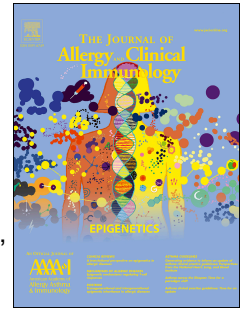


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Intestinal archaea inversely associated with childhood asthma

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Conflicts of Interest Statement

All authors declare no conflicts of interest.

Abstract

Background:

Methanogenic archaea are a key part of the gut microbiota alongside bacteria. However there is comparatively little research on the role of archaea in health.

Objective:

As in-vitro and animal experiments have demonstrated immunological effects of archaea, we hypothesised that intestinal exposure to archaeal species would influence the risk of asthma and other allergic diseases. We present the first human study connecting gut archaea with childhood asthma.

Methods:

We performed a cross-sectional analysis nested within the Dutch KOALA Birth Cohort Study. DNA from two common intestinal archaeal species, *Methanosphaera stadtmanae* and *Methanobrevibacter smithii*, was quantified in faecal samples from 472 children at school age, using qPCR.

Our primary outcome was parent-reported asthma at 6-10 years. Secondary outcomes were questionnaire-reported eczema, total serum IgE levels, sensitisation to aero- and food-allergens and lung function (FEV1/FVC).

Associations between the presence/absence of each archaeal species and outcome were assessed with logistic or linear regression models, adjusted for potential confounders.

Results:

Presence of *M. stadtmanae* was significantly associated with a lower risk of asthma, adjusted OR 0.32 (0.08 – 0.98). In addition, asthma risk decreased monotonically across three categories of increasing *M. stadtmanae* abundance (adjusted p-for-trend = 0.035). We also observed a non-significant tendency for less eczema and IgE sensitisation amongst children with *M. stadtmanae*. *M. smithii* was not associated with any outcome.

Conclusion:

Further longitudinal and experimental research is needed to explore whether archaea could be directly linked to asthma risk, or if archaeal abundance is indicative of other health-relevant variation in microbiota composition.

Key Messages

- We detected the methanogenic archaeon *Methanosphaera stadtmanae* significantly less frequently and in lower abundance in the faecal samples of asthmatic children than for non-asthmatic children
- In combination with previous laboratory evidence on the immunogenicity of this archaeal species our findings suggest that archaea should not be overlooked in future studies of the gut microbiota, asthma and allergic disease

Capsule summary

Methanosphaera stadtmanae, an immunogenic archaeal species found in the human gut microbiome, was detected less frequently in children with asthma. Gut archaea colonisation in childhood may be relevant to asthma and allergy risk.

Key Words

Archaea; Childhood Asthma; Allergy; Eczema; Gut Microbiota; Cross-sectional; qPCR

Abbreviations

qPCR = quantitative polymerase chain reaction

rRNA = ribosomal ribonucleic acid

FEV1 = forced expiratory volume in 1 second

FVC = forced vital capacity

BMI = body mass index

IgE = immunoglobulin E

OR = odds ratio

CI = confidence interval

ISAAC = International Study of Asthma and Allergies in Children

To the editor:

Both laboratory and epidemiological studies have demonstrated the importance of the gut microbiota in immunological development, childhood asthma and allergic disease pathophysiology (1). Whilst most studies have focused on bacteria, the methanogenic archaea that live alongside them are relatively understudied. Archaea are prokaryotic microorganisms belonging to their own domain of life, distinct from Bacteria and Eukaryota.

Methanobrevibacter smithii and *Methanosphaera stadtmanae* are the most abundant archaeal species in the gut (2). Bang and Blais-Lecours demonstrated that *M. stadtmanae* is particularly immunogenic, in human cells and mice (3, 4). We accordingly hypothesised that archaea, like bacteria, may play a role in childhood asthma or allergic disease risk, and in this letter we present the first epidemiological evidence linking an archaeal species, *Methanosphaera stadtmanae*, with lower asthma risk.

