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Microbial secondary metabolites in homes in association with moisture damage and asthma

Abstract We aimed to characterize the presence of microbial secondary metabolites in homes and their association with moisture damage, mold, and asthma development. Living room floor dust was analyzed by LC-MS/MS for 333 secondary metabolites from 93 homes of 1-year-old children. Moisture damage was present in 15 living rooms. At 6 years, 8 children had active and 15 lifetime doctor-diagnosed asthma. The median number of different metabolites per house was 17 (range 8–29) and median sum load 65 (4–865) ng/m^2. Overall 42 different metabolites were detected. The number of metabolites present tended to be higher in homes with mold odor or moisture damage. The higher sum loads and number of metabolites with loads over 10 ng/m^2 were associated with lower prevalence of active asthma at 6 years (aOR 0.06 (95% CI <0.001–0.96) and 0.05 (<0.001–0.56), respectively). None of the individual metabolites, which presence tended ($P < 0.2$) to be increased by moisture damage or mold, were associated with increased risk of asthma. Microbial secondary metabolites are ubiquitously present in home floor dust. Moisture damage and mold tend to increase their numbers and amount. There was no evidence indicating that the secondary metabolites determined would explain the association between moisture damage, mold, and the development of asthma.

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Practical Implications

This paper describes the most comprehensive characterization of microbial toxins and other secondary microbial metabolites in indoor environment until today. It demonstrates, for the first time, the ubiquitous presence of secondary microbial metabolites in homes even in the absence of moisture damage or mold and their potentially protective association with the development of asthma. In essence, our findings implicate that increased exposure to secondary microbial metabolites does not explain the elevated risk of asthma development in homes with moisture damage and mold.

Introduction

Exposure to moisture damage and mold at home is associated with considerably increased risk for upper and lower respiratory tract morbidity including exacerbation and development of asthma (Mendell et al., 2011; World Health Organization, 2009). With roughly half of the houses in United States affected with damp-

ness or mold, as many as one in five asthma cases may be attributable to these exposures (Mudarri and Fisk, 2007).

The causal agents are unknown but connection to moisture damage associated enhanced microbial growth, and related exposures, seems plausible (Mendell et al., 2011). The related exposures include those to airborne cell wall components, spores, volatile organic compounds, and secondary microbial metabolites such as mycotoxins. Many secondary metabolites are known to exhibit cytotoxic, irritant, and immunomodulatory effects and as such are evident candidates for causal agents (World Health Organization, 2009). In murine asthma model, mycotoxins have been shown to exacerbate airway hyper-reactivity, inflammation, and remodeling both by inhalation and ingestion (Miller et al., 2010; Schutze et al., 2010).

We have previously shown that secondary metabolites are present in severely moisture- and mold-damaged residential houses and that moisture damage in schools is associated with their increased abundance (Peitzsch et al., 2012; Taubel et al., 2011). In this study, we evaluated for the first time the presence of microbial secondary metabolites in a more representative sample of Finnish homes including both homes with and those without moisture damage and mold. We also explored whether microbial secondary metabolites might be able to explain the higher incidence of asthma in homes with moisture damage and mold, as earlier observed in the present birth cohort (Karvonen et al., 2014).

Methods

Study design

The study population included children from LUKAS2 birth cohort study. All pregnant women with estimated delivery at Kuopio University Hospital between May 2004 and May 2005 were invited to participate in LUKAS2 at week 32 of gestation (Karvonen et al., 2009). The cohort consists of 228 children and has extensive follow-up protocol (Keski-Nisula et al., 2010; Von Mutius and Schmid, 2006).

Data on asthma outcome were obtained from parent-reported questionnaires. Doctor-diagnosed asthma at least once or asthmatic bronchitis more than once by the age of 6 years was termed as 'asthma ever' and that with medication or wheezing at the age of 6 years as 'active asthma'. The only inclusion criterion was availability of quantitatively representative dust sample, defined as ≥250 mg of sieved dust, from living room at the age of 1 year. Families living in apartment buildings were excluded. On this basis, 93 index children were included. Living room was defined as the area where the family spends most of their time after dinner before going to sleep (see supporting information for details).

