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Microbial diversity in homes and the risk of allergic rhinitis and inhalant atopy in two European birth cohorts

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ABSTRACT

Background: Microbial exposures in early childhood direct the development of the immune system and their diversity may influence the risk of allergy development. We aimed to determine whether the indoor microbial diversity at early-life is associated with the development of allergic rhinitis and inhalant atopy.
Methods: The study population included children within two birth cohorts: Finnish rural-suburban LUKAS (N = 312), and German urban LISA from Munich and Leipzig study centers (N = 248). The indoor microbiota diversity (Chao1 richness and Shannon entropy) was characterized from floor dust samples collected at the child age of 2–3 months by Illumina MiSeq sequencing of bacterial and fungal DNA amplicons. Allergic rhinitis and inhalant atopy were determined at the age of 10 years and analyzed using logistic regression models.
Results: High bacterial richness (aOR 0.19, 95%CI 0.09–0.42 for middle and aOR 0.12, 95%CI 0.05–0.29 for highest *vs*. lowest tertile) and Shannon entropy were associated with lower risk of allergic rhinitis in LISA, and similar trend was seen in LUKAS. We observed some significant associations between bacterial and fungal diversity measured and the risk of inhalant atopy, but the associations were inconsistent between the two cohorts. High bacterial diversity tended to be associated with increased risk of inhalant atopy in rural areas, but lower risk in more urban areas. Fungal diversity tended to be associated with increased risk of inhalant atopy only in LISA.
Conclusions: Our study suggests that a higher bacterial diversity may reduce the risk of allergic rhinitis later in childhood. The environment-dependent heterogeneity in the associations with inhalant atopy – visible here as inconsistent results between two differing cohorts - suggests that specific constituents of the diversity may be relevant.

Authors' contributions

Heidi Hyytiäinen: Formal analysis, Data curation, Writing – original draft, Visualization. Pirkka V Kirjavainen: Methodology, Investigation, Writing – review & editing. Martin Täubel: Conceptualization, Methodology, Resources, Data curation, Writing – review & editing. Pauli Tuoresmäki: Investigation, Resources, Data curation, Visualization, Writing – review & editing. Lidia Casas: Investigation, Resources, Data

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1. Introduction

The prevalence of allergic rhinitis in the population varies by geographic location from 10% to 40%, thus being the most common allergic respiratory disease (Wise et al., 2018). It can negatively affect sleep, tolerance of physical exercise, productivity or cognitive performance (Wise et al., 2018), and increase risk of sinusitis (Huang 2000). In addition, both allergic rhinitis and inhalant atopy during childhood can predispose to asthma later in life (Rochat et al., 2010; Wang et al., 2009). Thus, it is important to identify key determinants affecting the development of allergic rhinitis and atopy.

Years of epidemiological research have established inverse associations between growing up in farming environment and the risk of asthma, hay fever and atopy, in which contact with farm animals and consumption of raw milk have been especially linked with protection against asthma and allergy (von Mutius and Vercelli 2010). Previously microbial diversity (Ege et al., 2011), and more recently, farm-like indoor microbiota (Kirjavainen et al., 2019) has been shown to offer protection from asthma, but not from atopy, and the latter, irrespectively of living in farming or other environments. In a large multicenter RHINE III study, growing up on farm and urban-rural gradient were inversely associated with allergic rhinitis in adulthood, which was suggested to be related to microbial diversity in early childhood (Christensen et al., 2016).

Most of the studies utilizing high throughput sequencing (HTS) approaches have explored the link between indoor microbial exposure and asthma (Birzele et al., 2017; Dannemiller et al., 2014; Dannemiller et al., 2016; Karvonen et al., 2019; O'Connor et al., 2018; Stein et al., 2016) and atopy (Kirjavainen et al., 2019; Lynch et al., 2014; Tischer et al., 2016). In a study by Tischer et al. (2016) using data from the urban LISA Munich sub-cohort and pre-HTS DNA-profiling methods, high fungal Simpson diversity index in early childhood was inversely associated with inhalant atopy at 6 years of age, but this effect attenuated towards the age of 10 years. In a birth cohort study with high-risk urban children in the US, bacterial richness was associated with atopy at the age of 3 years (Lynch et al., 2014), but no such association was found at the age of 7 years (O'Connor et al., 2018). In a cross-sectional study using DNA fingerprinting method, bacterial diversity in mattress dust was inversely associated with inhalant atopy in children aged 6–12 years (Valkonen et al., 2015).

There are no earlier studies utilizing HTS methods that have investigated allergic rhinitis as an outcome. A cross-sectional school study from Europe found that total viable molds and fungal quantitative polymerase chain reaction (qPCR) increased the risk of rhinitis past 12 months (Simoni et al., 2011). In contrast, one sub-urban study among adults with 5 years follow-up found an inverse association between diversity (the number of detected 19 qPCRs) and rhinitis only among random sample of 196 observations, however, no associations were found within atopic or non-atopic subgroups (Juel Holst et al., 2020).

In this study we aimed to gain understanding on the relationship between early-life microbial diversity in house dust and the development of allergic rhinitis and inhalant atopy later in childhood. In order to define the homogeneity of the associations in different environments and populations, we conducted analyses in a Finnish rural-suburban (LUKAS) and a German urban (LISA) birth cohort studies. We used 16 S and ITS amplicon sequencing for a thorough characterization of the bacterial and fungal diversity in house dust collected at early life.

2. Materials and methods

Study population and design. LUKAS, a Finnish, rural-suburban birth cohort consists of children born in Middle and Eastern Finland: the first half of the study population (children were born between September 2002 and May 2004, N = 214) is part of a European birth cohort (PASTURE) among farmers with cattle and non-farmers (von Mutius et al., 2006), while the second half of the cohort is an extension to LUKAS study with unselected children born between May 2004 and May 2005 (LUKAS2, N = 228) (Karvonen et al., 2009). Dust samples were collected from living room floors/rugs at the age of 2 months. Questionnaires on socioeconomic factors were collected from parents during pregnancy, and on environmental exposures and health ages 2, 12, and 18 months, annually between ages 2–6 years, and at the age of 10.5 years. The study protocol was approved by the Research Ethics Committee of the Hospital District of Northern Savo, Kuopio, Finland (Karvonen et al., 2009). A written informed consent was obtained from the parents.

LISA is a German birth cohort comprising term-born, healthy children with normal birthweight. Pregnant women, whose children were born between 1997 and 1999 in Munich, Leipzig, Wesel and Bad Honnef, were recruited (n = 3094) (Heinrich et al., 2002). House dust samples from child's bedroom floor (see details below) were collected in Leipzig and Munich populations (n = 2443). Questionnaires on environmental exposures, health and socioeconomic factors were collected from parents at child's birth and ages 6, 12, 18, 24 months, 4, 6, 10 and 15 years. The present study population was selected based on a case-cohort design originally for asthma or ADHD analyses, with at least 3 out of 4 follow-ups between ages 4 and 15 years, and dust samples collected during early life; detailed information on this case-cohort design is provided elsewhere (Casas et al., 2019). For the present analysis, participants with available information on sequencing data and 10-year outcomes were included. A description of the selection of the present study populations are provided in the Supplemental Material (Supplemental figure S1). All study protocols were approved by the local ethics committees (Department of Medicine (LMU), Medical Faculty of the University of Leipzig, Bavarian Board of Physicians and Board of Physicians of Saxony, respectively).