Data collection

We conducted cross-sectional analyses using faecal samples available at 6-10 years for a subset of children participating in the KOALA Birth Cohort Study in the Netherlands, as illustrated in supplementary figure 1. The KOALA study, described in detail elsewhere (5), consists primarily of children ($n = 2359$) whose mothers were recruited as healthy pregnant women from an ongoing prospective cohort study on pregnancy-related pelvic girdle pain. Additionally an “alternative recruitment group” of 496 women were recruited through advertisements in organic food shops, anthroposophic doctors and midwives, Steiner schools, and dedicated magazines. The KOALA study was approved by the Medical Ethical Committee of the Maastricht University/University Hospital of Maastricht and the National Ethical Committee for Medical Research. All parents signed informed consent forms.

We requested childhood faecal samples from 1432 families, targeting those who had already provided other biosamples, such as maternal and child blood samples. We also oversampled for 27 asthmatics from within the KOALA cohort, to increase statistical power. We received 672 samples, and excluded samples with transport times over 4 days, leaving 472 available for analysis. We quantified *M. smithii* and *M. stadtmanae* in these samples using qPCR targeting regions of the 16S rRNA genes specific for these species (5).

All 472 children had data on asthma and eczema diagnoses, symptoms and medication use from up to four parentally-completed questionnaires spanning 6 to 10 years of age. 355 children had total and specific serum IgE measurements (with one missing for food-allergens) and 337 had spirometric lung function data. These blood samples and spirometric readings were taken during home visits at 6-10 years by trained research nurses. Serum specific IgE levels defined aeroallergen and food-allergen sensitisation outcomes, and normalised FEV1/FVC ratio was used as a continuous lung function outcome. Asthma diagnoses were based on modified ISAAC questionnaires, requiring a reported physician diagnosis, as well as reported recent symptoms or medication use. Further details of data collection protocols and outcome definitions are included in the supplement.

Analyses

Asthma and eczema are overrepresented in the study population relative to the whole KOALA cohort, as was expected from the oversampling of asthmatic children (16.7% versus 10.4% and 34.1% versus 28.5%, supplementary table S1). A larger proportion of the study population (24.8%) are from the alternative recruitment group than in the overall KOALA cohort (17.4%).

This was also expected, as the alternative recruits are more likely than the conventional recruits to have early-life biosamples such as infant blood, because KOALA's alternative recruitment program began at approximately the same time as the biosampling program. Early-life biosample availability was a criterion for inclusion in the childhood faecal sampling program.

Further description of the study population can be found in supplementary table S3.

M. stadtmanae and *M. smithii* were detectable in 8.3% and 78.2% of samples respectively (6). As the abundance distributions of both *M. stadtmanae* and *M. smithii* followed an apparent trimodal pattern (supplementary figure 1), we also classified participants into three abundance categories.

We tested for associations between the presence of each archaeal species and the risk of our primary outcome asthma, using logistic regression models adjusted for multiple potential confounders (table 1). We adjusted for recruitment group and faecal sampling round in all analyses. No significant interaction between recruitment group and archaeal exposure was found, so pooling these groups was considered appropriate. Analyses involving serum IgE data were also adjusted for analytic batch number.

The vast majority of participants had no missing covariate data. 7 covariates had ≤ 5 missings, which we imputed with the most common category or mean value. Organic dairy consumption was imputed for 19 children, using the categories predicted by a regression model based on prior dietary and lifestyle information.

Our results indicate that the presence of *M. stadtmanae* is associated with a lower risk of asthma at 6-10 years of age, adjusted OR = 0.32 (0.08 – 0.98), $p = 0.045$ (table 1). Furthermore the risk of asthma showed a monotonic decrease over the three categories of *M. stadtmanae* abundance (adjusted $p = 0.036$ for linear trend over the *M. stadtmanae* abundance categories, table 2). No significant interaction was found between *M. stadtmanae* exposure and parental asthma history ($p = 0.495$), suggesting that the association does not significantly differ by parental asthma status.

