Indoor bacterial microbiota and development of asthma by 10.5 years of age

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Background: Early-life indoor bacterial exposure is associated with the risk of asthma, but the roles of specific bacterial genera are poorly understood.
Objective: We sought to determine whether individual bacterial genera in indoor microbiota predict the development of asthma.
Methods: Dust samples from living rooms were collected at 2 months of age. The dust microbiota was characterized by using Illumina MiSeq sequencing amplicons of the bacterial 16S ribosomal RNA gene. Children (n = 373) were followed up for ever asthma until the age of 10.5 years.
Results: Richness was inversely associated with asthma after adjustments ($P = .03$). The phylogenetic microbiota composition in asthmatics patients' homes was characteristically different from that in nonasthmatic subjects' homes ($P = .02$, weighted UniFrac, adjusted association, permutational multivariate analysis of variance, PERMANOVA-S). The first 2 axis scores of principal coordinate analysis of the weighted UniFrac distance matrix were inversely associated with asthma. Of 658 genera detected in the dust samples, the relative abundances of 41 genera correlated ($r > |0.4|$) with one of these axes. *Lactococcus* genus was a risk factor for asthma (adjusted odds ratio, 1.36 [95% CI, 1.13-1.63] per interquartile range change). The abundance of 12 bacterial genera (mostly from the Actinomycetales order) was associated with lower asthma risk ($P < .10$), although not independently of each other. The sum relative abundance of these 12 intercorrelated genera was significantly protective and explained the majority of the association of richness with less asthma.
Conclusion: Our data confirm that phylogenetic differences in the microbiota of infants' homes are associated with subsequent asthma risk and suggest that communities of selected bacteria are more strongly linked to asthma protection than individual bacterial taxa or mere richness. (J Allergy Clin Immunol 2019;■■■:■■■-■■■.)

Key words: *Asthma development, children, diversity, environment,* Lactococcus *species*

Microbial exposures early in life can have a dual role in asthma development. Early-life viral infections predispose to asthma and also bacterial infections, and airway colonization by potential respiratory bacterial pathogens can have a similar influence.[1] On the other hand, it is well recognized that microbial exposure *in utero* and early life appear to be essential in instructing adaptive and regulated immune system responses to other environmental elements, such as allergens, particles, and viruses.[2]

Accordingly, intimate exposure to environments rich in microbes, such as those associated with traditional farming practices, might decrease the risk of asthma and other allergic diseases.[3] Earlier epidemiologic studies on home microbial exposure and asthma were based on characterization of exposure through measures of general microbial markers,[4-7] such as

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Abbreviations used
aOR: Adjusted odds ratio
CE: Cell equivalent
MaAsLin: Multivariate association with linear model
PCoA: Principal coordinate analysis
PERMANOVA-S: Permutational multivariate analyses of variance
qPCR: Quantitative PCR

endotoxin in dust samples (reviewed by Doreswamy and Peden[8]). We have previously shown in this cohort that the quantity of exposure to bacterial and fungal cell-wall components in early life has a bell-shaped association with asthma at the age of 6 years.[9] Studies with DNA-based methods have indicated that the asthma-protective characteristics might include diversity[10-13] or, more specifically, diversity within certain taxa and a lack of predisposing microbes.[14-17] However, it remains unclear whether there are specific individual taxa in the indoor microbiome that are independently associated with reduced asthma risk.

The overall objective of this study was to identify individual bacterial genera from the early-life indoor environment that are associated with the development of asthma until the age of 10.5 years. We also tested whether the protective association between high bacterial diversity and asthma is independent of the contributing microbes, as has been hypothesized.

METHODS

The study population consisted of children born in middle and eastern Finland: the first half of the study population (n = 214) belonged to a European birth cohort (Protection Against Allergy Study in Rural Environments [PASTURE])[18] among farmers and nonfarmers, whereas the second half of the cohort consisted of unselected children (n = 228).[19] Pregnant women who gave birth between September 2002 and May 2005 were recruited. The selection procedure has been described earlier, and the study protocol was approved by a local ethics committee in Finland.[19] Written informed consent was obtained from the parents.

Follow-up

The children were followed up with questionnaires,[19] as described in the Methods section in this article's Online Repository at www.jacionline.org. *Ever asthma* was defined as first parent-reported doctor-diagnosed asthma and/or second diagnoses of asthmatic (or obstructive) bronchitis. *Current asthma* was defined as ever asthma, with use of asthma medication and/or reported wheezing symptoms in the past 12 months at the 10.5-year follow-up. Wheezing phenotypes were created by using latent class analyses (see the Methods section in this article's Online Repository).

House dust samples

House dust samples were sequenced from 394 living room floor dust samples. The protocols for dust collection at 2 months of age and analyses of general microbial markers have been described previously.[7,9] The protocol for sequencing (V4 region of the 16S rRNA),[20] data processing, and measuring the relative abundances and quantitative PCR (qPCR; assay targeting the 16S rRNA gene) are described in the Methods section in this article's Online Repository. Bacterial richness (a measure of the number of different operational taxonomic units in each sample) and Shannon diversity (abundance and evenness of the taxa in each sample) indices were calculated within samples. The "load" of the bacterial genus (ie, expressed as cell equivalents [CEs] per square meter) was calculated by multiplying relative abundance with total bacteria (CEs per milligram) in that sample, as measured by using qPCR and amounts of dust, and dividing by sampling area (square meters).