Moisture and mold damage assessment

Walk-through home inspection records were available from 83 of the homes with adequate dust sample. The median age of the child at inspection was 5.7 (range 1.1–25.0) months. The moisture damage and mold assessment was performed by a civil engineer as described earlier using a pre-designed checklist and supported by surface moisture measurements (Karvonen et al., 2009; Pekkanen et al., 2007). In brief, the civil engineer(s) inspected the homes for the signs of moisture in the surfaces and the structures and graded them using a 6-point 'need for repair' estimation scale and the area of the damage: class 2 meant a repair of surface materials needed; class 3 meant a repair of structural components needed; and classes 4 and 5 meant more extensive repair needed. 'No damage' was defined as no need for repair (class 0) or only cosmetic repair (classes 1). 'Major damage' was defined as either a need for repair of surface materials (class 2) with the area of damage ≥1 m^2 or a need for repair of structural components (class 3) with the area of damage ≥0.1 m^2, or a need for more extensive repair (classes 4 or 5). Other damage was classified as 'minor damage' (Karvonen et al., 2009; Pekkanen et al., 2007). During the home inspection, observed visible mold was also recorded in each room and categorized into three classes: no mold, spots of mold, and visible mold.

Dust sampling and metabolite analysis

All the study homes had smooth floors (i.e., no wall-to-wall carpets) with or without a rug. The dust samples were collected by the occupant to a nylon sampling sock by vacuuming an area of 1 m^2 from a rug for two minutes (n = 88) or in the absence of a rug, an area of 4 m^2 from a smooth floor for two minutes (n = 2). From 3 samples, this information was not available. The dust samples were homogenized by sieving through sterile strainer, dried in desiccator, and stored at −20°C until analysis.

A 100 mg subsample of dust was used for the secondary metabolite analysis. Metabolites were extracted and diluted from dust using acetonitrile/water/acetic acid solution. The analysis was performed as described before but with expanded range of detectable microbial secondary metabolites (333) and a more sensitive LC-MS/MS system (see supplement of the complete list of analytes (Vishwanath et al., 2009). In brief, we used Agilent 1290 Series HPLC System (Agilent, Waldbronn, Germany) coupled to a QTrap 5500 equipped with Turbo Ion Spray ESI source (Applied Biosystems, Foster City, CA, USA) in connection with a Gemini® C18 column, 150 × 4.6 mm i.d., 5 μm particle size protected by a C18 security guard cartridge, 4 × 3 mm i.d. (all from Phenomenex, Torrance, CA, USA). A methanol/water gradient containing 1% acetic acid

and 5 mM NH_4Ac was used at 1 ml/min. Data acquisition was performed in the scheduled multiple reaction monitoring (sMRM) mode in both positive and negative polarity using two separate chromographic runs per sample. Confirmation of the identity of the investigated analytes was obtained through acquiring 2 sMRM transitions (except for moniliformin and 3-nitropropionic acid, which yield only one detectable fragment ion) and comparison of the intensity ratio and LC retention time to an authentic standard. Quantification was performed based on linear, $1/\times$ weighed calibration curves deriving from serial dilutions of a multi-analyte standard.

Statistical analyzes

Kruskal–Wallis test was used to analyze whether the amount of individual metabolites, their sum amounts, or total numbers were different between houses that did or did not have moisture damage or moisture damage with visible mold at specific locations. Fisher's exact test with and without Bonferroni correction was used to study associations in the presence of individual metabolites with moisture damage and mold and development of asthma. The presence was categorized based on presence or absence or tertiles of load depending on the metabolite prevalence.

The association between the total numbers and amount of metabolites and development of asthma was assessed by logistic regression models. Firth's penalized likelihood-based bias-adjusted estimates were used due to the relatively small sample size (see supplement for details) (Firth, 1993; Heinze and Schemper, 2002). Also, the association between moisture damage and moisture damage with visible mold, asthma development and total metabolite numbers and amount was exploratively studied with logistic regression with the initial adjustments. All analyses were performed using SAS Enterprise Guide 5.1 (SAS Institute Inc., Cary, NC, USA).

