f1, Der 1 and geometric mean (95% CI) total dust weight by study centre

Centre	n = 353	Total Der p1 (μg)	Total Der f1 (μg)	Total Der 1 (μg)	Mattress dust weight (g)	Floor dust weight (g)
Pune, India	33	0.02 (0.01–0.04)	0.07 (0.03–0.16)	0.10 (0.04–0.23)	0.002 (0.001–0.004)	0.004 (0.002–0.007)
Kerala, India	36	0.06 (0.03–0.12)	0.01 (0.01–0.01)	0.06 (0.03–0.12)	0.01 (0.01–0.03)	0.02 (0.01–0.04)
Neyveli, India	36	0.25 (0.14–0.45)	0.01 (0.01–0.02)	0.26 (0.15–0.48)	0.03 (0.02–0.04)	0.22 (0.14–0.34)
Hong Kong	36	0.03 (0.02–0.05)	0.05 (0.03–0.10)	0.07 (0.04–0.14)	0.01 (0.01–0.02)	0.01 (0.01–0.02)
Klang Valley, Malaysia	35	0.08 (0.04–0.15)	0.03 (0.02–0.04)	0.10 (0.05–0.20)	0.01 (0.01–0.02)	0.01 (0.01–0.01)
Chiang Mai, Thailand	36	12.80 (6.79–24.15)	3.73 (1.53–9.10)	22.14 (11.83–41.45)	1.29 (0.90–1.86)	0.39 (0.26–0.59)
Fukuoka, Japan	35	0.77 (0.33–1.77)	0.54 (0.24–1.20)	2.10 (0.99–4.46)	0.02 (0.01–0.04)	0.04 (0.02–0.10)
Valdivia, Chile	36	8.29 (3.92–17.51)	0.01 (0.01–0.02)	8.30 (3.93–17.55)	0.52 (0.26–1.04)	0.63 (0.43–0.93)
Santiago, Chile	36	0.07 (0.03–0.14)	0.01 (0.01–0.01)	0.07 (0.03–0.15)	0.31 (0.19–0.53)	0.22 (0.15–0.32)
Wellington, New Zealand	34	1.66 (0.73–3.77)	0.01 (0.01–0.02)	1.98 (0.95–4.12)	0.04 (0.02–0.08)	0.06 (0.04–0.09)

Table 3. The change in symptom prevalence among International Study of Asthma and Allergies (ISAAC) participants per unit change in the geometric mean highest (floor or mattress) allergen