For the secondary outcomes, *M. stadtmanae* also appeared to be associated with a slightly lower likelihood of eczema, aeroallergen and food-allergen sensitisation, but these associations did not reach statistical significance (table 1). *M. stadtmanae* was not associated with either of the continuous outcomes, total serum IgE or FEV1/FVC z-score (table S4). We found no significant associations between *M. smithii* and any outcome (tables 1 & S4).

We also conducted a complete case analysis, which produced similar results (table S5).

Additionally, to check if the oversampling of asthma cases in the second sampling round had introduced any bias we repeated the analyses with only the 372 children sampled in the first round (table S6). Similar odds ratios were observed in this subgroup, e.g. for *M. stadtmanae* presence and asthma: OR 0.39 (0.06 - 1.60). The lower precision observed was expected from the smaller sample size and fewer asthma cases, and this sensitivity analysis otherwise showed no evidence of any bias introduced by the oversampling.

Interpretation

Due to the low prevalence of *M. stadtmanae* and cross-sectional study design our results should be interpreted with caution. Although bacterial species not examined in our analysis might confound this association, we speculate that a mechanistic link between *M. stadtmanae* and allergic disease appears plausible. Prior evidence demonstrates the airway and gut immunogenicity of *M. stadtmanae* in mouse models and human cells (3, 7). Epidemiological evidence also indicates a timing-dependent relationship between asthma risk and exposure to farm environments (8), where *M. stadtmanae* is present (9). However, farm exposure has also been associated with risk of atopy, which our study does not show.

Exposure to immunogenic bacterial species in infancy has been shown to be tolerogenic and reduce asthma risk (1). Our study identifies an association between *M. stadtmanae* presence at school-age and lower asthma risk, leading us to speculate that early-life archaeal exposures may also be tolerogenic. We therefore encourage further research into the immunological roles of archaea.

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Main text tables

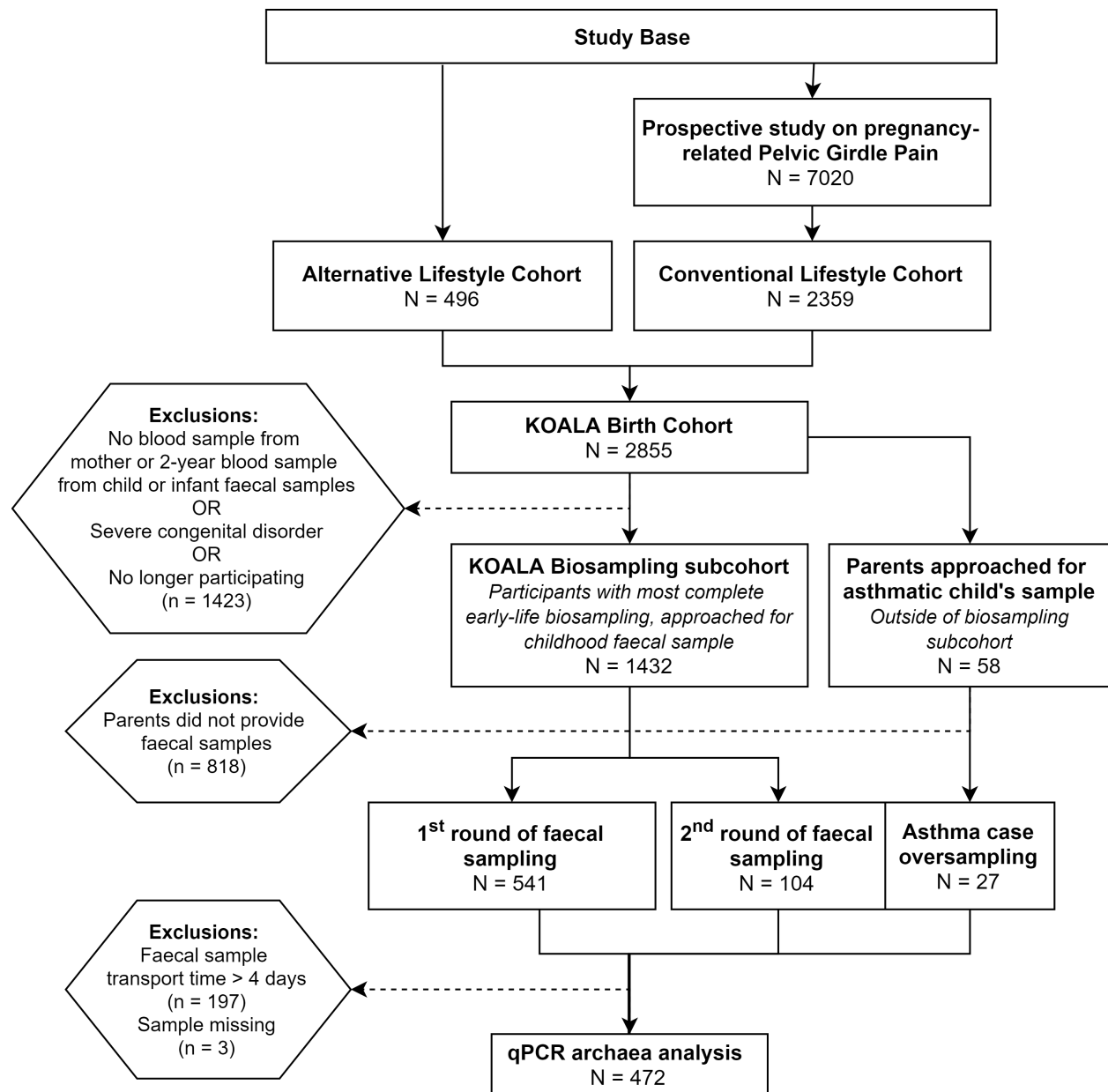
Table 1: Results table for presence of archaea (yes/no)