Results

Metabolite profile overview

Overall, 42 different metabolites were detected in the floor dust samples (Figure 1). A maximum of 29 different metabolites were detected in one house without any moisture damage or mold, while the median for all houses was 17 (range 8–29). The median sum load of the metabolites was 65 (range 4–865) ng/m^2 and concentration 139 (range 10–2949) ng/g. Six metabolites, namely brevianamide

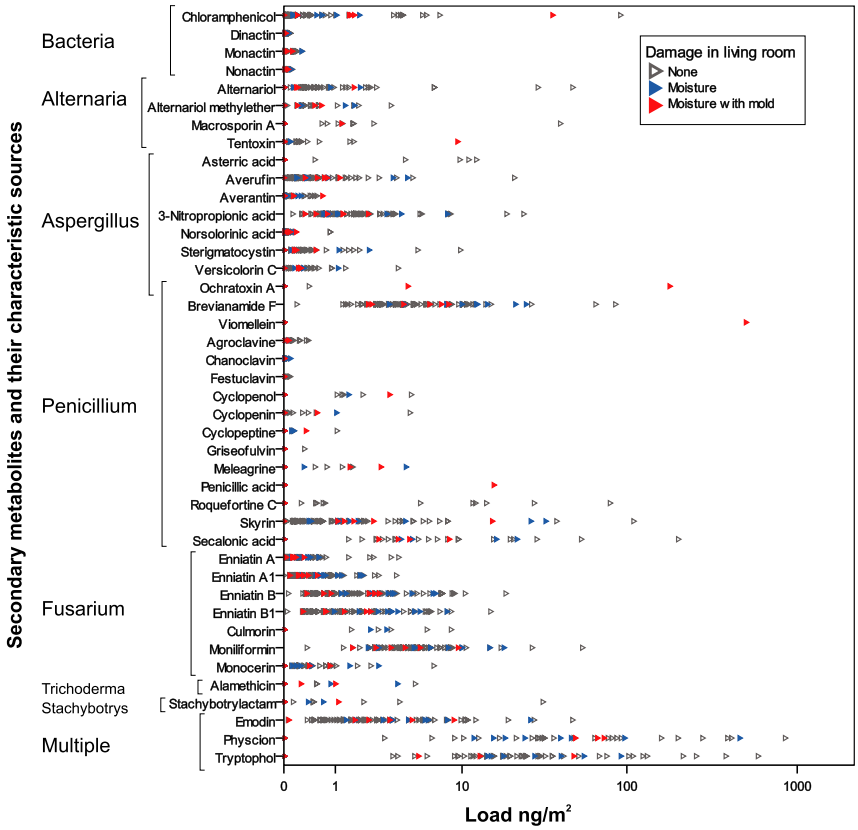

Fig. 1 Overview of the abundance of the microbial secondary metabolites in homes with or without moisture damage or moisture damage with visible mold

F, moniliformin, emodin, enniatins B and B1, and 3-nitropropionic acid were detected in all houses and enniatins A and A1 in all but one house. The metabolite loads were strongly correlated between different individual metabolites (Table S1). The strongest correlations were seen within metabolite families, such as ennitatins, nactins, and sterigmatocystin precursors that are commonly produced by the same microbial species and have partially overlapping biosynthesis pathways.

Metabolite profile in houses with moisture damage

Any moisture damage was detected in 15 living rooms and moisture damage with mold in six living rooms of the 83 inspected homes (Table 1). Major moisture damage or visible mold anywhere within the indoor space was detected in 33 and 17 homes, respectively (Table S2).