Inhalant atopy. Venous blood samples were collected from 270 children at the age of 10.5 years (mean age 10.46 years, SD 0.31) in LUKAS and from 181 children at the age of 10 years in LISA (mean age 10.20, SD 0.21). In LUKAS, samples were analyzed for allergen-specific immunoglobulin E (sIgE) against 13 inhalant allergens (Mediwiss Analytic, Moers, Germany): two house dust mites (*Dermatophagoides pteronyssinus* and *D. farinae*), 7 pollens (alder, birch, European hazel, grass pollen mixture, rye, mugwort and plantain), cat, horse and dog dander, and the mold *Alternaria alternata*. In LISA, specific serum IgE concentrations were assayed by the CAP-RAST FEIA system (Pharmacia Diagnostics, Freiburg Mediwiss Analytic, Moers, Germany) according to the manufacturer's instructions. First, a screening test (SX1) was used to test inhalant allergic sensitization and then, if positive, specific allergens against 8 inhalant allergens were analyzed: house dust mite (*D. pteronyssinus)*, four pollens (timothy grass, birch, rye, and mugwort), cat and dog dander, and the mold *Cladosporium herbarum*. Inhalant atopy was defined as sensitization against at least one of the 13 or 8 tested specific inhalant allergens (≥0.70 kU/l) in LUKAS or in LISA, respectively.

Allergic rhinitis. The definition of allergic rhinitis was based on

sensitization to at least one specific inhalant allergen (≥3.50 kU/L) and parent reported rhinoconjunctivitis, which was based on positive answers to both of the following two The International Study of Asthma and Allergies in Childhood (ISAAC, 1998) questions in the 10-year questionnaires: "During the last 12 months, has your child had attacks of sneezing or runny, blocked or itchy nose without having a cold?" and "Has your child ever had itchy or runny eyes along with these nose symptoms?". Higher threshold for inhalant atopy (≥3.50 kU/L) was selected for the definition of allergic rhinitis in order to increase specificity, to exclude subjects for example with vasomotor rhinitis.

Dust samples. In LUKAS study, dust samples were collected from 422 homes when the children were 2 months of age. The dust sample was vacuumed from living room floor using a nylon dust sampling sock (Karvonen et al., 2014). Sample processing and DNA extraction were done from 401 samples which had enough dust left (≥10 mg), and these processed have been previously described in detail (Karvonen et al., 2019; Kirjavainen et al., 2019).

In LISA, the microbial exposure was evaluated from child's bedroom floor (N = 284) and living room floor (N = 142) dust samples collected at the age of 3 months. Samples were collected on ALK filters with a vacuum cleaner. Sampling and processing of house dust samples in the LISA cohort as well as DNA extraction has been described in detail earlier (Casas et al., 2019). In brief, the floor dust samples were collected at the child's age of 3 months from the child's bedroom, and part of the houses also form living rooms, onto ALK filters with a vacuum cleaner, stored at −20 °C and shipped to the analyzing laboratory at Finnish Institute for Health and Welfare, Kuopio, on dry ice in 2016. After the dust samples were homogenized through a sterile strainer to remove larger particles (same procedure as in LUKAS cohort samples) and aliquoted, DNA was extracted from a target amount of 20 mg of dust, using bead-milling technique on MiniBeadbeater-16 (Biospec Products, Inc. USA) for mechanical cell disruption (Haugland et al., 2002), and Chemagic DNA Plant-Kit (PerkinElmer chemagen Technologie GmbG, Germany) for cleaning the extracted DNA. The DNA was stored at −20 °C and shipped on dry ice to the sequencing service provider. Due to lower number of samples from living room, bedroom floor samples were used for health analyses.

Amplicon sequencing. Targeted DNA amplification using primers targeting the V4 region of the bacterial 16 S rRNA gene (Caporaso et al., 2011) and the fungal ITS1 region (Smith and Peay 2014) and amplicon sequencing on Illumina MiSeq® v3 platform producing 300bp paired end reads was performed, and processing were performed as previously described (Casas et al., 2019; Jayaprakash et al., 2017).

Bioinformatic analyses. Sequence processing was performed separately for both birth cohorts with similar fashion. In brief, sequence processing largely relied on QIIME (Quantitative Insights Into Microbial Ecology) (Caporaso et al., 2011). Negative reagent controls were included in DNA extraction, PCR, sequencing and sequence processing to identify contaminants and to inform sequence number threshold for inclusion of samples and rarefaction in diversity calculations. In LUKAS, samples with less than 2150 sequences for bacteria (n = 7) and 2700 sequences for fungi (n = 19) were excluded from the analysis; in LISA, the respective values were 1026 (n = 5) and 996 (n = 9) for bacteria and fungi. The same values were used as rarefaction values for calculation of alpha diversity measures in QIIME, and presented as a mean of ten iterations. The bacterial and fungal richness in the samples was estimated using the Chao1 richness estimate predicting the number of different OTUs detectable in a sample. Shannon diversity index was calculated as an estimate of 'true' taxa diversity taking into account both richness and evenness of individual OTU's (homogeneity of abundance) in a sample.

Statistical analyses. All analyses were conducted separately for both birth cohorts. Differences in the diversity indices in relation to outcomes were tested with Mann-Whitney *U* test and weighted Mann-Whitney *U* test in LUKAS and LISA, respectively. Comparisons of prevalence of outcomes, microbial diversity indices and differences of living environments were tested with Chi square, Spearman's rank correlation, Kruskal-Wallis, and post hoc Scheffe test. Adjusted associations between each microbial diversity index and the outcomes were tested with logistic regression models in LUKAS and weighted logistic regression in LISA using cohort-specific tertiles as cut offs. Based on the original case-cohort study design in LISA, observations in the random sample were weighted with 1/(250/1353) and observations in the asthma and ADHD-enriched samples were not weighted. Due to probability difference between selection process, the observations from the random sample were weighted with 5.412 (multiplied by 5.412) and the observations that belonged to the enrichment groups were weighted with 1 (i.e. no weighting was performed) in Mann-Whitney U statistic test and weighted logistic regression analyses. Results remained unchanged when the models were rerun without farm home samples (n = 2) or when three participants, who had received immunotherapy for allergic rhinitis but were categorized as no allergic rhinitis, were excluded from the analyses of allergic rhinitis in LISA. In LISA, diversity indices between samples, which were taken from bedroom and living room floors (N = 120 with both samples), were compared using paired T-test and Spearman rank correlation.

Models were adjusted for a set of a priori selected confounders, which included gender, parental atopy (either mother or father reporting asthma, eczema or hay fever, if at least one of the parents were answered the question) and number of older siblings in both cohorts; maternal education, living on a farm and cohort in LUKAS; and parental education (the highest level of either parent) and study center (Munich, Leipzig) in LISA. Additional confounders (birth weight, mode of delivery, season of dust sampling, environmental tobacco smoke exposure, dog or cat ownership, duration of breastfeeding, day care attendance under in age 1 years or less, maternal age at delivery, building structure of the house, and mold or moisture damage in the home) were tested in the model with bacterial richness and allergic rhinitis separately in both cohorts: confounders, which changed the estimates more than 10% were included in the final adjusted models. Thus, the final models included all a priori selected confounders and the following additional confounders: season of dust sampling in both cohorts; the number of different pet species indoors in LUKAS; and age of the mother during delivery in LISA.