Statistical analyses

Statistical analyses are described in more detail in the Methods section in this article's Online Repository. Generalized UniFrac-based principal coordinate analysis (PCoA) was performed with QIIME, and the first 6 axis scores (eigenvalues > 1) were used in the analyses. The adjusted association of bacterial composition and ever asthma was studied by using permutational multivariate analysis of variance (PERMANOVA-S).[21]

Kruskal-Wallis or *t* tests were used for comparing the relative abundances of taxa in homes of asthmatic (ever asthma) and nonasthmatic children. For multivariate models, the variables were ln-transformed (natural logarithm +1, except diversity indices) and divided by interquartile range. Discrete-time hazard models, generalized estimating equations, and multinomial logistic regression were used for analyzing asthma, respiratory symptoms, and wheezing phenotypes, respectively. The results are presented as adjusted odds ratios (aORs) and their 95% CIs.

Oligotyping analysis was performed for the *Lactococcus* genus by using entropy positions to increase taxonomic resolution.[22] Multivariate association with linear models (MaAsLin)[23] was run by using all of the most abundant taxa (mean relative abundance, >0.1%) from the phylum level to the genus level.

All models were adjusted for follow-up time, study cohort, living on a farm, and well-known risk factors for asthma (maternal history of allergic diseases, sex, number of older siblings, and smoking during pregnancy). Two selected models were carefully tested for 25 additional confounding factors,[7] but none of these potential confounders changed the estimates of exposure by greater than 10% and thus were not included in the analyses. At the age of 3 years, the majority (80%) of the children still lived in the same house. Data were analyzed by using SAS 9.3 for Windows (SAS Institute, Cary, NC).

RESULTS

Of the 442 children, 394 (89.1%) had data on the bacterial microbiota in dust samples, and 373 (94.6%) of those had sufficient data to assess asthma until the age of 10.5 years and information on covariates. By the age of 10.5 years, 69 (18.5%) children had ever asthma, and 29 (7.8%) had current asthma at 10.5 years.

Bacterial diversity in the homes of asthmatic patients and nonasthmatic subjects

The bacterial richness and Shannon diversity index were lower in the homes of children with ever asthma than in the homes of nonasthmatic subjects (Fig 1). When the models were adjusted for confounding factors, bacterial richness was inversely associated with ever asthma, and the Shannon index qualified as a trend ($P = .03$ and $P = .12$, respectively; Table I).

UniFrac-based weighted PCoA axis scores and asthma

The overall microbiota composition between asthmatic patients and nonasthmatic subjects was significantly different, as indicated by weighted UniFrac β-diversity analysis ($P = .02$, adjusted association, PERMANOVA-S). The first 2 PCoA axis scores (PCoA1 and PCoA2) explained 36% of the variance in the weighted UniFrac dissimilarity distance matrix (Fig 2). The PCoA1 and PCoA2 axis scores were inversely associated with ever asthma (Table I). The first axis score appeared to reflect the ratio of Firmicutes and Proteobacteria in the samples, with

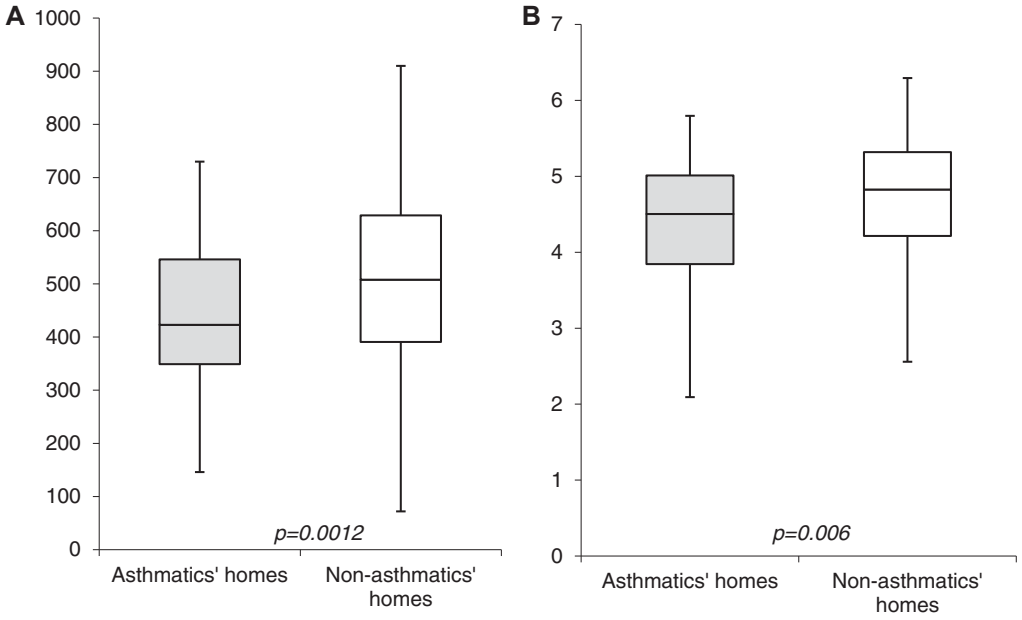


FIG 1. Box plots of bacterial richness **(A)** and the Shannon diversity index **(B)** in homes of children with asthma ever *(gray boxes)* and in homes of nonasthmatic children *(white boxes)*. Richness is the number of different operational taxonomic units in a sample. *Box plots* present minimum, first quartile, median, third quartile, and maximum values. *P* values are from *t* tests.