The total load of metabolites in living room floor dust (ng/m^2) tended to be increased if there was moisture damage in the living room or mold odor anywhere indoors during the site visit (Table 1). The total number of different metabolites tended to also be higher in these cases, especially the number of metabolites present at loads higher than 0.1 ng/m^2. The trends were less evident or absent if the moisture damage was present elsewhere than in the living room, where the dust sample was taken, and no trends were seen in association with presence of visible mold anywhere indoors (Table S2). Total metabolite loads tended to be higher also in houses where the occupant reported moisture or mold damage, but not in houses where the damages had been repaired as compared to houses with no reported damage (Table S2).

The presence or load of 13 individual metabolites tended ($P < 0.2$) to be increased, and the presence of two metabolites (monactin and roquefortine C) decreased in association with moisture damage in the sampling space (Table 2). However, none of these changes were significant after Bonferroni correction.

Analyses using metabolite concentration in dust instead of loads per floor area or exclusion of suspected outdoor-associated metabolites from the sum variables returned comparable trends as those presented in Table 1 (results not shown). The metabolites classified as outdoor-associated were enniatins B and B1, chloramphenicol, emodin, skyrin, and 3-nitropropionic acid, which in our previous study were detected in particulate matter of large volume outdoor samples (Täubel et al., 2013).

Metabolite profile in association with asthma

At 6 years, 15 of the 93 children included in the study had ever been diagnosed with asthma (asthma ever) and in 8 it was active.

Higher total sum load of metabolites (ng/m^2) and higher numbers of different metabolites with loads over 10 ng/m^2 at 1 year were significantly associated with lower risk for active asthma at 6 years of age (Table 3). The protective trend was seen also with other markers of metabolite numbers and with asthma ever, but the associations did not reach statistical significance. Accordingly, when the association between moisture

Table 1 Association of microbial secondary metabolites in living room floor dust at 1 year with moisture damage and mold assessed during home visit

				Total number of metabolites[c] at different load cutoffs (ng/m^2)											
	Total load[a] (ng/m^2)			>0			>0.1			>1		>10		>100	
	N[b]	Median	95%	N	Median	95%	N[d]	Median	95%	Median	95%	Median	95%	Median	95%
Living room															
Moisture damage															
No	65	55	562	67	17	25	65	14	19	7	12	1	4	0	1
Yes	15	97	803	15	20	28	15	16	25	10	18	2	6	0	2
P-value	0.12			0.23				0.03		0.03		0.04		0.62	
Moisture damage with mold															
No	74	60	636	76	17	25	74	14	21	7.5	16	1.5	4	0	1
Yes	6	97	803	6	19	28	6	17.5	25	9	18	1	5	0	2
P-value	0.84			0.53				0.35		0.46		0.65		0.97	
Whole indoor space															
Mold odor (site visit)															
No	77	59	636	80	17	25	77	14	24	7	16	1	4	0	1
Yes	3	145	637	3	22	24	3	20	21	14	17	3	6	0	1
P-value	0.09			0.07				0.04		0.02		0.05		0.54	

[a]Total load of metabolites represents the sum load (ng/m^2) of all detected metabolites.

[b]N = number of homes.

[c]Total number of metabolites represents the sum of different metabolites, which were detected in floor dust in amounts exceeding the given load cutoffs.

[d]The *N* is the same for all cutoffs above 0.1 ng/m^2. The information on the vacuumed area was missing from 3 homes and for these the load cutoffs could not be calculated.

Table 2 Suggestive associations ($P \leq 0.2$)[a] of individual metabolites with moisture damage or mold in living room and with asthma