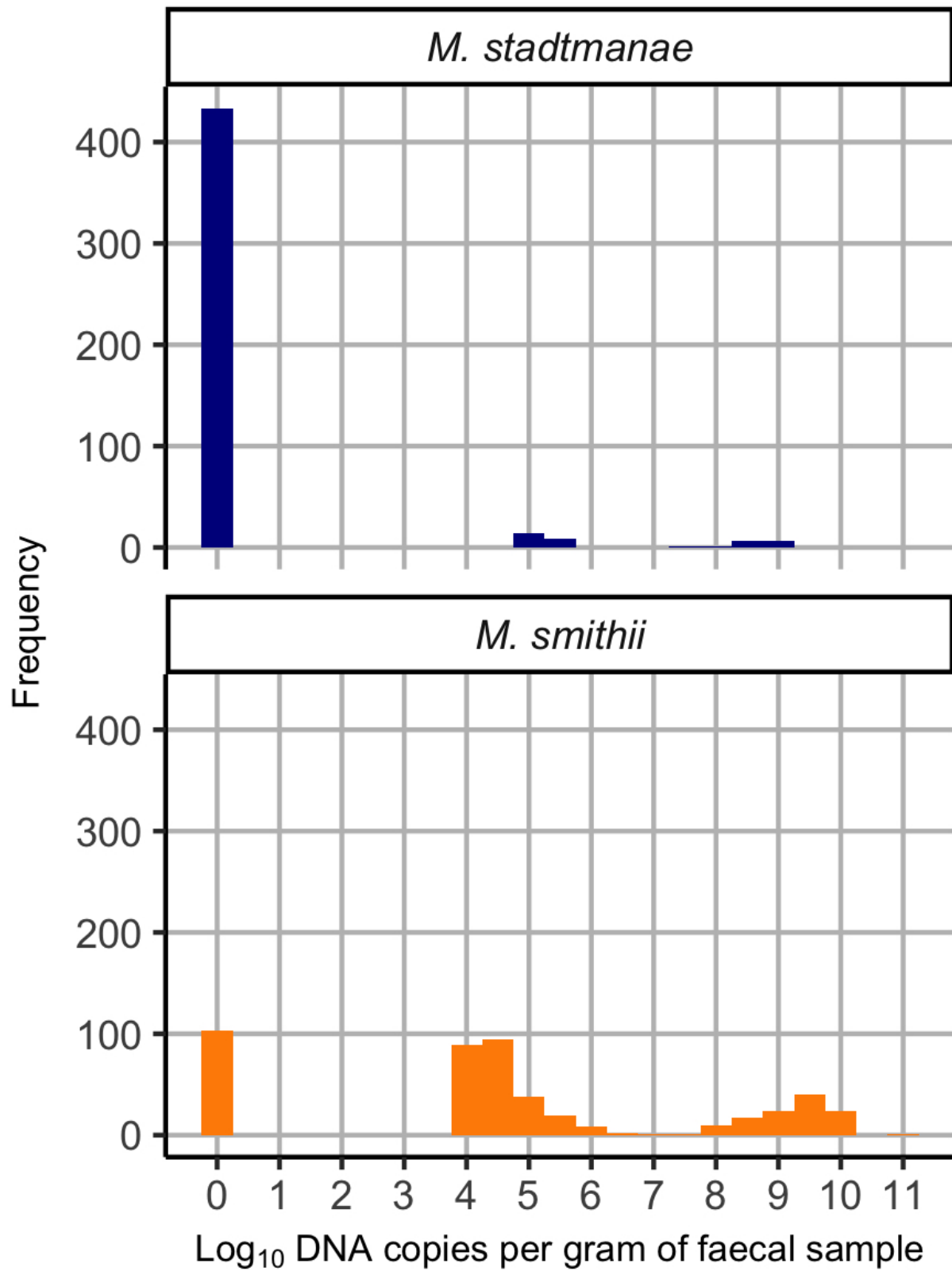
Exposure	Outcome	Sample size	OR (95% CI) crude*	OR (95% CI) adjusted*
<i>M. stadtmanae</i>	Asthma	472	0.38 (0.10 - 1.10)	0.32 (0.08 - 0.98)
	Eczema	472	0.72 (0.34 - 1.46)	0.68 (0.30 - 1.42)
	Aeroallergen sensitised	355	1.04 (0.45 - 2.30)	0.83 (0.32 - 2.02)
	Food allergen sensitised	354	0.82 (0.27 - 2.09)	0.69 (0.21 - 1.95)
<i>M. smithii</i>	Asthma	472	1.17 (0.63 - 2.27)	1.09 (0.56 - 2.20)
	Eczema	472	1.38 (0.86 - 2.25)	1.32 (0.81 - 2.18)
	Aeroallergen sensitised	355	0.96 (0.57 - 1.63)	1.05 (0.59 - 1.89)
	Food allergen sensitised	354	1.03 (0.56 - 1.94)	1.30 (0.68 - 2.59)