	6–7 yr		13–14 yr	
	Prevalence change (95% CI)	p	Prevalence change (95% CI)	p
Wheeze prevalence (written questionnaire)				
Der p1 (μg/g)	0.68 (−0.54 to 1.91)	0.24	1.08 (0.10–2.06)	0.03
Der p1 (μg/m^2)	0.12 (−1.12 to 1.36)	0.83	0.46 (−0.98 to 1.90)	0.48
Der f1 (μg/g)	0.07 (−5.65 to 5.79)	0.98	0.13 (−5.85 to 6.11)	0.96
Der f1 (μg/m^2)	−0.43 (−4.82 to 3.96)	0.83	1.05 (−4.65 to 6.75)	0.68
Der 1 (μg/g)	0.42 (−0.31 to 1.16)	0.22	0.64 (0.02–1.26)	0.05
Der 1 (μg/m^2)	0.02 (−0.72 to 0.76)	0.96	0.27 (−0.65 to 1.18)	0.52
Wheeze prevalence (video questionnaire)				
Der p1 (μg/g)	–	–	0.76 (−0.18 to 1.71)	0.10
Der p1 (μg/m^2)	–	–	0.21 (−1.05 to 1.48)	0.70
Der f1 (μg/g)	–	–	0.22 (−5.19 to 5.63)	0.93
Der f1 (μg/m^2)	–	–	0.48 (−3.95 to 4.91)	0.80
Der 1 (μg/g)	–	–	0.43 (−0.14 to 0.99)	0.11
Der 1 (μg/m^2)	–	–	0.11 (−0.63 to 0.85)	0.72
4+ asthma attacks				
Der p1 (μg/g)	0.16 (−0.27 to 0.59)	0.41	0.29 (−0.02 to 0.60)	0.07
Der p1 (μg/m^2)	0.09 (−0.27 to 0.44)	0.58	0.09 (−0.31 to 0.50)	0.61
Der f1 (μg/g)	0.57 (−0.98 to 2.13)	0.42	0.45 (−1.24 to 2.14)	0.56
Der f1 (μg/m^2)	0.15 (−1.13 to 1.43)	0.80	0.43 (−1.23 to 2.10)	0.56
Der 1 (μg/g)	0.15 (−0.09 to 0.39)	0.19	0.20 (0.01–0.38)	0.04
Der 1 (μg/m^2)	0.04 (−0.17 to 0.26)	0.66	0.07 (−0.20 to 0.34)	0.56
Wheeze limiting speech				
Der p1 (μg/g)	0.06 (−0.18 to 0.30)	0.59	0.16 (−0.17 to 0.48)	0.30
Der p1 (μg/m^2)	−0.02 (−0.22 to 0.19)	0.86	0.02 (−0.34 to 0.38)	0.90
Der f1 (μg/g)	−0.13 (−1.01 to 0.74)	0.74	−0.24 (−1.53 to 1.05)	0.68
Der f1 (μg/m^2)	−0.17 (−0.88 to 0.54)	0.59	0.09 (−1.34 to 1.52)	0.89
Der 1 (μg/g)	0.03 (−0.11 to 0.17)	0.64	0.07 (−0.13 to 0.26)	0.45
Der 1 (μg/m^2)	−0.02 (−0.14 to 0.10)	0.75	0.02 (−0.22 to 0.25)	0.87

The video questionnaire was shown to 13–14 yr olds only in all centres in this study except Valdivia, Chile and Kerala, India.

attacks among children at 6–7-yr age group also strengthened.

After adjustment for GNI per capita, the effect of Der p1 remained essentially unchanged but Der 1 effects reduced, all becoming non-significant (data not shown). Additional analysis showed that Der f1 concentration, but not total Der f1, was positively related to GNI per capita, whereas Der p1 was not (data not shown).

Determinants of Der p1 and Der f1 on mattresses and floors were examined after adjustment for the fixed effects of possible confounders and the random effects of country, centre within country and school within centre. Table 4 shows that the factors determining mattress Der p1 and Der f1 concentrations differ. Reported dampness in the bedroom was a clear determinant of high Der p1, and kapok mattresses a strong determinant of high Der f1. Similar effects were found for total Der p1 and total Der f1 (data not shown). The concentrations of floor Der p1 and Der f1 (Table 5) also appear to be independently predicted by different determinants. While in the presence of carpet strongly predicted floor Der p1 concentrations the association was much weaker for Der f1 concentrations. Findings were similar for total Der p1 and Der f1 except the association between carpets and Der f1 strengthened, becoming significant.

Discussion

We found a positive association for the concentration of dust mite allergen (Der p1 and Der 1) with asthma symptom prevalence and having at least four attacks of asthma among 13–14 yr olds. These results should be interpreted with caution as ecological associations are poor predictors of causative relationships (16) and the findings are based on only 10 data points. The lower significance for wheeze measured by the video reflects the fact that this data was available for only eight centres. However, the prevalence data from ISAAC is based on large sample sizes with prevalence point estimates for each centre likely to have high accuracy.