			Damage								Asthma							
			Moisture				Mold				Ever[c]				Active[d]			
Characteristic source	Metabolite	Tertile/presence	*N*[b]	%	*P*	↑↓	*N*	%	*P*	↑↓	*N*	%	*P*	↑↓	*N*	%	*P*	↑↓
Bacteria	Chloramphenicol	1st	26	4	0.02	↑	–				–				–			
		2nd	27	19														
		3rd	27	33														
	Cyclopeptine	No	73	16	0.12	↑	–				–				–			
		Yes	7	43														
	Monactin	No	50	24	0.15	↓	–				58	17	0.20	↓	–			
		Yes	30	10							31	6						
Aspergillus	Averantin	No	–				–				66	18	0.03	↓	66	12	0.11	↓
		Yes									23	0			23	0		
	Ochratoxin A	No	–				77	5	0.01	↑	–				–			
		Yes					3	67										
	Viomellein	No	–				79	6	0.08	↑	–				–			
		Yes					1	100										
Aspergillus and *Penicillium*	Brevinamid F	1st	26	12	0.08	↑	–				–				–			
		2nd	27	11														
		3rd	27	33														
Penicillium	Meleagrin	No	73	15	0.02	↑	73	5	0.08	↑	–				–			
		Yes	7	57			7	29										
	Physcion	No	47	9	<0.01	↑	–				–				–			
		Yes	33	33														
	Roquefortine C	No	69	22	0.11	↓	–				78	10	0.04	↑	–			
		Yes	11	0							11	36						
	Secalonic acid D	No	54	13	0.07	↑	54	4	0.08	↑	–				–			
		Yes	26	31			26	15										
	Skyrin	1st	26	4	0.04	↑	–				–				–			
		2nd	27	22														
		3rd	27	30														
	Penicillic acid	No	–				79	6	0.08	↑	–				–			
		Yes					1	100										
Fusarium	Enniatin A	1st	26	4	0.04	↑	–				–				–			
		2nd	27	30														
		3rd	27	22														
	Enniatin A1	1st	26	8	0.12	↑	–				–				–			
		2nd	27	19														
		3rd	27	30														
	Moniliformin	1st	–				–				–				29	17	0.16	↓
		2nd													30	3		
		3rd													30	7		
Trichoderma viride	Alamethicin	No	73	15	0.02	↑	73	5	0.08	↑	–				–			
		Yes	7	57			7	29										

Arrows visualize whether increased (↑) or decreased (↓) presence/abundance of the metabolite is associated with presence of moisture damage or mold or increased risk of asthma ever or active asthma. Dash (–) indicates lack of suggestive association.

[a]As defined by Fisher's exact test without Bonferroni correction.

[b]N = number of homes.

[c]Doctor-diagnosed asthma at least once or asthmatic bronchitis more than once by the age of 6 years.

[d]Asthma ever and medication or wheezing at the age of 6 years.

damage and mold with active asthma was adjusted for total sum load or total number of different metabolites in the floor dust, the odds ratios increased further (Table 4).

In the analyses with individual metabolites, the risk of asthma ever by 6 years tended to be higher if roquefortine C was detected and lower if monactin and/or averantin were detected in floor dust of their homes (Table 2). Lower risk of active asthma was seen in children from houses where moniliformin and averantin were detected. None of these associations were significant after Bonferroni correction.

Analyses using metabolite concentration in dust instead of loads per floor area returned comparable trends as those presented in Table 3 (results not shown). Similarly, the exclusion of the suspected outdoor-associated metabolites from the sum had little effect on the results, except for decrease in the risk estimate for active asthma at 6 years in association with metabolite loads over 1 ng/m^2 at 1 year (data not shown).

Table 3 Association between number and load of microbial secondary metabolites in living room floor dust at 1 year of age and asthma development by 6 years