* The crude model is adjusted only for study design-related variables: faecal sampling round and recruitment group. The fully adjusted model also included: birth mode and place; antibiotic exposure; farm animal exposure; cat/dog exposure; BMI z-score; maternal age at delivery; maternal education; parental atopy; sibling atopy; sex; organic dairy consumption; and abundance of the other archaeal species.

Table 2: *M. stadtmanae* abundance and asthma risk; sample size = 472.

<i>M. stadtmanae</i> abundance	N	Asthma Prevalence %	OR (95% CI) crude	OR (95% CI) adjusted
Absent	433	17.3	1.00 (reference)	1.00 (reference)
Low abundance	23	13.0	0.37 (0.08 - 1.35)	0.43 (0.08 - 1.73)
High abundance	16	6.2	0.40 (0.02 - 2.03)	0.20 (0.01 - 1.18)
<i>p</i> -value for linear association with interval-scale abundance categories			<i>p</i> = 0.096	<i>p</i> = 0.036





Supplementary figures

Supplementary figure 1: Participant flowchart illustrating the source of the children included in this study.

Supplementary Figure 2: Histograms showing the trimodal distributions of 16S rRNA gene copies per gram of sample for *M. stadtmanae* and *M. smithii*. DNA copies /gram faeces shown on a base 10 logarithmic scale.