In contrast, Der f1 did not predict asthma symptom or severity prevalence. It is interesting that in nearly all centres *Dermatophagoides pteronyssinus* is the predominant mite species

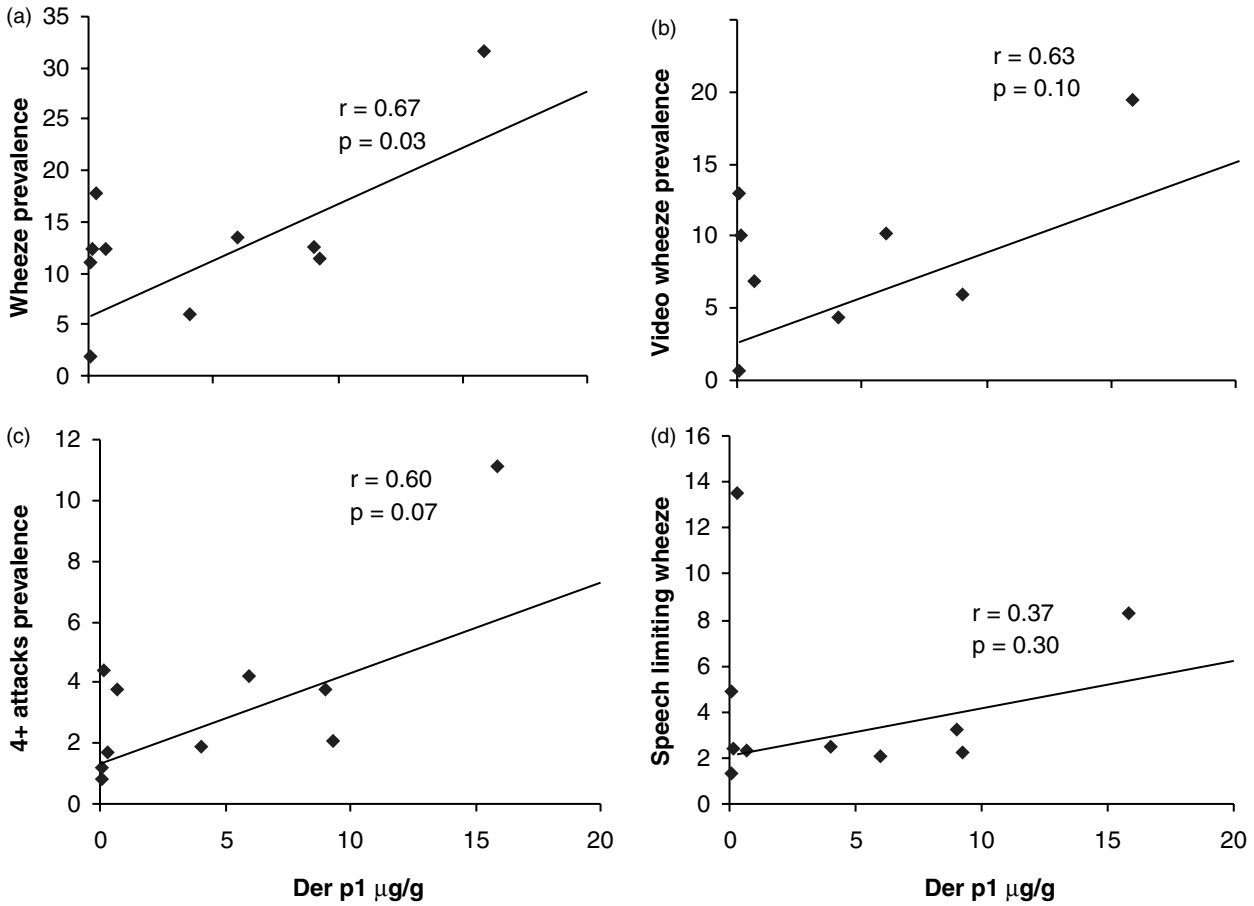

Fig. 2. (a) Highest Der p 1 μg/g by wheeze prevalence at 13–14 yr. (b) Highest Der p 1 μg/g by video wheeze prevalence at 13–14 yr. (c) Highest Der p 1 μg/g by prevalence of 4+ attacks of asthma at 13–14 yr. (d) Highest Der p 1 μg/g by prevalence of speech limiting wheeze at 13–14 yr.