TABLE I. Associations between richness, Shannon index, the first 2 axis scores (PCoA1 and PCoA2) and development of ever asthma until the age of 10.5 years and current asthma

	Ever asthma		Current asthma	
	aOR (95% CI)	*P* value	aOR (95% CI)	*P* value
Richness	0.61 (0.39-0.95)	.03	0.55 (0.27-1.12)	.10
Shannon index	0.77 (0.55-1.07)	.12	0.76 (0.45-1.30)	.32
PCoA1	0.74 (0.57-0.98)	.03	0.76 (0.50-1.16)	.20
PCoA2	0.75 (0.55-1.02)	.07	0.59 (0.36-0.98)	.04

aORs are expressed as interquartile range changes in the estimate (ln-transformed in axis scores). PCoA1 indicates the first axis score of weighted UniFrac-based PCoAs. PCoA2 indicates the second axis score of weighted UniFrac-based PCoAs. Discrete-time hazard models are adjusted for follow-up time, cohort, living on a farm, sex, maternal history of allergic diseases, maternal smoking during pregnancy, and number of older siblings. The number of subjects at the beginning of the survey/total number of observations in the analyses/number of outcomes in the ever asthma model (n = 373/2387/69, respectively) and in the current asthma model (n = 310/2333/29, respectively) are shown.

negative correlation with Firmicutes and positive correlation with Proteobacteria abundance at the phylum level. The second axis score appeared to reflect diversity and Actinobacteria abundance seen as a positive correlation with both (Fig 3). The fourth most common phylum, Bacteroidetes, had a weak positive correlation with both axis scores (Fig 3). There were no significant associations between the other 4 PCoA axis scores (eigenvalue > 1) and ever asthma (see Table E1 in this article's Online Repository at www.jacionline.org).

Phylum and genus levels in homes of asthmatic children and nonasthmatic children

At the phylum level, the relative abundance of Firmicutes was greater and that of Actinobacteria was lower in the homes of asthmatic children than in the homes of nonasthmatic children (see Fig E1 in this article's Online Repository at www.jacionline.org). At the genus level, the relative abundances of *Lactococcus* species (Firmicutes) and *Streptococcus* species (Firmicutes) were greater, but the relative abundance of *Sphingomonas* species (Proteobacteria) was lower in the homes of asthmatic patients than the homes of nonasthmatic subjects (Fig 4). Consistent with results on richness, the combined relative abundance of the rest of the genera (mean relative abundance, <1%) was lower in the homes of asthmatic children than in the homes of nonasthmatic children (43.0% vs 47.8%, respectively; $P < .001$).

Bacterial genera and asthma

Of 658 detected bacterial genera, 139 bacterial genera had a mean relative abundance of greater than 0.1%, and they were studied further. Forty-one of the 139 genera correlated ($r > |0.4|$) with either or both PCoA1 and PCoA2 axis scores (see Table E2 in this article's Online Repository at www.jacionline.org). After adjustments, the relative abundances of the 12 genera were inversely ($P < .1$) associated with the development of ever asthma and *Lactococcus* species was positively ($P = .001$) associated with the development of ever asthma (Fig 5). *Lactococcus* species (median relative abundance, 3.9%) was the only genus that was associated with greater risk of ever asthma after correction for multiple testing (Bonferroni). High positive correlation coefficients (mostly $r = 0.5$-0.8) were found within the relative abundances of these 12 genera, except for *Brevibacterium* species and other genera within the Dermabacteraceae family, which had clearly lower correlation coefficients (see Fig E2). When the negative associations of the 12 genera and the positive association of *Lactococcus* species were mutually adjusted in the model of ever asthma, only the positive association of *Lactococcus* species