Three level living environment variable was created using information on inclusion criteria (farm/non-farm, but rural) in the LUKAS1; and with a question inquired from the pregnant mother in LUKAS2: "Do you live in a 1) center of a town, 2) suburban area, 3) built-up area in the country side (village or village community), or 4) rural areas in the country side?" In LUKAS2, positive answers to options 1 or 2 were recoded as living in suburban areas, and positive answer to options 3 or 4 as rural areas. Due to relatively low number of observations and cases in the stratified analyses of three living environments in LUKAS (especially with allergic rhinitis: amongst the 96 children living on farms and 56 children from suburban areas in LUKAS, only 9 and 7 children had allergic rhinitis, respectively), no multivariate logistic regression models were performed, and the inhalant atopy models were performed without adjusting for the number of different pet species.

The study populations in the bacterial and fungal sequencing analyses were 395 and 382 in LUKAS, and 284 and 260 in LISA, respectively, and in the analyses with bacterial and/or fungal sequencing data, allergic rhinitis and/or inhalant atopy, and the confounders, were 312 in LUKAS and 248 in LISA. Out of 312 children, 88% at the age of 2 years, and 74% at the age of 6 years, lived in the same house as from where the dust sample was collected at the age of 2 months (mean age 2.2 months, SD 0.8) in LUKAS. The respective figures in LISA were 80% and 57%, but this information was only available from families participating through the Munich study center (N = 147). The mean age for dust sampling was 3.2 months (SD 0.6) in LISA. The data were analyzed using SAS 9.3 SAS for Windows (Institute Inc., Cary, NC, USA) and R version 3.6.0 (R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria, https://www.R-project.org/).

3. Results

The prevalence of allergic rhinitis and atopy were 11.5% and 46% in LUKAS and 10% and 42% in LISA, respectively (Table 1).

The most prevalent sensitization against specific inhalant allergens varied between cohorts, and the biggest difference between cohorts was found with sensitization against *Dermatophagoides pteronyssinus* (3% in LUKAS and 25% in LISA) (Supplement Table S1). Among LUKAS population, 31% of the children lived in farm houses, whereas LISA homes were mainly located in urban environment (Table 1). The number of older siblings was higher in LUKAS than in LISA. The prevalence of allergic rhinitis and inhalant atopy were lower in LUKAS children who lived in farms compared to rural and suburban areas, but the differences were not statistically significant. Description of the levels of bacterial and fungal diversity metrics in both cohorts are presented in Supplemental Table S2.

3.1. Comparison between living environments in LUKAS

Bacterial richness and Shannon entropy and fungal richness were significantly different in farm, rural and suburban houses (Kruskal-Wallis test, $p < 0.001$): the highest levels were measured in farm homes, then rural homes and the lowest in suburban homes (Supplemental figure S2). In the post hoc tests, the levels of these diversity indices in house dust were significantly higher in farm houses than in rural or suburban homes, and only fungal richness was significantly higher in rural (non-farm) homes than in suburban homes (Scheffe post hoc test, *p-value* < 0.05). Fungal Shannon entropy did not differ between three different groups of living environment (Kruskal-Wallis test, $p = 0.41$).

Table 1
Description of the two study populations.

		LUKAS (N = 312)		LISA (N = 248)	
		n	%	n	%
Inhalant atopy	Yes	123	45.6	76	42.0
Allergic rhinitis	Yes	36	11.5	25	10.2
Gender	Girls	156	50.0	113	45.6
Parental history of allergic diseases	Yes	230	73.7	144	58.1
Number of older siblings	None	101	32.4	142	57.3
	One	109	34.9	83	33.5
	≥2	102	32.7	23	9.3
Season of dust sampling	Winter	84	26.9	65	26.2
	Spring	92	29.5	63	25.4
	Summer	80	25.6	54	21.8
	Autumn	56	18.0	66	26.6
Cohort (LUKAS)/Center (LISA)	LUKAS2/ Munich	153	49.0	162	65.3
Living on a farm	Yes	96	30.8	2	0.9
Number of pet species indoors	None	123	39.4	173	70.0
	One	124	39.7	53	21.5
	≥2	65	20.8	21	8.5
Maternal age during delivery (years)	<29.5	12	38.5	78	21.5
	29.5–33.5	92	29.5	93	37.5
	>33.5	100	32.0	77	31.1
Education level		65	20.8	178	71.8

Footnote: Data from bacterial and fungal sequencing missing n = 1 and n = 11 in LUKAS and n = 4 and n = 7 in LISA, respectively. Inhalant atopy at 10 years was defined as any tested 13 or 8 inhalant allergen ≥0.70 kU/L in LUKAS and LISA, respectively. The number of missing information with inhalant atopy was 42 in LUKAS and 67 in LISA. Allergic rhinitis was based on parent reported rhinitis symptoms and positive result in inhalant atopy (≥3.50 kU/L). The information on allergic rhinitis was missing in 2 observations in LISA. Parental history of allergic diseases was defined as either parent reported of asthma, allergic rhinitis and/or atopic eczema. Cohort is referring to LUKAS and center to LISA. Living on a farm was enquired at birth in LUKAS and at the age of 4 years in LISA (missing information on 14 observations in LISA). The information on the number of pet species is missing from one observation in LISA. Education level: maternal academic education in LUKAS and high parental education level in LISA.

3.2. Sampling locations within the home in LISA

In a subgroup of homes in LISA, dust samples were taken from both bedroom and living room floors (N = 120). Bacterial but not fungal diversity was significantly lower in bedroom than living room samples (Supplemental Table S3). Accordingly, the correlation between the diversity measures between bedroom and living room were lower for the bacterial ($r = 0.39$ and 0.36) than fungal richness and Shannon entropy ($r = 0.55$ and 0.58, respectively).

3.3. Bacterial diversity in house dust versus allergy outcomes

In unadjusted (Fig. 1 and Supplement Table S4) and adjusted (Table 2) analyses, the measures of bacterial richness and Shannon entropy in the early-life house dust were significantly lower in the homes of children with allergic rhinitis than in the homes of children without allergic rhinitis at 10 years of age in LISA. No significant differences were found in LUKAS, though the associations had the same direction as in LISA. Due to low number of children with allergic rhinitis in farms and suburban areas, only unadjusted stratified analyses were performed: no significant differences were found between levels of bacterial diversity in the homes of children with allergic rhinitis than in the homes of children without allergic rhinitis in three living areas (Supplemental figure S3).

Few consistent associations were seen with atopy. Bacterial richness in house dust tended to be inversely associated with inhalant atopy in LISA, but not in LUKAS, and the middle tertile of Shannon entropy was positively associated in LUKAS, but not in LISA (Table 2). In stratified analyses in LUKAS, the associations between bacterial Shannon entropy in dust samples and inhalant atopy in children were different depending on whether they had grown up on farms, rural (non-farm) or suburban areas (p-value for interaction term, $p = 0.11$, Table 3). Bacterial Shannon entropy was positively associated with the risk of inhalant atopy in rural children and similar tendency was seen among farm children while in suburban children the association was inverse (Table 3). Similar suggestion was found with bacterial richness.