Table 4. Adjusted* ratios (95% CI) showing associations for bedroom factors with concentration of mattress Der p1 and Der f1

	Der p1 (μg/g)	p	Der f1 (μg/g)	p
Mattress type				
Foam	1.00		1.00	
Inner sprung	1.02 (0.36–2.88)	0.97	0.84 (0.35–1.99)	0.69
Kapok	1.55 (0.34–6.97)	0.57	14.95 (4.85–46.11)	<0.0001
Other	0.72 (0.25–2.08)	0.55	1.53 (0.65–3.59)	0.33
Mattress age				
>10 yr vs. ≤ 10 yr	1.42 (0.37–5.43)	0.61	1.12 (0.36–3.50)	0.84
Mattress cover	1.62 (0.43–6.07)	0.48	0.44 (0.16–1.20)	0.11
Bedroom				
Window condensation†	0.84 (0.36–1.95)	0.68	0.50 (0.25–1.00)	0.05
Damp patches†	3.53 (1.41–8.83)	0.007	1.57 (0.73–3.38)	0.25
Visible mould†	0.73 (0.29–1.89)	0.52	0.74 (0.33–1.65)	0.45
Windows glazed	0.29 (0.03–3.16)	0.86	3.21 (0.43–24.01)	0.25
Family size				
>1 vs. 1 child	1.38 (0.58–3.27)	0.46	0.55 (0.27–1.14)	0.11

*Adjusted for all variables in the table plus random terms for country, centre within country and school within centre.
†Present vs. absent over the past year.

Table 5. Adjusted* ratios (95% CI) showing associations for living room factors with concentration of floor Der p1 and Der f1

	Der p1 (μg/g)	p	Der f1 (μg/g)	p
Living room				
Window condensation†	0.74 (0.38–1.45)	0.38	0.93 (0.52–1.66)	0.80
Damp patches†	1.44 (0.64–3.20)	0.38	1.14 (0.58–2.26)	0.70
Visible mould†	0.73 (0.29–1.83)	0.50	0.88 (0.40–1.94)	0.76
Windows glazed	0.21 (0.05–0.92)	0.04	1.04 (0.29–3.77)	0.95
Carpet				
Yes vs. No	10.43 (5.13–21.19)	<0.0001	1.73 (0.95–3.17)	0.07
>10 yr old	2.82 (0.75–10.65)	0.13	1.03 (0.33–3.18)	0.96
Family size				
>1 vs. 1 child	0.47 (0.23–0.95)	0.04	1.01 (0.56–1.84)	0.97

*Adjusted for all variables in the table plus random terms for country, centre within country and school within centre.
†Present vs. absent over the past year.

and may therefore be more important in predicting asthma symptoms. Geometric mean values of Der f1 were based on low numbers with detectable Der f1 in half the centres (Table 1).

Der f1 concentration was, however, significantly associated with GNI per capita, and after adjustment for GNI per capita the effects of Der 1 (which includes Der f1) concentration on symptom and severity prevalence reduced. This is not surprising as Japan has the highest concentrations of Der f1 and the highest GNI per capita.

There was no association for the total amount of Der p1 or Der f1 in the dust sample and asthma symptom prevalence.

The small amount of dust collected from most centres suggests that there may have been methodological problems due to the collection device or investigator technique that may have biased the findings towards the null hypothesis.