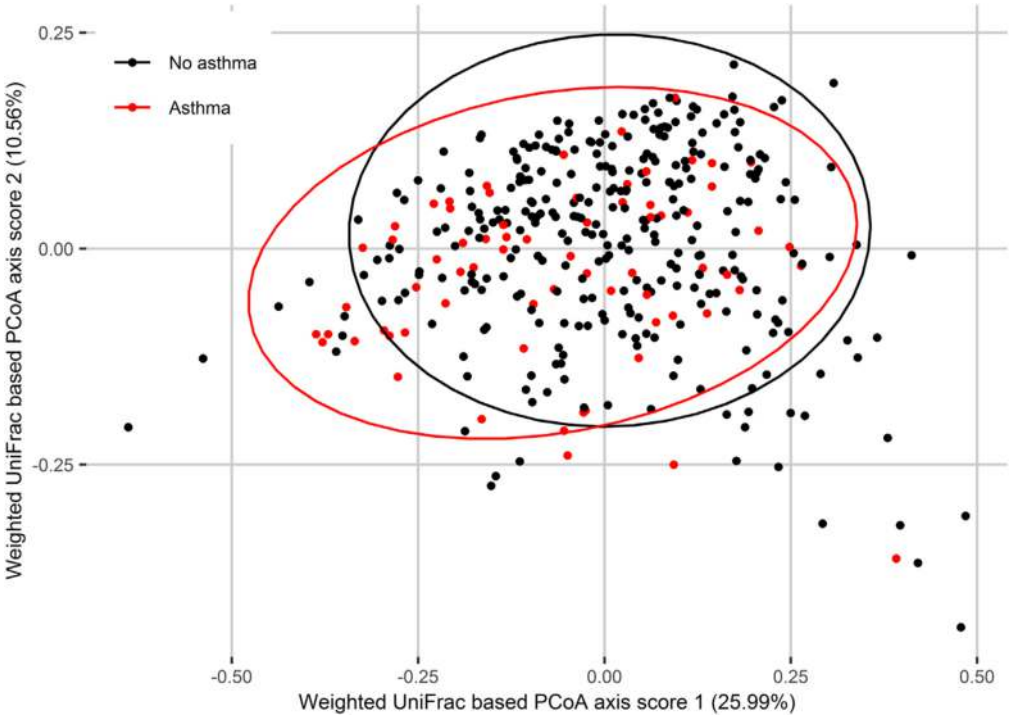


FIG 2. Plot of PCoA1 and PCoA2 axis scores by ever asthma status. PCoA1 represents the first and PCoA2 represents the second axis scores from weighted UniFrac-based PCoAs: children with ever asthma *(red dots)* and nonasthmatic subjects *(black dots)*. Percentages of variance explained by axis scores are shown in parentheses. *Red* and *black ellipses* represent 95% CIs from *t* tests for children with ever asthma and nonasthmatic children, respectively.

remained significant (see Fig E3 in this article's Online Repository at www.jacionline.org).

The relative abundances of the 12 protective genera were thus added up into a new variable because of their high intercorrelation. The sum abundance of the 12 protective genera (median relative abundance, 5.2%) was dose-dependently associated with ever asthma (aOR of 0.48 [95% CI, 0.26-0.85; $P = .01$] for the middle tertile and aOR of 0.31 [95% CI, 0.15-0.63; $P = .001$] for the highest tertile; compared with the lowest tertile). The sum abundance of the 12 protective genera and the *Lactococcus* genus were independent predictors for having ever asthma (data not shown). Associations with current asthma were largely similar (data not shown).

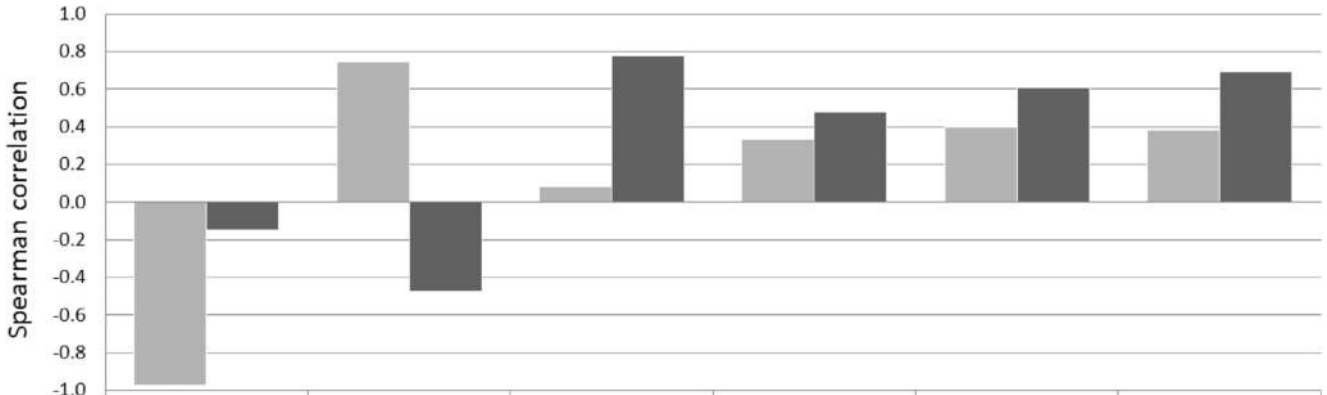
The predisposing association between the relative abundance of *Lactococcus* species and the inverse association of the sum abundance of the 12 protective genera with ever asthma were independent of bacterial richness, the Shannon index, amounts of dust, endotoxin, $LPS_{10:0\text{-}16:0}$, and muramic acids (see Table E3 in this article's Online Repository at www.jacionline.org). The sum abundance of the 12 protective genera explained 61% of the association between richness and ever asthma (see Table E4 in this article's Online Repository at www.jacionline.org). Environmental and behavioral determinants associated with reduced signals of asthma predisposing to *Lactococcus* species abundance and an increase in asthma protection–associated microbes included animal and farm contacts, timber structures, age of the house, and natural ventilation (see the Results section and Table E5 in this article's Online Repository at www.jacionline.org).