Metabolite abundance at 1 year	Doctor-diagnosed asthma at 6 years					
	Ever[a]			Active[b]		
	N[c]	%	aOR[d] (95% CI)	*N*	%	aOR[e] (95% CI)
Total sum load of different metabolites (ng/m^2)						
1st tertile (4–40 ng)	29	17	1.00	29	17	1.00
2nd tertile (43–104 ng)	30	13	1.06 (0.15–6.50)	30	7	0.08 (<0.001–1.37)
3rd tertile (110–865 ng)	30	10	0.90 (0.13–6.42)	30	3	0.06 (<0.001–0.96)
P-value			0.98			0.09
Number of different metabolites above specified load cutoffs (ng/m^2)						
$N_{metabolites} > 0$						
1st tertile (8–15)	33	18	1.00	33	12	1.00
2nd tertile (16–18)	30	17	0.85 (0.15–4.66)	30	13	1.38 (0.14–13.27)
3rd tertile (19–29)	29	7	0.37 (0.04–2.47)	29	3	0.12 (0.002–1.32)
P-value			0.56			0.16
$N_{metabolites} > 0.1$						
1st tertile (5–12)	31	19	1.00	31	13	1.00
2nd tertile (13–15)	27	11	0.92 (0.14–4.85)	27	7	1.10 (0.14–8.17)
3rd tertile (16–25)	31	10	0.65 (0.07–5.54)	31	6	0.22 (0.006–2.53)
P-value			0.91			0.39
$N_{metabolites} > 1$						
1st tertile (1–6)	27	22	1.00	27	19	1.00
2nd tertile (7–8)	31	10	0.81 (0.11–5.34)	31	3	0.36 (0.01–3.93)
3rd tertile (9–18)	31	10	0.67 (0.11–3.75)	31	6	0.33 (0.02–2.73)
P-value			0.89			0.44
$N_{metabolites} > 10$						
No	17	24	1.00	17	24	1.00
Yes	72	11	0.61 (0.10–3.96)	72	6	0.05 (<0.001–0.56)
P-value			0.57			0.01
$N_{metabolites} > 100$						
No	72	14	1.00	72	10	1.00
Yes	17	12	0.84 (0.12–4.68)	17	6	0.49 (0.04–3.76)
P-value			0.81			0.49

[a]Doctor-diagnosed asthma at least once or asthmatic bronchitis more than once by the age of 6 years.
[b]Asthma ever and medication or wheezing at the age of 6 years.
[c]N = number of homes.
[d,e]All the models are adjusted for farming status, gender, maternal history of allergic disease (hay fever, atopic, dermatitis and/or asthma), smoking during pregnancy, the number of older siblings, paternal education level, total living area, house type (detached or row house), time from last vacuuming before dust collection as well as for age of the house, cat ownership, and season at dust collection.

Table 4 Association between moisture damage and mold[a] in the living room early in life and risk of having active asthma at 6 years of age with or without adjustments for metabolite abundance

Adjustment for metabolites	Active asthma at 6 years[b]					
		Moisture damage			Mold	
	N	%	aOR[c] (95% CI)	*P*	aOR[c] (95% CI)	*P*
Unadjusted	81	10	1.37 (0.23–6.52)	0.70	3.85 (0.57–23.10)	0.17
Adjusted for sum load[d]	79	9	2.79 (0.38–22.91)	0.27	5.82 (0.77–47.41)	0.09
Adjusted for number of different metabolites[e]	81	10	2.03 (0.32–12.79)	0.42	7.62 (0.92–86.11)	0.07

[a]Moisture damage or moisture damage with visible mold.
[b]Doctor-diagnosed asthma at least once or asthmatic bronchitis more than once by the age of 6 years with medication or wheezing at 6 years.
[c]All models adjusted for farming status, gender, maternal history of allergic disease (hay fever, atopic dermatitis, and/or asthma), smoking during pregnancy, and the number of older siblings and in addition to [d]total sum load or [e]number of different metabolites categorized into tertiles.

Discussion

This study has three major findings. First, secondary microbial metabolites are ubiquitously present, albeit in relatively low concentrations, in the primary living space of residential houses even in absence of any signs of moisture damage or mold. Second, the total load and number of different metabolites are moderately increased by moisture damage. Third, such increase does not seem to have an adverse, but rather protective, association with asthma development in children.

We have previously shown that floor dust from all houses with severe moisture damage contains microbial secondary metabolites, but this is the first study to demonstrate their presence also in all samples from houses without moisture damage or mold (Taubel et al., 2011). In the present study, we used more sensitive analytical instrumentation, larger dust sample amounts, and targeted wider list of metabolites than earlier. This explains why several metabolites were detected more frequently in the present than previous study.