3.4. Fungal diversity and allergy outcomes

The fungal richness and Shannon entropy were neither associated with allergic rhinitis nor with inhalant atopy in either cohort in unadjusted analyses (Supplement Table S4). After adjustments, no dose-dependent associations were found with allergic rhinitis in both cohorts, however, the middle tertiles of fungal richness and Shannon entropy were positively associated with inhalant atopy only in LISA (Table 2). In stratified analyses in LUKAS, the associations between fungal Shannon entropy and inhalant atopy in children tended to be different depending on the living environment (*p*-value for interaction term, $p = 0.12$, Table 3). Fungal Shannon entropy tended to be positively associated with the risk of inhalant atopy in suburban children, but had an opposite tendency with farm or rural children (Table 3).

3.5. Sensitivity analyses

Compared to results with bacterial and fungal richness and Shannon entropy with allergic rhinitis, the results were very similar when rhinoconjunctivitis was analyzed (without inhalant atopy restriction) in LUKAS and LISA (Supplemental Table S5). Only associations between fungal Shannon entropy and rhinoconjunctivitis were different in three environments in LUKAS (interaction term, p-value *0.001*): protective in farms and suburban areas, but were a risk factor for children in rural

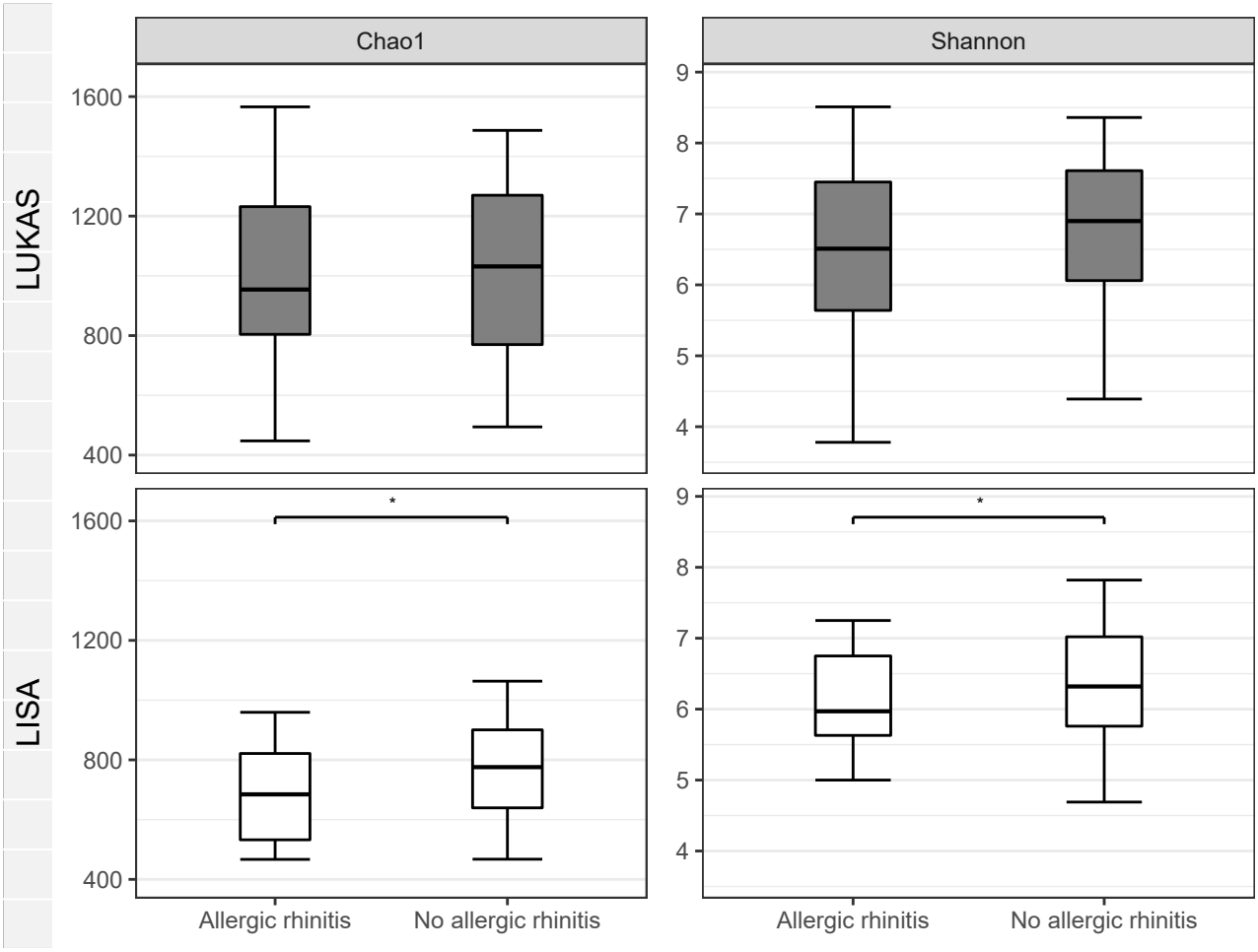

Fig. 1. Comparisons between the levels of bacterial richness (Chao1) and Shannon entropy in dust samples collected from homes of children with or without allergic rhinitis at the age of 10 years in LUKAS (grey boxes) and LISA (white boxes) cohorts.
The boxplots present 5th percentile, first quartile, median, third quartile, and 95th percentile. An asterisk shows statistically significant difference ($p = 0.001$ for richness and *0.009* for Shannon entropy) from weighted Man-Whitney U –test. Allergic rhinitis LUKAS, bacteria: n/N = 35/311 and fungi 35/301 and respectively in LISA 24/242 and 22/23. Associations with microbial richness/diversity were analyzed within each cohort and visualized separately because the absolute richness and diversity values are not fully comparable between cohorts due to separate sample analysis and sequence processing steps.

areas (data not shown). When different cut-offs were used in the inhalant atopy (i.e. ≥0.35 or ≥3.50kU/L) analyses, the results with bacterial diversity results were more robust (i.e. showing same direction in the associations) than fungal diversity results (data not shown). In LISA, the results did not change when analyses were rerun only in random sample (i.e. without weighting, data not shown).

When excluding children who moved to a new home before the age of 2 years from the analyses, the estimates and p-values were closely similar compared to the original models of bacterial and fungal diversity and both outcomes in LUKAS and LISA (data not shown). Neither did moving prior to the age of 6 years have influence on the results in LUKAS. Unfortunately we cannot perform similar sensitivity analyses among Munich participants because almost half of them moved prior to the age of 6 years making the subgroup of non-movers too small for statistical analyses, however, no differences were found in the levels of bacterial and fungal diversities between non-movers and movers before the age of 6 years in the LISA (weighted Mann Whitney *U* test, p-values >0.1) (data not shown).

4. Discussion

In the present study we showed in two separate study populations that high bacterial diversity in early-life home was associated with protection from allergic rhinitis later in life. The effect was significant only in the urban LISA cohort in Germany, although similar suggestion was observed also in the rural-suburban LUKAS cohort in Finland. No significant associations were found between fungal diversity and allergic rhinitis. Few consistent associations were seen with inhalant atopy. Bacterial diversity was associated with increased odds of inhalant atopy only in rural areas in LUKAS, whereas it tended to be opposite in suburban or urban areas in both cohorts. Fungal diversity, on the other hand, tended to be associated with increased risk of inhalant atopy only in LISA.