Bacterial exposure and wheezing phenotypes

In analyses of wheezing phenotypes (based on latent class analyses) during the first 6 years of life, no associations were found between the relative abundance of *Lactococcus* species, the sum abundance of the 12 protective genera, diversity indices, and transient wheeze, which is mostly related to infection in early age (see Table E6 in this article's Online Repository at www.jacionline.org). Numbers of cases in the late-onset and persistent wheeze groups were small, and associations were toward the same directions than with ever asthma but clearly weaker. However, there was a tendency toward inverse associations between the sum of 12 protective genera and late-onset and persistent wheeze ($P < .20$). When exploring associations with respiratory symptoms, similar but mostly nonsignificant associations, as with asthma ever, were found, except for *Lactococcus* species, which had weaker associations with wheezing (see Table E7 in this article's Online Repository at www.jacionline.org).

Oligotypes of *Lactococcus* species and asthma

Oligotyping analysis was performed with *Lactococcus* genus, and 10 oligotypes were created to increase taxonomic resolution for the finding of the taxon being associated with ever asthma (see Table E8 in this article's Online Repository at www.jacionline.org). Most of the sequences belonged to the GGCCAAGGA oligotype (95% of all sequences), which had the greatest mean relative abundance (7.2%) and correlated with relative abundance of the *Lactococcus* genus and operational taxonomic unit number 1100972 ($r = 0.99$). For this oligotype, the 2 best Basic Local



	Firmicutes	Proteobacteria	Actinobacteria	Bacteroidetes	Richness	Shannon index
PCoA1	-0.97	0.75	0.08	0.33	0.40	0.38
PCoA2	-0.15	-0.47	0.78	0.48	0.60	0.69

FIG 3. Spearman rank correlation coefficients between the first 2 axis scores, PCoA1 *(light gray columns)* and PCoA2 *(dark gray columns)*, and the 4 most abundant bacterial phyla, richness, and Shannon index.

Alignment Search Tool hits from the National Center for Biotechnology Information database were uncultured bacterial clone 1714 and *Lactococcus lactis* (lactis gene for 16S rRNA) with 100% similarity (identity and coverage). Relative abundances of each of the 10 oligotypes were positively associated with the development of ever asthma after adjustments ($P < .15$). Correlations among the 10 oligotypes ranged from 0.28 to 0.70 (mostly >0.45). When the relative abundances of 10 oligotypes were simultaneously adjusted, none of them were significantly associated with ever asthma (data not shown).

Loads of bacterial genera, total bacterial qPCR, and asthma

Associations with ever asthma were slightly weaker when loads of the bacterial genera (ie, expressed as CEs per square meter) were used instead of relative abundance, except for the *Lactococcus* genus, for which the estimate was stronger (see Table E9 in this article's Online Repository at www.jacionline.org). Correlations between the relative abundances of sequences of the 13 bacterial genera and their loads were fairly high (r = 0.57-0.79). Total bacterial qPCR was not associated with ever asthma or current asthma (data not shown).

MaAsLin

MaAsLin identified the relative abundance of 9 taxa that were significantly associated with ever asthma after multiple testing was taken into account (q value < 0.05). The strongest association was found with *Lactococcus* species (see Table E10 in this article's Online Repository at www.jacionline.org). For the rest of the taxa, other genera within the Microbacteriaceae family, which was one of 12 protective genera, were also identified.

DISCUSSION

The present study suggests that phylogenetic differences in the early home indoor microbiota composition precede asthma development, and this association is not explained by bacterial richness alone. Of 658 genera detected in dust samples, only the relative abundance of *Lactococcus* genus was determined as an independent risk factor for asthma. Twelve bacterial genera (mostly from the order Actinomycetales) were identified as protective. The sum of the relative abundance of these 12 protective genera was significantly protective and explained the majority of the association of richness with less asthma.

We found a similar inverse association between bacterial richness and asthma, as has been reported in 2 recent cross-sectional studies from rural areas.[10,12] Another nested case-control study with children at high risk of allergy from an urban environment by Lynch et al[14] found a similar association between bacterial richness and atopy and recurrent wheeze together with atopy but not wheeze by itself at the age of 3 years. In contrast, a study among asthmatic patients showed that high levels of bacterial richness in homes was associated with more severe asthma symptoms compared with homes with low bacterial richness in house dust.[24] This might be explained by the notion that bacterial richness might have different importance to asthma severity than to asthma development, something that has been found earlier with high endotoxin exposure.[25] Thus our findings support earlier observations that a diverse environmental microbial exposure at early age through ingestion, inhalation, and/or the skin might be essential for stimulating immune development to respond appropriately to other environmental elements.[2]