Supplementary Tables

Supplementary table S1: Outcome prevalence table

	Study population (children with archaea data)	Faecal samples requested	KOALA cohort
Number	472	1432	2855
Asthma (%)	79 (16.7)	118 (10.5)	224 (10.4)
<i>missing</i>	0	311	702
Eczema (%)	161 (34.1)	333 (29.7)	614 (28.5)
<i>missing</i>	0	311	703
Aeroallergen sensitised (%)	117 (33.0)	145 (29.5)	163 (31.5)
<i>missing</i>	117	941	2338
Food allergen sensitised (%)	71 (20.1)	106 (21.6)	115 (22.3)
<i>missing</i>	118	942	2339
Total IgE / ln(kU/L) [mean (SD)]	4.38 (1.75)	4.36 (1.75)	4.41 (1.76)
<i>missing</i>	117	941	2338
FEV1/FVC (mean (SD))	0.85 (0.06)	0.86 (0.06)	0.86 (0.06)
<i>missing</i>	135	930	2335

Supplementary table S2: Outcome prevalence table split by archaeal presence/absence

	<i>M. stadtmanae</i>		<i>M. smithii</i>	
	Not-detected	Detected	Not-detected	Detected
Number	433	39	103	369
Asthma (%)	75 (17.3)	4 (10.3)	18 (17.5)	61 (16.5)
Eczema (%)	150 (34.6)	11 (28.2)	30 (29.1)	131 (35.5)
Aeroallergen sensitised (%)	107 (32.8)	10 (34.5)	29 (34.1)	88 (32.6)
<i>Missing</i>	107	10	107	10
Food allergen sensitised (%)	66 (20.3)	5 (17.2)	17 (20.0)	54 (20.1)
<i>Missing</i>	108	10	108	10
Total IgE / ln(kU/L) [mean (SD)]	4.37 (1.75)	4.40 (1.84)	4.06 (1.72)	4.48 (1.75)
<i>Missing</i>	107	10	107	10
FEV1/FVC [mean (SD)]	0.85 (0.06)	0.85 (0.07)	0.85 (0.05)	0.86 (0.06)
<i>Missing</i>	122	13	122	13

Supplementary table S3: Full population characteristics table

	Study population (children with archaea data)	Faecal samples requested	KOALA cohort
Number	472	1432	2855
Age (years) at archaea sample / mean (SD)	7.35 (0.82)	-	-
BMI at age of sample or 6-8yo / mean (SD)	15.64 (1.68)	15.43 (1.51)	15.45 (1.58)
<i>Missing</i>	2	465	1074
Maternal age (years) at due date / mean (SD)	32.72 (3.73)	32.54 (3.85)	32.03 (3.81)
<i>Missing</i>	0	20	23
Faecal sample given in second sampling round (%)	100 (21.2)	-	-
Alternative recruitment group (%)	117 (24.8)	402 (28.1)	496 (17.4)
Maternal education level (%)			
<i>Other or Missing</i>	18 (3.8)	76 (5.3)	164 (5.7)
<i>Low</i>	34 (7.2)	105 (7.3)	289 (10.1)
<i>Middle</i>	171 (36.2)	487 (34.0)	1060 (37.1)
<i>High</i>	249 (52.8)	764 (53.4)	1342 (47.0)
Female (%)	228 (48.3)	698 (48.8)	1385 (48.6)
<i>Missing</i>	0	3	8
Parental atopy, at least 1 parent atopic (%)	297 (63.3)	832 (60.4)	1578 (59.1)
<i>Missing</i>	3	54	184
Older sibling atopy (%)			
<i>No older siblings</i>	188 (39.8)	599 (42.2)	1273 (45.8)
<i>Older siblings without atopy</i>	183 (38.8)	569 (40.1)	1078 (38.8)
<i>Older siblings with atopy</i>	101 (21.4)	250 (17.6)	428 (15.4)
<i>Missing</i>	0	14	76
Exposure to tobacco smoke in first trimester (%)	184 (39.1)	535 (37.9)	1268 (44.8)
<i>Missing</i>	1	21	24