This is, to our knowledge, the first prospective study to investigate the associations of allergic rhinitis and both fungal and bacterial exposure using high-throughput sequencing for an accurate determination of the indoor microbiota. Sampling was performed at early childhood, i.e. during the crucial developmental phase of the immune system. Children growing up in farm environments are exposed to high bacterial richness (Kirjavainen et al., 2019) and it has been consistently shown that allergic rhinitis is less common among children who grow up on farms (von Mutius and Vercelli 2010), although the role and importance of microbial exposure in this association is not established. Our study is the first prospective study to provide evidence for an association between allergic rhinitis and bacterial exposure. Earlier studies have shown inverse associations between high exposure to microbial cell wall markers, such as endotoxin (Braun-Fahrländer et al., 2002; Celedón et al., 2007) and allergic rhinitis in both rural and urban environments in

Table 2
Adjusted associations between bacterial and fungal diversity indices and inhalant atopy and allergic rhinitis at the age of 10.5/10 years in LUKAS and LISA.

		Allergic rhinitis							Inhalant atopy						
		LUKAS				LISA			LUKAS				LISA		
Bacterial diversity	Tertile	N	%	aOR (95%CI)	*p*	N	%	aOR (95%CI)	*p*	N	%	aOR (95%CI)	*p*	N	%	aOR (95%CI)	*p*
Richness	Lowest	99	13.1	1		314	12.3	1		88	48.9	1		237	35.1	1	
	Middle	107	9.4	0.49 (0.18, 1.32)	*0.16*	307	3.7	**0.19 (0.09, 0.42)**	***<0.0001***	89	41.6	0.84 (0.43, 1.65)	*0.61*	209	37.8	0.99 (0.62, 1.58)	*0.96*
	Highest	105	11.4	0.82 (0.24, 2.84)	*0.76*	331	2.8	**0.12 (0.05, 0.29)**	***<0.0001***	92	45.7	1.60 (0.66, 3.87)	*0.29*	242	29.1	0.71 (0.44, 1.15)	*0.16*
Shannon entropy	Lowest	104	13.5	1		328	10.4	**1**		89	42.7	1		258	33.5	1	
	Middle	107	10.3	0.86 (0.35, 2.13)	*0.74*	288	3.3	**0.16 (0.07, 0.35)**	***<0.0001***	92	52.2	**1.98 (1.04, 3.78)**	***0.04***	177	35.7	0.76 (0.48, 1.20)	*0.24*
	Highest	100	10.0	0.86 (0.28, 2.65)	*0.78*	337	4.7	**0.27 (0.13, 0.55)**	***<0.0001***	89	40.9	1.62 (0.70, 3.73)	*0.26*	254	32.8	0.91 (0.58, 1.42)	*0.68*
Fungal diversity																	
Richness	Lowest	99	11.1	1		279	4.8	1		89	48.3	1		192	27.6	1	
	Middle	97	10.3	0.85 (0.32, 2.44)	*0.75*	303	4.9	0.99 (0.43, 2.28)	*0.99*	82	39.0	0.65 (0.34, 1.25)	*0.20*	197	35.9	**1.90 (1.11, 3.25)**	***0.02***
	Highest	105	13.3	1.02 (0.39, 2.67)	*0.96*	345	4.6	0.78 (0.35, 1.76)	*0.56*	89	49.4	1.15 (0.58, 2.26)	*0.69*	286	32.9	1.40 (0.87, 2.23)	*0.16*
Shannon entropy	Lowest	99	10.1	1		293	6.1	1		86	44.2	1		198	27.2	1	
	Middle	100	13.0	1.10 (0.43, 2.82)	*0.84*	303	5.2	0.98 (0.46, 2.13)	*0.97*	88	44.3	0.81 (0.43, 1.55)	*0.53*	219	34.6	**1.93 (1.19, 3.13)**	***0.007***
	Highest	102	11.8	1.18 (0.45, 3.11)	*0.73*	331	3.1	0.50 (0.21, 1.21)	*0.12*	86	48.8	1.04 (0.54, 1.99)	*0.90*	257	34.1	1.54 (0.94, 2.53)	*0.09*

Footnote: N number of observations in a class, and % percentage of observations of the outcome in a class. Weighted frequencies presented in LISA. aOR adjusted odds ratios, (95%CI) their 95% confidence intervals and p-values are from adjusted logistic and weighted logistic regression models in LUKAS and LISA, respectively. Models are adjusted for maternal education, cohort, living on a farm, gender, parental history of allergic diseases, older siblings, season for dust sampling and the number of different pet species at home in LUKAS; and for parental education, gender, the history of parental allergic diseases, center, older siblings, mother age at birth and season for dust sampling in LISA.

cross-sectional and prospective studies. However, in some cross-sectional urban/rural studies such associations have not been seen at all or they have been only suggestive (Gehring et al., 2002, 2008; Marinho et al., 2007; Moniruzzaman et al., 2012). Thus, our findings on the importance of bacterial diversity support earlier observations that high environmental bacterial exposure at early age may be essential for stimulating immune development. Our findings also indicate that the protective effect of high bacterial diversity on the development of allergic rhinitis can be achieved not only on farms but also in urban environments.

We observed that the protective effect of bacterial diversity against development of allergic rhinitis was more pronounced in the German urban study compared to Finnish rural-suburban cohort. The results were unchanged when only random sample was used in the analyses in German cohort and thus, the observed associations were not result of the weighting in the models. Unfortunately, the numbers of children with allergic rhinitis were too low to perform stratified multivariable analyses in suburban and farm environments in the Finnish cohort, and we may have also been underpowered to show any difference in the unadjusted analyses either. Another explanation could be that children growing up on farms with cattle may be misclassified by exposure more than children who grow up on suburban or urban areas, because their true exposure – considering short term visits in or in vicinity of stables for example - can be 5- to 7-fold higher than measured in house dust, as previously reported by Schram et al. (2005). Therefore, an effect of early-life home microbiota on allergic disease development may be more difficult to observe in this sub-population, as has been hypothesized also in earlier studies (Valkonen et al., 2015).

Present study did not find association between fungal diversity and allergic rhinitis, which has not been studied earlier in children. Due to currently limited and inconsistent results with fungal exposure from the present study with two cohorts, further prospective studies are needed to discover the role of fungal diversity in the development of allergic rhinitis.

Few consistent associations were seen with inhalant atopy between cohorts. Bacterial diversity was associated with increased risk of inhalant atopy only in rural areas in LUKAS, whereas it tended to be opposite in suburban or urban areas. This is in accordance with some previous reports where bacterial richness has been inversely associated with atopy in urban children at 3 years of age (Lynch et al., 2014), but not in farm children at school age (Ege et al., 2011). In a study based on DNA fingerprinting, bacterial Shannon diversity in mattress dust was inversely associated with inhalant atopy in children aged 6–12 years, and the protective effect was stronger in non-farm children although visible in the whole study population (Valkonen et al., 2015). Even if methods for determination of bacterial richness from house dust varied between studies, bacterial richness seems to have stronger protective effect on atopy in urban areas than in farms where the overall microbial exposure is much higher than in urban environments.