The phylogenetic composition of the microbiota was significantly different in house dust of asthmatic patients and nonasthmatic subjects, as found with the PERMANOVA-S analysis method, which uses UniFrac distance, a measure of similarity and dissimilarity of the bacterial composition between samples. Of the 12 protective genera identified, 7 were from the order Actinomycetales, which are found in outdoor environmental sources (eg, soil, fresh water, and compost). The 12 genera were intercorrelated, and thus it was not surprising that individual genera were not associated with asthma protection independently from each other, although their sum abundance was associated. Whether the 12 protective genera had a common source or distinct functional influence on asthma development remains unclear. Interestingly, the association between bacterial richness and asthma was largely explained by the sum abundance of the 12 protective genera but not by the low relative abundance of *Lactococcus* species. This suggests that particular compositions of bacterial exposure, the source of which is outdoors, better predict the

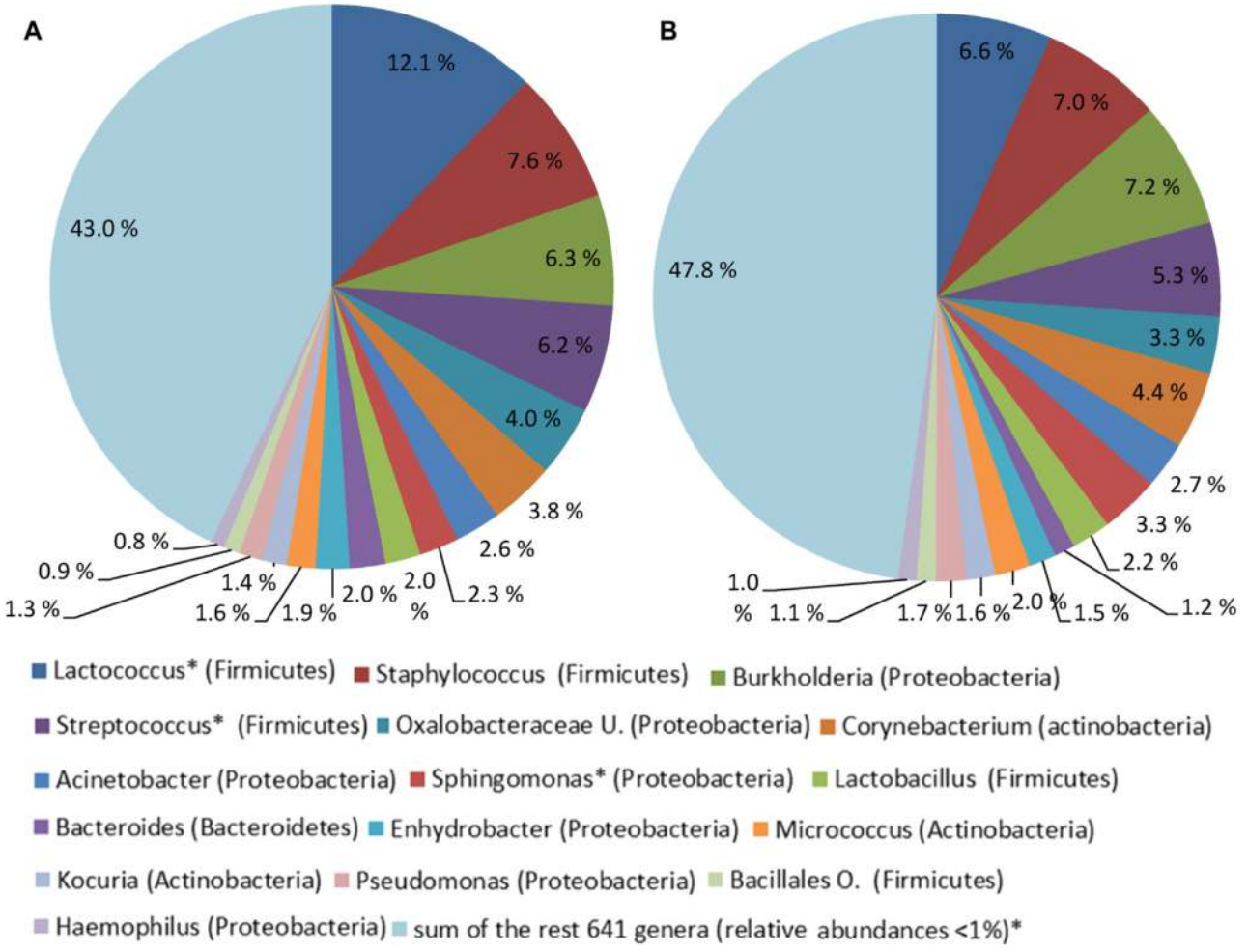


FIG 4. Relative abundances of the bacterial genera in living room dust (at age 2 months) from homes of children with ever asthma **(A)** and without asthma **(B)**. The 641 genera with a mean relative abundance of less than 1% in the whole data set are combined in the sum variable. Phylum names are shown in parenthesis. *U,* "Unassigned" genus within a family; *O,* "other" genus within a family. *$P < .05$, Kruskal-Wallis test.

development of asthma than overall bacterial richness. However, the taxa that were identified and combined in the present study should be confirmed by other studies in different environments and in different geographic areas, and their potential protective functions should be explored.