In the present study, increased number of metabolites and especially number of metabolites above specified load cutoffs were associated with moisture damage and mold odor. This supports our comparable observation in European school buildings and extends the finding to residential houses (Peitzsch et al., 2012). Individual metabolites associated with moisture damage or moisture damage with mold included physcion, skyrin, meleagrin, ochratoxin A, enniatin A, and alamethicin (Table 2). In line with this, previously the detection of meleagrin and physcion was exclusive to moisture-damaged schools in Finland and that of alamethicin to moisture-damaged schools in all study countries (Peitzsch et al., 2012). Also ochratoxin A and enniatin A have been detected in moisture-damaged homes but previously without comparison to undamaged homes (Polizzi et al., 2009; Taubel et al., 2011). In contrast, we did not detect metabolites, such as beauvericin and equisetin, that have been present in some floor dust samples from homes needing major repair actions (Taubel et al., 2011). We also did not detect the notorious macrocyclic trichothecene secondary metabolites of *Stachybotrys chartarum*, such as satratoxins and verrucarol. These compounds have been detected in dust samples from severely damaged buildings and highlighted as potential causal agents of the adverse health effects (Bloom et al., 2007, 2009; Gottschalk et al., 2008; Peitzsch et al., 2012). However, *S. chartarum* is relatively rarely detected in association with moisture damage in the Nordic countries. Previous studies indicate that only about 10% of mois-

ture-damaged houses (i.e., 1–2 houses in this study) are likely to harbor macrocyclic trichothecene-producing *S. chartarum* strains (Andersen et al., 2002; Pietarinen et al., 2008). Some of our samples (11%) did contain stachybotrylactam, another toxic metabolite potentially of *Stachybotrys* origin, but its presence was neither associated with moisture damage or mold, nor asthma. Similarly, sterigmatocystin and its precursors have been common isolates in moisture-damaged homes (Bloom et al., 2007; Engelhart et al., 2002; Polizzi et al., 2009) and were frequently detected also in the present study but without specific association with moisture damage or mold or asthma predisposing effect.

In the present study, none of the individual secondary metabolites were detected frequently and exclusively enough in association with moisture damage or mold to promote them as potential biomarkers of moisture damage or mold. This is in line with what we have previously reported from airborne settled dust in schools (Peitzsch et al., 2012).

Perhaps the most notable and intriguing finding in this study was the lack of direct, but tendency for inverse, association between the microbial secondary metabolites and the development of asthma. There are no earlier studies combining broad spectrum metabolite characterization with prospective data on asthma. In the only previous epidemiological study on measured mycotoxin exposure and asthma, which we are aware of, the presence of verrucarol in Malaysian secondary schools was not associated with the risk of doctor-diagnosed asthma in a cross-sectional analysis. Instead, there was a modest but significant protective association with daytime breathlessness (Cai et al., 2011).

Whether the seemingly protective association in our study is indeed due to the metabolites per se or rather reflects correlation between total metabolite and total microbial loads, conditions where the metabolites are produced or the small number of asthma cases included in the analyses can only be speculated upon.

The asthma-protective potential of early life microbial exposures is supported by mechanistic studies and epidemiological evidence and has also been seen in the present birth cohort (Karvonen et al., 2014; Von Mutius and Vercelli, 2010). The determinants of protective microbial exposure are not known. Therefore, it cannot be ruled out that low dose exposure to microbial secondary metabolites, simultaneously via airways and gut, could have positive influence, for example, via immunomodulation. Mechanistic studies indicate that at higher levels, the influence is more likely to be adverse (Miller et al., 2010; Rand et al., 2011; Schutze et al., 2010) although the metabolites in these previous studies were mostly different than what we found in the house dust. Metabolite numbers and loads may also, and perhaps more probably, be surrogate markers of the diversity and load of the indoor microbiota, which in turn may protect from asthma. However, the amount of dust, which correlates highly with the total microbial load (Karvonen et al., 2014), did not explain the finding, as there were no marked differences in the results whether concentration or load of toxins was used.