Birth mode and place (%)			
<i>Home vaginal delivery</i>	220 (47.1)	617 (46.0)	1189 (44.9)
<i>Hospital vaginal delivery</i>	199 (42.6)	572 (42.7)	1151 (43.4)
<i>Hospital C-section delivery</i>	48 (10.3)	151 (11.3)	311 (11.7)
<i>Missing</i>	5	92	204
Breastfeeding duration (%)			
<i>Never breastfed</i>	56 (11.9)	177 (12.7)	446 (15.9)
<i>Up to 3 months</i>	138 (29.2)	409 (29.3)	934 (33.2)
<i>4 to 6 months</i>	93 (19.7)	258 (18.5)	503 (17.9)
<i>> 6 months</i>	185 (39.2)	553 (39.6)	925 (32.9)
<i>Missing</i>	0	35	47
Regular organic dairy consumption (%)			
<i>≥1 per week at age 6-10</i>	91 (20.1)	237 (23.7)	354 (17.8)
<i>Missing</i>	19	433	922
Early antibiotic exposure (%)			
<i>Antibiotics taken ≥1 time in first 2 years of life</i>	251 (53.2)	712 (51.9)	1418 (51.4)
<i>Missing</i>	0	61	98
Early farm animal visit (%)			
<i>≥1 Farm visit during first year of life</i>	299 (63.8)	844 (63.6)	1631 (63.8)
<i>Missing</i>	3	105	297
Early cat or dog exposure (%)			
<i>Pet present at home or day-care during first 2 years of life</i>	224 (47.6)	699 (51.2)	1419 (52.0)
<i>Missing</i>	1	66	125
Day-care or host parent attendance (%)			
<i>Any reported attendance during first 2 years of life</i>	310 (65.8)	916 (67.2)	1767 (64.8)
<i>Missing</i>	1	68	128

Supplementary table S4: Linear model results for continuous outcomes Total serum IgE and FEV1/FVC Z-scores

Archaea species	Outcome measure	Sample size	β estimate (95% CI) crude*	β estimate (95% CI) adjusted
<i>M. stadtmanae</i>	Total serum IgE	355	0.073 (-0.599, 0.745)	0.015 (-0.677, 0.708)
	FEV1/FVC Z-score	337	0.035 (-0.304, 0.375)	0.106 (-0.243, 0.454)
<i>M. smithii</i>	Total serum IgE	355	0.393 (-0.036, 0.822)	0.341 (-0.092, 0.774)
	FEV1/FVC Z-score	337	0.037 (-0.178, 0.253)	0.027 (-0.190, 0.244)
*crude model adjusted only for recruitment group and faecal sampling round or IgE analytic batch number.				

Supplementary table S5: Main results for complete case analysis

Exposure	Outcome	Sample size	Crude OR (95% CI)	Adjusted OR (95% CI)
<i>M. stadtmanae</i>	Asthma	441	0.26 (0.06 - 0.86)	0.21 (0.04 - 0.72)
	Eczema	441	0.72 (0.32 - 1.50)	0.66 (0.28 - 1.43)
	Aeroallergen sensitisation	337	1.13 (0.48 - 2.53)	0.92 (0.35 - 2.27)
	Food-allergen sensitisation	336	0.86 (0.28 - 2.21)	0.74 (0.22 - 2.11)
<i>M. smithii</i>	Asthma	441	1.27 (0.67 - 2.53)	1.21 (0.60 - 2.53)
	Eczema	441	1.52 (0.93 - 2.54)	1.52 (0.92 - 2.59)
	Aeroallergen sensitisation	337	0.92 (0.54 - 1.57)	0.93 (0.52 - 1.70)
	Food-allergen sensitisation	336	1.00 (0.55 - 1.91)	1.25 (0.65 - 2.50)

Supplementary table S6: Main results for sensitivity analysis in children from first faecal sampling round only

Exposure	Outcome	Sample size	Adjusted OR (95% CI)
<i>M. stadtmanae</i>	Asthma	372	0.39 (0.06 - 1.60)
	Eczema	372	0.63 (0.23 - 1.54)
	Aeroallergen sensitisation	262	0.40 (0.10 - 1.32)
	Food-allergen sensitisation	261	0.39 (0.05 - 1.72)
<i>M. smithii</i>	Asthma	372	1.30 (0.56 - 3.32)
	Eczema	372	2.01 (1.11 - 3.83)
	Aeroallergen sensitisation	262	1.30 (0.64 - 2.71)
	Food-allergen sensitisation	261	2.12 (0.92 - 5.46)