This study revealed a genus of gram-positive bacteria, *Lactococcus* species (belonging to the Firmicutes phylum and Streptococcaceae family), that increased the risk of asthma independently of microbial diversity. *Lactococcus* species is the most prevalent genus in raw and pasteurized cow's milk,[26] is used in manufacturing of fermented dairy products, and is also found in soil. In the oligotyping analyses the vast majority of the sequences of *Lactococcus* species were allocated to one specific oligotype (GGCCAAGGA) that had the strongest effect on asthma development and that, based on the Basic Local Alignment Search Tool analyses, might refer to *Lactococcus lactis*. Although there is a small but growing literature on early-life environmental microbial exposures and development of wheeze and asthma in children, no previous study has shown an association with *Lactococcus* species. A previous study[27] found in a murine and experimental model that exposure to the *Lactococcus lactis* G121 strain along with another bacterial strain, *Acinetobacter lwoffii* F78, which were both isolated from farm stables, prevented experimental allergic asthma in mice. The gram-positive *L lactis* G121 especially activated cells through nucleotide-binding oligomerization domain-containing protein 2 and Toll-like receptor 2. In our study we observed that the *Lactococcus* genus and its oligotypes were significant risk factors for asthma. Because of similar associations between *Lactococcus* species and ever asthma among children from farms and nonfarms, it is unlikely that farm milk is the source of *Lactococcus* species in the present study. However, our sequencing analyses method was not designed to enter into the strains/species level with confidence, which is a general weakness of the amplicon sequencing method. Whether the *Lactococcus* species is a true risk factor for asthma or a proxy of other predisposing factors, such as a particular lifestyle or nutrition, remains to be determined in experimental and other epidemiologic studies, including quantitative and specific detection (eg, by using qPCR) of *Lactococcus* species.