Furthermore, although the concentration of allergen is not expected to be related to the dust weight, we have shown that there is a positive association between dust weight and allergen concentration in this and other studies (17) when the amount of dust per m^2 collected is low. As many of the samples in this study with undetectable levels of allergen also had very low dust weights, we cannot be sure whether with more dust, allergen would have been detected. In support of this, previous geometric mean dust weights from beds (0.5 g) and living room floors (1.1 g) and Der p1 levels from beds (46.6 μg/g) and living room floors (25.5 μg/g) in Wellington were much higher (17). Der p1 levels have also been previously reported as much higher in Hong Kong mattresses (8.83 μg/g) and lounges (1.27 μg/g) (18). Nevertheless, the dust collection device and the method of collection were standardized across all centres suggesting that, although the absolute levels may be suppressed, the relative differences between centres are valid. Furthermore, all allergen measurements were conducted in the same laboratory in Wellington, New Zealand.

Asthma symptoms are defined in ISAAC as the presence of wheeze in the last 12 months. It is widely recognized that wheeze can have both an allergic and non-allergic basis, with allergen exposure affecting allergic wheeze only. The lack of any association for Der p1 on wheeze among 6–7-yr age group children in this study may be explained by a higher prevalence of non-allergic wheeze in this age group.

Our comparison across geographically and culturally diverse regions contrasts with findings of other studies that have shown similar asthma symptom prevalence despite different levels of dust mite allergen in different climatic zones within Australia (2, 10) and France (11). However, as dust mite allergen level varies more widely across countries than within countries associations may be evident in the cross-country comparison that are not evident for within-country comparisons.

We need to consider whether dust mite allergen levels in the present study are a marker for risk factors that differ in prevalence across centres. Economic development, measured using Gross National Product (now GNI) per capita, has been shown to be positively associated with allergic disease prevalence across countries (19) and may also be associated with lifestyle factors that enhance the proliferation of dust mites, such as closed homes and carpets. In the present study, both the highest Der 1 allergen level (23.3 μg/g) and the highest prevalence of symptoms (31.6% by written questionnaire) were in Wellington, New Zealand, with Fukuoka, Japan having the second to highest Der 1 allergen level (20.3 μg/g) and a moderate symptom prevalence (13.4% by written questionnaire). However, controlling for GNI per capita only affected associations that involved Der f1.

The cross-sectional nature of the study is a limitation as early allergen exposure is likely to be the more important time period to influence the immune system towards an allergic Th2 response.

The analysis of allergen level and symptom prevalence (Table 3) involved multiple comparisons, some of which may have been significant by chance.

Dermatophagoides pteronyssinus depends on a minimum relative humidity (55%) for survival (20) and our finding that reported damp in the bedroom was the strongest risk factor for Der p1 confirms findings from many other studies (17, 21). As humidity is an important factor determining the distribution of *D. pteronyssinus* within countries, it is difficult to explain why the more tropical high-humidity centres in this study often had very low levels of Der p1. The collection of dust during winter is unlikely to have resulted in lower levels of allergen as countries with high year-round humidity have shown no seasonal variation in levels (22) or, in the Klang Valley, higher *D. pteronyssinus* mite counts have been reported in January (23). However, the low levels of *D. pteronyssinus* in tropical regions may be due to the more prevalent *Blomia tropicalis* occupying the same ecological niche (23, 24). Our failure to measure allergens from this mite may have underestimated the total house dust mite allergen load in tropical countries. In Thailand, however, *D. pteronyssinus* has been reported to be the most abundant species with *B. tropicalis* relatively unimportant (25). Our data confirms high levels of allergen from the

Dermatophagoides group in this country but with kapok mattresses, the strongest predictor of high levels of the allergen from *Dermatophagoides farinae*.

The influence of carpets on Der p1 is well established (17, 26) and our finding of Der p1 concentrations 10 times higher in the presence of carpet is therefore not unexpected. The association of Der f1 with carpet was unexplainably weaker.

This exploratory study is the first to compare the association between dust mite allergen level and asthma symptom prevalence across multiple countries. Although ecological analyses are inherently flawed, our findings strongly suggest the need for further international comparative studies of infants followed prospectively in centres with variable levels of dust mite allergen.

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