Supplementary material

Methodological details

Data collection protocols

Faecal samples were collected from participating children between the ages of 6 and 10. The parents were sent faeces collection tubes with a spoon attached to their lids and collection instructions (Sarstedt, Nümbrecht, Germany). Samples were collected at home visit or sent to the laboratory by mail and upon arrival were 10-fold diluted in peptone/water (Oxoid CM0009) containing 20% (vol/vol) glycerol (Merck, Darmstadt, Germany) and stored at -80°C until further analysis.

DNA was isolated from the samples with a combination of Repeated-Bead-Beating and column purification, which is effective for archaeal DNA recovery. DNA concentration and purity was assessed with a Nanodrop 1000 spectrophotometer (Thermo Fisher Scientific, Wilmington). *M. smithii* and *M. stadtmanae* were enumerated using a real-time quantitative polymerase chain reaction (qPCR) protocol, using an Applied Biosystems Prism 7900 sequence detection system. This is described in detail by [Mbakwa et al. 2015](#). The lower limits of detection were 3.81 and 4.82 log₁₀ 16S rRNA gene copies/g faeces for *M. smithii* and *M. stadtmanae*, respectively.

Children were visited at home between the ages of 6 and 10 for blood sample collection, lung function testing and BMI measurement. Venous blood samples were collected and stored at -80°C until analysis. Samples were analysed for total IgE using RIA, and specific IgE against hen's egg, cow's milk, peanut, birch pollen, grass pollen, cat, dog, and house dust mite allergens by the radioallergosorbent test. The detection limit was 0.50 IU/mL for total IgE and 0.10 IU/mL for specific IgE.

Trained research assistants assessed lung function during the home visits. They used the handheld Medikro Spirostar USB spirometer (Medikro, Kuopio, Finland) according to the American Thoracic Society/European Respiratory Society (ATS/ERS) guidelines. Lung function testing was performed after 10 minutes rest in an upright, seated position. Each child performed forced expiration until three acceptable curves were achieved. Curves were considered acceptable upon meeting the reproducibility criteria defined by the ATS/ERS.

Outcome definitions

Identification of prevalent asthma and eczema cases was based on parentally completed questionnaires adapted from the International Study of Asthma and Allergies in Childhood questionnaire (ISAAC), which have been extensively validated ([Büchtele et al. 2010](#)).

Asthma was our primary outcome of interest. The presence of asthma was determined on four questionnaires taken between the ages of 6 and 10. For the present study, a child is classified as an asthma case upon satisfying the following criteria on at least one of the questionnaires: a reported doctor's diagnosis of asthma in combination with clinical symptoms or regular asthma medication use in the last 12 months.

Parental self-report of physician-diagnosed asthma has been shown to have good agreement with actual general practitioner records ([Cornish et al. 2014](#)), and our additional requirement of either recent medication use or recent symptoms is intended to ensure that these children currently have asthma.

Clinical symptoms were defined as at least one attack of wheeze or dyspnoea. Regular asthma medication use was defined as everyday use of short-acting bronchodilators or inhaled corticosteroids, according to the Dutch guidelines for treatment of bronchial asthma in children for a period of at least two months, or use associated with physical activity. Leukotriene receptor antagonist, LTRA, use was not included in our definition, but nevertheless all KOALA study

participants with reported LTRA prescriptions were categorised as asthmatic according to our definition.

For eczema, children were regarded as current cases if their parent reported an itchy rash coming or going over the last 12 months, on any of the four questionnaires. Children were not regarded as eczema cases if they reported only rash around the eyes and/or scalp scaling.

For the IgE sensitisation outcomes, children were considered sensitised if specific serum IgE levels were greater than 0.35 IU/mL against one or more allergen, separately for food-allergens and aeroallergens.