In our study, high fungal diversity in house dust tended to predispose for developing inhalant atopy in the urban cohort LISA. In the same cohort an inverse association has been reported between fungal Simpson diversity in early childhood and inhalant atopy at the age of 6 but not 10 years of age (Tischer et al., 2016). However, the study population was smaller than in the present study, including only Munich study center. Also, the association between fungal diversity and inhalant atopy reported by Tischer et al. (2016) was not dose-dependent but rather inverse U-shape. Partly inconsistent findings may be due to differences between sampled room (living room vs bedroom), and methods to characterize microbiome (tRELP vs 16 S rRNA/ITS amplicon) in the earlier and present report. Differences in calculating Simpson versus Shannon diversity indices do not explain the differences observed in the studies, because these two indices were highly correlated in our study ($r = 0.97$). Studies using earlier DNA-profiling methods detect only the more prevalent taxa in a sample and will provide generally lower resolution of richness compared to next generation sequencing, thus are

Table 3
Adjusted associations between bacterial and fungal diversity and inhalant atopy in LUKAS, stratified by living environment.

		Inhalant atopy									
		Living on farms			Living in rural areas			Living in suburbans			
Bacterial diversity	Tertile	N	%	aOR (95%CI)	N	%	aOR (95%CI)	N	%	aOR (95%CI)	*p*
Richness	Lowest	3	33.3	1	61	44.3	1	24	62.5	1	
	Middle	18	38.9	2.26 (0.14, 35.54)	58	44.8	1.34 (0.57, 3.15)	13	30.8	**0.09 (0.01, 0.70)**	
	Highest	65	38.5	1.43 (0.11, 18.70)	20	65.0	**3.63 (1.05, 12.56)**	7	57.1	0.74 (0.07, 7.92)	*0.26*
Shannon entropy	Lowest	4	25.0	1	64	39.1	1	21	57.1	1	
	Middle	19	63.2	5.67 (0.4, 79.77)	58	51.7	1.83 (0.84, 3.98)	15	40.0	0.59 (0.12, 2.90)	
	Highest	63	31.8	1.43 (0.12, 17.5)	17	64.7	**3.86 (1.06, 14.11)**	8	62.5	2.09 (0.24, 18.44)	***0.11***
Fungal diversity											
Richness	Lowest	17	47.1	1	47	46.8	1	25	52.0	1	
	Middle	25	32.0	0.28 (0.06, 1.29)	45	40.0	0.88 (0.36, 2.16)	12	50.0	0.80 (0.14, 4.48)	
	Highest	39	41.0	0.48 (0.12, 1.97)	44	56.8	1.95 (0.72, 5.31)	6	50.0	0.26 (0.02, 4.09)	*0.87*
Shannon entropy	Lowest	30	43.3	1	39	48.7	1	17	35.3	1	
	Middle	28	28.6	0.37 (0.11, 1.26)	46	45.7	0.76 (0.30, 1.89)	14	71.4	5.11 (0.73, 36.04)	
	Highest	23	47.8	0.81 (0.22, 2.96)	51	49.0	0.78 (0.31, 1.96)	12	50.0	1.98 (0.28, 14.17)	***0.12***

Footnote: N number of observations in a class, and % percentage of observations of the outcome in a class. aOR adjusted odds ratios, (95%CI) their 95% confidence intervals from adjusted logistic regression models for maternal education, cohort, gender, parental history of allergic diseases, older siblings, and season for dust sampling. p-values for interaction term. Significant associations ($p < 0.05$) are indicated in bold.

methodologically not fully comparable to the current study.

The main strength of our study is the relatively large sample size and utilizing two birth cohort studies from different countries. To our knowledge this is the first HTS study on early-life microbial exposure and allergic rhinitis later in childhood and the first study to report results from two cohorts including children from both urban and rural environments. It should be noted that the laboratory method of determining specific IgE against inhalant allergens and statistical methods were different in the two cohorts. However, in sensitivity analyses, the results were fairly robust when different cut offs were used, and when the results were rerun only using random sample in the LISA. Another limitation is that we used information on the symptoms of allergic rhinitis at the age of 10 years and thus cannot exclude those who had earlier onset with remission. Also, we cannot rule out the possibility that some of the children may have received immunotherapy and could have been misclassified as children with no allergic rhinitis in the LUKAS study, but based on the results from the LISA where only three out of fourteen participants who had received immunotherapy were categorized as having no allergic rhinitis, this seems unlikely or at least to be a very rare event. Due to practical and financial reasons, microbial exposure in early life was evaluated from dust samples collected from floors or rugs rather than active air samples. We have previously shown that the microbial composition in carpet dust may not fully reflect the inhalation exposure of the infant due to differences in resuspension of different microbial taxa from floor dust (Hyytiäinen et al., 2018). However, children are exposed to environmental microbes also through other exposure routes (dermal contact, ingestion) for which floor dust could be a decent surrogate sample (Kirjavainen et al., 2019). Lastly, we are unclear whether different methods of collecting dust samples (nylon dust socks vs. ALK) in these cohorts would impact on the microbial diversity in dust due to no one has performed validation in the context of amplicon sequencing, although these methods have been previously compared for allergen and endotoxin measurements (Wickens et al., 2004): higher dust sample amounts was collected with the nylon sock compared to the ALK filter cassette.

In conclusion, our study suggests that higher bacterial diversity in early-life home may reduce the risk of allergic rhinitis later in childhood. The environment-dependent heterogeneity in the associations with inhalant atopy suggests that specific constituents of the measured diversity may be relevant, but future studies will need to follow-up on this indication.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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References

Birzele, L.T., Depner, M., Ege, M.J., Engel, M., Kublik, S., Bernau, C., Loss, G.J., Genuneit, J., Horak, E., Schloter, M., Braun-Fahrländer, C., Danielewicz, H., Heederik, D., von Mutius, E., Legatzki, A., 2017. Environmental and mucosal microbiota and their role in childhood asthma. Allergy 72, 109–119. https://doi.org/10.1111/all.13002 ([doi]).

Braun-Fahrländer, C., Riedler, J., Herz, U., Eder, W., Waser, M., Grize, L., Maisch, S., Carr, D., Gerlach, F., Bufe, A., Lauener, R.P., Schierl, R., Renz, H., Nowak, D., von Mutius, E., Allergy and Endotoxin Study Team, 2002. Environmental exposure to endotoxin and its relation to asthma in school-age children. N. Engl. J. Med. 347, 869–877. https://doi.org/10.1056/NEJMoa020057 ([doi]).

Caporaso, J.G., Lauber, C.L., Walters, W.A., Berg-Lyons, D., Lozupone, C.A., Turnbaugh, P.J., Fierer, N., Knight, R., 2011. Global patterns of 16S rRNA diversity at a depth of millions of sequences per sample. Proc. Natl. Acad. Sci. U. S. A. 108 (Suppl. 1), 4516–4522. https://doi.org/10.1073/pnas.1000080107 [doi].

Casas, L., Karvonen, A.M., Kirjavainen, P.V., Täubel, M., Hyytiäinen, H., Jayaprakash, B., Lehmann, I., Standl, M., Pekkanen, J., Heinrich, J., 2019. Early life home microbiome and hyperactivity/inattention in school-age children, 019-53527-1 Sci. Rep. 9, 17355. https://doi.org/10.1038/s41598-019-53527-1 [doi].