By definition, secondary metabolites do not have essential physiological role in the growth of the source organism and are usually produced in response to suboptimal, competitive, or changing growth conditions. Thus, the secondary metabolite abundance and its inverse correlation to health may also be indicative of specific growth conditions within the residence or particular type of moisture damage such as one on frequently drying or nutrient-deprived building material or one populated by competing microbial species.

Providing reliable inhalation exposure assessment to the secondary metabolites based on floor dust analysis is questionable. A rough estimate of the potential magnitude is presented for the purposes of this discussion using previously described means of exposure assessment, which is based on estimated amount of dust particles inhaled by children at home (0.3 mg/day) (Oomen and Lijzen, 2004). Assuming the metabolite concentrations for floor and airborne dust are the same, the total chronic exposure of a child by inhalation would be around 3×10^{-3} ng/kg bw/day with median and up to 6×10^{-2} ng/kg/day with the maximum secondary metabolite concentrations observed in the present study. The respective oral exposures would be around 8 and 180 ng/kg bw/day, based on EPA estimate of 60 mg of dust ingestion per day by a 1- to 6-year-old child (USEPA, 2008). While these values may be underestimates for several reasons, such as limitations of vacuum sampling, it is notable that the estimated total exposures by inhalation are orders of magnitude smaller than 4 ng/kg bw/day at which immunomodulatory effects are starting to be seen in mice (Schutze et al., 2010). Sporadically and locally at mucosal surfaces, the concentrations may reach considerably higher levels than estimated above (Carey et al., 2012). Also, the estimated values through ingestion are considerably higher than by inhalation but compared to the amount of exposure to secondary metabolites through foods, these total amounts are marginal. For example, the daily intake of enniatins alone is estimated to be at the level of micrograms or even over hundred micrograms per day (Juan et al., 2013; Serrano et al., 2013).

This study has several strengths including prospective follow-up of well-described study population and objective civil engineer performed home inspection. Furthermore, the coverage of over 330 different analytes makes this the most comprehensive characterization of microbial secondary metabolites in indoor environment until today. Confidence on the representative metabolite profile is further enhanced

by the highly sensitive instrumentation and inclusion of only samples with substantial amount of dust (Scott et al., 2012). Still the conclusions of this study are obviously limited to the array of metabolites determined. Other obvious limitations of this study are the relatively small number of asthma cases and houses with visible mold. Also, the metabolites were determined only from a single site of the home and at single time point, while the secondary metabolite production is likely to fluctuate with time in changing conditions.

Vacuumed floor dust is not as established indicator of potential airway exposures as active airborne dust sampling or dust settled on upper surfaces (Noss et al., 2008). However, our unpublished data show high correlation between vacuumed floor dust and airborne settled dust for the total number of detected metabolites (Spearman's $\rho = 0.9$) and moderate correlation for total metabolite concentrations ($\rho = 0.4$). Also, the fact that associations between secondary metabolite loads in the floor dust and moisture damage and mold were seen in the present study attest to the relevance of vacuumed floor dust as sampling material for this type of analyses.

In conclusion, microbial secondary metabolites are ubiquitously present in the floor dust of all houses with or without moisture damage and mold. Our study indicates that the loads of the secondary metabolites may be moderately increased with moisture damage and mold. However, the metabolite abundance alone was, if anything, protective of asthma. Moreover, none of the individual metabolites, which tended to be increased in moisture- and mold-damaged houses, showed any trend for increasing the asthma risk. Thus, our results do not support the suggestion that the secondary microbial metabolites determined in this study would explain the association between moisture damage and mold and asthma development in children.

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Conflict of interest

The authors have no conflict of interest and financial interests relating to this article to disclose.

Supporting Information

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

Appendix S1. Supplementary information on the study design, list of metabolites determined and building the logistic regression models.

Table S1. Spearman rank correlations between different secondary metabolites detected at least in seven different houses.

Table S2. Abundance of microbial secondary metabolites in different parts of house at 1 year in association with moisture damage and mold within the same house.

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