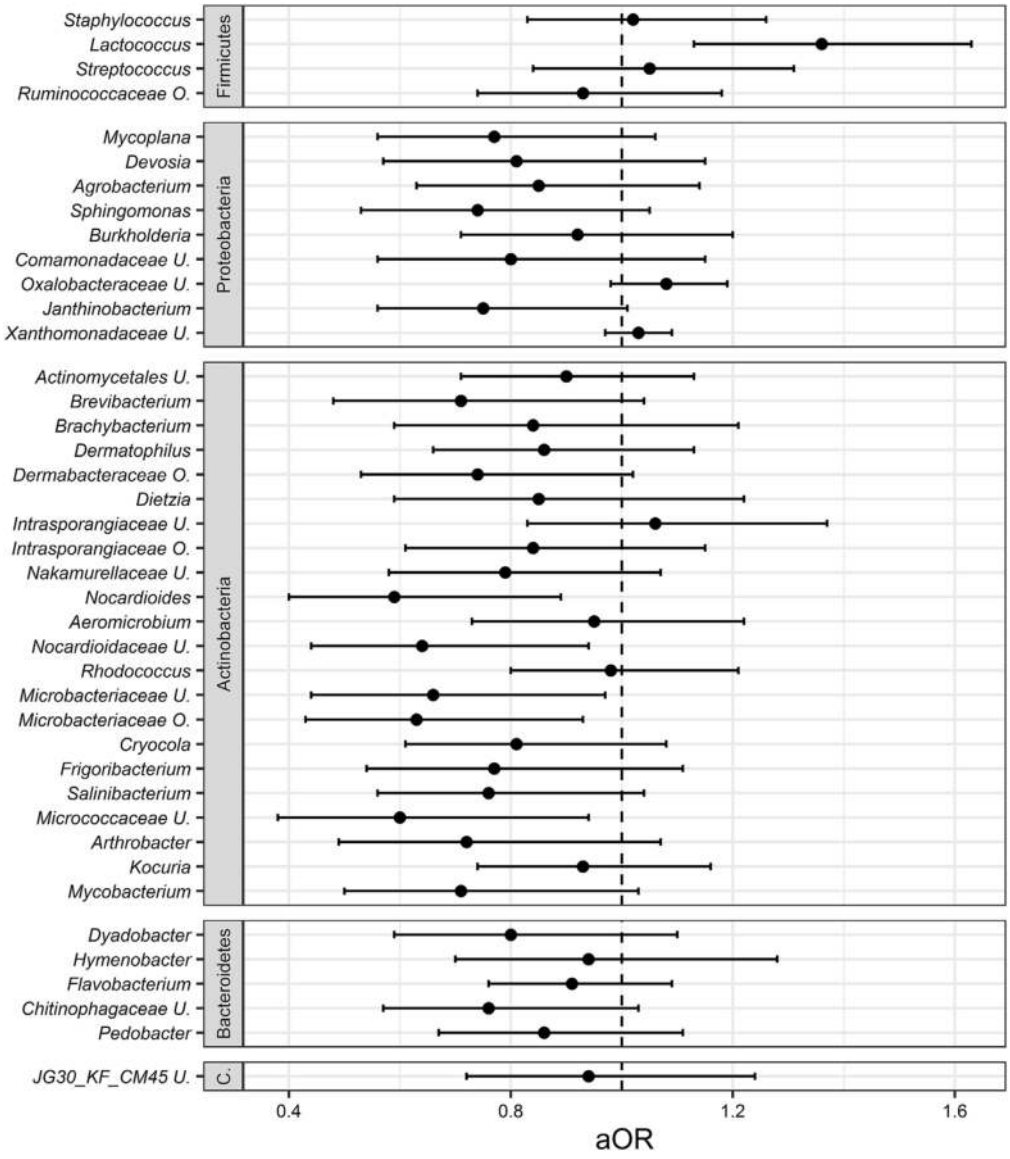


FIG 5. aORs (95% CIs) between the selected 41 genera and asthma ever. Genera have been ordered by phylum. aORs are expressed as interquartile range changes in the estimate (ln-transformed). Models are adjusted for follow-up time, living on a farm, cohort, sex, maternal history of allergic diseases, maternal smoking during pregnancy, and number of older siblings. *U*, "Unassigned" genus within a family; *O*, "other" genus within a family; *C*, Chloroflexi (phylum).

We have previously shown in this cohort that farm-like bacterial relative abundance patterns in indoor microbiota are associated with asthma protection by 6 years of age.[15] There was little overlap between the specific genera identified in the current study to be associated with asthma after adjustment for farming and the best predictors of the farm-like indoor microbiota composition identified in our previous study. However, there were phylogenetic similarities because both imply importance of high abundance of members within the Actinobacteria phylum. In contrast, there was little or no overlap among the 13 genera identified in the present study and taxa that have been associated with lower asthma risk in other previous studies.[10,12,14,16,17] In these studies minimal[10,12] to no[14,16,17] adjustments have been made for potential confounders, and comparability with our results is also influenced by other differences, including those in sampling material, microbiological determinations, outcomes, and study designs (eg, prospective vs cross-sectional). A commonality in the present study and some of the previous studies has been that rather than diversity as such, it is certain compositional aspects within bacterial diversity that explain associations between indoor bacterial exposure and asthma. Further studies aiming at functional profiling, such as through metagenomics, metabolomics, or experimental studies, are needed to characterize the potential asthma-protective properties that might be identified by the bacterial taxa described here.

Mechanisms behind the association between environmental microbial exposures in early life and asthma protection are not

well understood. Evidence from epidemiologic and experimental studies show that specific microbial exposures, such as those encountered in farming environments or homes with dogs, trigger receptors of the innate immunity,[28] might increase epithelial barrier function in the airways and the presence of immunosuppressive cells, suppress responsiveness toward microbial immunogens, and reduce allergen-induced airway inflammation.[15,16,29,30] Exposure to rich and diverse microbiota might have a positive effect on airway colonization, which might in turn defend against viral infections and thus contribute to asthma prevention.[31,32] In addition, there is evidence from murine models that exposure to microbes in house dust modulates intestinal microbiota and might, at least partially, mediate the effect on immune responses in the airways.[30,33] Whether this would also apply to human subjects remains unknown. There is evidence that environmental factors, such as dogs, can influence human gut microbiota composition,[30,34] but overall, this influence is thought to be limited.[1]

In the present study, few environmental and behavioral determinants such as increased animal contacts, natural as opposed to mechanical ventilation and timber structures were associated with increase in asthma protection associated microbes and with decrease in the asthma predisposing *Lactococcus* abundance. These and future findings from more focused studies could direct public health initiatives for asthma prevention. Such initiatives might be efficient ways to reduce the allergy and asthma burden, as indicated by the Finnish Allergy Program, which provided practical recommendations for behavior modification.[35]

The main strengths of the present study are the prospective birth cohort design with high participation rates and an extensive set of microbial exposure measurements, including high-resolution next-generation sequencing data, DNA-based targeted qPCR, and general microbial markers. Dust samples were collected from living room floors in early childhood, which has been shown to be an important time window for intensive maturation of the adaptive immunity.[3] Long-term active air sampling, which is the best way to assess exposure, is logistically and technically challenging in large cohorts, and thus surrogates of airborne microbial exposure are used almost exclusively.[36] Floor dust better represents the overall environmental exposures carried from outdoors to indoors than, for example, bed dust, which likely reflects the human-associated microbiota.[37] However, dust from floors/rugs will only be partially resuspended into the air with a size that is inhalable and thus only partially contributes to inhalation exposure. Recently, we have shown that the microbiota of floor dust are not fully consistent with the microbiota of infant breathing zone air, but the microbiota of bulk air in a room are also not fully representative of the particular infant breathing exposure on activities near the floor.[38] As noted earlier, oral ingestion exposure or exposure through the skin during the first years of life might be relevant as well.[2]

One weakness of our study is that the taxonomic resolution of the sequencing approach did not, in general, allow species-level identification. Future studies will have to implement metagenomics (shotgun sequencing) approaches or more targeted approaches, such as qPCR or chip-based hybridization techniques, once knowledge on specific targets has accumulated to overcome this restriction in taxonomic identification.

In conclusion, our data confirm that phylogenetic differences in home microbiota influence asthma risk and suggest that communities of selected bacteria are more strongly linked to asthma protection than individual bacterial taxa or richness.

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Key messages

- **Childhood asthma risk is affected by bacterial composition of the early-life home indoor microbiota.**
- **Communities of bacteria, rather than an individual taxon or overall bacterial diversity, are most strongly linked to asthma protection.**

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