Celedón, J.C., Milton, D.K., Ramsey, C.D., Litonjua, A.A., Ryan, L., Platts-Mills, T.A., Gold, D.R., 2007. Exposure to dust mite allergen and endotoxin in early life and asthma and atopy in childhood. J. Allergy Clin. Immunol. 120, 144–149. S0091-6749(07)00629-X [pii].

Christensen, S.H., Timm, S., Janson, C., Benediktsdóttir, B., Forsberg, B., Holm, M., Jogi, R., Johannessen, A., Omenaas, E., Sigsgaard, T., Svanes, C., Schlünssen, V., 2016. A clear urban-rural gradient of allergic rhinitis in a population-based study in Northern Europe. Eur. Clin. Respir. J. 3, 33463. https://doi.org/10.3402/ecrj.v3.33463 [doi].

Dannemiller, K.C., Gent, J.F., Leaderer, B.P., Peccia, J., 2016. Influence of housing characteristics on bacterial and fungal communities in homes of asthmatic children. Indoor Air 26, 179–192. https://doi.org/10.1111/ina.12205 [doi].

Dannemiller, K.C., Mendell, M.J., Macher, J.M., Kumagai, K., Bradman, A., Holland, N., Harley, K., Eskenazi, B., Peccia, J., 2014. Next-generation DNA sequencing reveals that low fungal diversity in house dust is associated with childhood asthma development. Indoor Air 24, 236–247.

Ege, M.J., Mayer, M., Normand, A.C., Genuneit, J., Cookson, W.O., Braun-Fahrlander, C., Heederik, D., Piarroux, R., von Mutius, E., GABRIELA Transregio 22 Study Group, 2011. Exposure to environmental microorganisms and childhood asthma. N. Engl. J. Med. 364, 701–709. https://doi.org/10.1056/NEJMoa1007302 [doi].

Gehring, U., Bischof, W., Fahlbusch, B., Wichmann, H.E., Heinrich, J., 2002. House dust endotoxin and allergic sensitization in children. Am. J. Respir. Crit. Care Med. 166, 939–944. https://doi.org/10.1164/rccm.200203-256OC [doi].

Gehring, U., Strikwold, M., Schram-Bijkerk, D., Weinmayr, G., Genuneit, J., Nagel, G., Wickens, K., Siebers, R., Crane, J., Doekes, G., Di Domenicantonio, R., Nilsson, L., Priftanji, A., Sandin, A., El-Sharif, N., Strachan, D., van Hage, M., von Mutius, E., Brunekreef, B., ISAAC Phase Two Study Group, 2008. Asthma and allergic symptoms in relation to house dust endotoxin: phase two of the international study on asthma and Allergies in childhood (ISAAC II). Clin. Exp. Allergy 38, 1911–1920. https://doi.org/10.1111/j.1365-2222.2008.03087.x [doi].

Haugland, R.A., Brinkman, N., Vesper, S.J., 2002. Evaluation of rapid DNA extraction methods for the quantitative detection of fungi using real-time PCR analysis. J. Microbiol. Methods 50, 319–323. S0167701202000374 [pii].

Heinrich, J., Bolte, G., Hölscher, B., Douwes, J., Lehmann, I., Fahlbusch, B., Bischof, W., Weiss, M., Borte, M., Wichmann, H.E., LISA Study Group, 2002. Allergens and endotoxin on mothers' mattresses and total immunoglobulin E in cord blood of neonates. Eur. Respir. J. 20, 617–623. https://doi.org/10.1183/09031936.02.02322001 [doi].

Huang, S.W., 2000. The risk of sinusitis in children with allergic rhinitis. Allergy Asthma Proc. 21, 85–88. https://doi.org/10.2500/108854100778250905 ([doi]).

Hyytiäinen, H.K., Jayaprakash, B., Kirjavainen, P.V., Saari, S.E., Holopainen, R., Keskinen, J., Hameri, K., Hyvärinen, A., Boor, B.E., Täubel, M., 2018. Crawling-induced floor dust resuspension affects the microbiota of the infant breathing zone, 0405-8 Microbiome 6, 25–2018. https://doi.org/10.1186/s40168-018-0405-8 ([doi]).

ISAAC, 1998. The international study of asthma and Allergies in childhood (ISAAC) steering committee: Worldwide variation in prevalence of symptoms of asthma, allergic rhinoconjunctivitis, and atopic eczema. Lancet 351, 1225–1232. S0140673697073029 [pii].

Jayaprakash, B., Adams, R.I., Kirjavainen, P., Karvonen, A., Vepsäläinen, A., Valkonen, M., Järvi, K., Sulyok, M., Pekkanen, J., Hyvärinen, A., Täubel, M., 2017. Indoor microbiota in severely moisture damaged homes and the impact of interventions. Microbiome 5, 138. https://doi.org/10.1186/s40168-017-0356-5 [doi].

Juel Holst, G., Pørneki, A., Lindgreen, J., Thuesen, B., Bønløkke, J., Hyvärinen, A., Elholm, G., Østergaard, K., Loft, S., Brooks, C., Douwes, J., Linneberg, A., Sigsgaard, T., 2020. Household dampness and microbial exposure related to allergy and respiratory health in Danish adults. Eur. Clin. Respir. J. 7, 1706235. https://doi.org/10.1080/20018525.2019.1706235 [doi].

Karvonen, A.M., Hyvärinen, A., Rintala, H., Korppi, M., Täubel, M., Doekes, G., Gehring, U., Renz, H., Pfefferle, P.I., Genuneit, J., Keski-Nisula, L., Remes, S., Lampi, J., von Mutius, E., Pekkanen, J., 2014. Quantity and diversity of environmental microbial exposure and development of asthma: a birth cohort study. Allergy 69, 1092–1101. https://doi.org/10.1111/all.12439 [doi].

Karvonen, A.M., Hyvärinen, A., Roponen, M., Hoffmann, M., Korppi, M., Remes, S., von Mutius, E., Nevalainen, A., Pekkanen, J., 2009. Confirmed moisture damage at home, respiratory symptoms and atopy in early life: a birth-cohort study. Pediatrics 124, e329–338. https://doi.org/10.1542/peds.2008-1590 [doi].

Karvonen, A.M., Kirjavainen, P.V., Täubel, M., Jayaprakash, B., Adams, R.I., Sordillo, J. E., Gold, D.R., Hyvärinen, A., Remes, S., von Mutius, E., Pekkanen, J., 2019. Indoor bacterial microbiota and development of asthma by 10.5 years of age. J. Allergy Clin. Immunol. 144, 1402–1410. S0091-6749(19)31033-4 [pii].

Kirjavainen, P.V., Karvonen, A.M., Adams, R.I., Täubel, M., Roponen, M., Tuoresmäki, P., Loss, G., Jayaprakash, B., Depner, M., Ege, M.J., Renz, H., Pfefferle, P.I., Schaub, B., Lauener, R., Hyvärinen, A., Knight, R., Heederik, D.J.J., von Mutius, E., Pekkanen, J., 2019. Farm-like indoor microbiota in non-farm homes protects children from asthma development. Nat. Med. 25, 1089–1095. https://doi.org/10.1038/s41591-019-0469-4 [doi].

Lynch, S.V., Wood, R.A., Boushey, H., Bacharier, L.B., Bloomberg, G.R., Kattan, M., O'Connor, G.T., Sandel, M.T., Calatroni, A., Matsui, E., Johnson, C.C., Lynn, H., Visness, C.M., Jaffee, K.F., Gergen, P.J., Gold, D.R., Wright, R.J., Fujimura, K., Rauch, M., Busse, W.W., Gern, J.E., 2014. Effects of early-life exposure to allergens and bacteria on recurrent wheeze and atopy in urban children. e12 J. Allergy Clin. Immunol. 134, 593–601. https://doi.org/10.1016/j.jaci.2014.04.018 [doi].

Marinho, S., Simpson, A., Lowe, L., Kissen, P., Murray, C., Custovic, A., 2007. Rhinoconjunctivitis in 5-year-old children: a population-based birth cohort study. Allergy 62, 385–393. ALL1294 [pii].

Moniruzzaman, S., Hägerhed Engman, L., James, P., Sigsgaard, T., Thorne, P.S., Sundell, J., Bornehag, C.G., 2012. Levels of endotoxin in 390 Swedish homes: determinants and the risk for respiratory symptoms in children. Int. J. Environ. Health Res. 22, 22–36. https://doi.org/10.1080/09603123.2011.588322 [doi].

O'Connor, G.T., Lynch, S.V., Bloomberg, G.R., Kattan, M., Wood, R.A., Gergen, P.J., Jaffee, K.F., Calatroni, A., Bacharier, L.B., Beigelman, A., Sandel, M.T., Johnson, C. C., Faruqi, A., Santee, C., Fujimura, K.E., Fadrosh, D., Boushey, H., Visness, C.M., Gern, J.E., 2018. Early-life home environment and risk of asthma among inner-city children. J. Allergy Clin. Immunol. 141, 1468–1475. S0091-6749(17)31204-6 [pii].

Rochat, M.K., Illi, S., Ege, M.J., Lau, S., Keil, T., Wahn, U., von Mutius, E., Multicentre Allergy Study (MAS) group, 2010. Allergic rhinitis as a predictor for wheezing onset in school-aged children. e2 J. Allergy Clin. Immunol. 126, 1170–1175. https://doi.org/10.1016/j.jaci.2010.09.008 [doi].

Schram, D., Doekes, G., Boeve, M., Douwes, J., Riedler, J., Ublagger, E., von Mutius, E., Budde, J., Pershagen, G., Nyberg, F., Alm, J., Braun-Fahrländer, C., Waser, M., Brunekreef, B., PARSIFAL Study Group, 2005. Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children–the PARSIFAL Study. Allergy 60, 611–618. ALL748 [pii].

Simoni, M., Cai, G.H., Norback, D., Annesi-Maesano, I., Lavaud, F., Sigsgaard, T., Wieslander, G., Nystad, W., Canciani, M., Viegi, G., Sestini, P., 2011. Total viable molds and fungal DNA in classrooms and association with respiratory health and pulmonary function of European schoolchildren. Pediatr. Allergy Immunol. 22, 843–852. https://doi.org/10.1111/j.1399-3038.2011.01208.x [doi].

Smith, D.P., Peay, K.G., 2014. Sequence depth, not PCR replication, improves ecological inference from next generation DNA sequencing. PloS One 9, e90234. https://doi.org/10.1371/journal.pone.0090234 ([doi]).

Stein, M.M., Hrusch, C.L., Gozdz, J., Igartua, C., Pivniouk, V., Murray, S.E., Ledford, J.G., Marques dos Santos, M., Anderson, R.L., Metwali, N., Neilson, J.W., Maier, R.M., Gilbert, J.A., Holbreich, M., Thorne, P.S., Martinez, F.D., von Mutius, E., Vercelli, D., Ober, C., Sperling, A.I., 2016. Innate immunity and asthma risk in amish and hutterite farm children. N. Engl. J. Med. 375, 411–421. https://doi.org/10.1056/NEJMoa1508749 [doi].

Tischer, C., Weikl, F., Probst, A.J., Standl, M., Heinrich, J., Pritsch, K., 2016. Urban dust microbiome: impact on later atopy and wheezing. Environ. Health Perspect. 124, 1919–1923. https://doi.org/10.1289/EHP158 [doi].

Valkonen, M., Wouters, I.M., Täubel, M., Rintala, H., Lenters, V., Vasara, R., Genuneit, J., Braun-Fahrländer, C., Piarroux, R., von Mutius, E., Heederik, D., Hyvärinen, A., 2015. Bacterial exposures and associations with atopy and asthma in children. PloS One 10, e0131594. https://doi.org/10.1371/journal.pone.0131594 ([doi]).

von Mutius, E., Schmid, S., PASTURE Study Group, 2006. The PASTURE project: EU support for the improvement of knowledge about risk factors and preventive factors for atopy in Europe. Allergy 61, 407–413. ALL1009 [pii].

von Mutius, E., Vercelli, D., 2010. Farm living: effects on childhood asthma and allergy. Nat. Rev. Immunol. 10, 861–868. https://doi.org/10.1038/nri2871 [doi].

Wang, J., Visness, C.M., Calatroni, A., Gergen, P.J., Mitchell, H.E., Sampson, H.A., 2009. Effect of environmental allergen sensitization on asthma morbidity in inner-city asthmatic children. Clin. Exp. Allergy 39, 1381–1389. https://doi.org/10.1111/j.1365-2222.2009.03225.x [doi].

Wickens, K., Lane, J., Siebers, R., Ingham, T., Crane, J., 2004. Comparison of two dust collection methods for reservoir indoor allergens and endotoxin on carpets and mattresses. Indoor Air 14, 217–222. https://doi.org/10.1111/j.1600-0668.2004.00253.x.

Wise, S.K., Lin, S.Y., Toskala, E., Orlandi, R.R., Akdis, C.A., Alt, J.A., Azar, A., Baroody, F. M., Bachert, C., Canonica, G.W., Chacko, T., Cingi, C., Ciprandi, G., Corey, J., Cox, L. S., Creticos, P.S., Custovic, A., Damask, C., DeConde, A., DelGaudio, J.M., Ebert, C.S., Eloy, J.A., Flanagan, C.E., Fokkens, W.J., Franzese, C., Gosepath, J., Halderman, A., Hamilton, R.G., Hoffman, H.J., Hohlfeld, J.M., Houser, S.M., Hwang, P.H., Incorvaia, C., Jarvis, D., Khalid, A.N., Kilpeläinen, M., Kingdom, T.T., Krouse, H., Larenas-Linnemann, D., Laury, A.M., Lee, S.E., Levy, J.M., Luong, A.U., Marple, B.F., McCoul, E.D., McMains, K.C., Melén, E., Mims, J.W., Moscato, G., Mullol, J., Nelson, H.S., Patadia, M., Pawankar, R., Pfaar, O., Platt, M.P., Reisacher, W., Rondón, C., Rudmik, L., Ryan, M., Sastre, J., Schlosser, R.J., Settipane, R.A., Sharma, H.P., Sheikh, A., Smith, T.L., Tantilipikorn, P., Tversky, J.R., Veling, M.C., Wang, Y., Westman, M., Wickman, M., Zacharek, M., 2018. International consensus statement on allergy and rhinology: allergic rhinitis. Int. Forum. Allergy Rhinol. 8, 108–352. https://doi.org/10.1002/alr.22073 [doi].