

***Bifidobacterium pseudocatenulatum* is associated with atopic eczema: A nested case-control study investigating the fecal microbiota of infants**

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Background: Exposure to specific bacterial bowel commensals may increase/reduce the risk of atopic diseases.

Objective: To compare fecal bacterial communities of young infants with/without eczema.

Methods: Nested case-control study. Infants age 3 to 6 months with eczema (cases, n = 37) and without (controls, n = 24) were matched for sex, age, feeding (breast/bottle/mixed/solids), ethnicity. Information was collected on maternal/infant antibiotic exposure, feeding, gastrointestinal symptoms, family history of allergy. Eczema severity scoring was used (Severity Scoring of Atopic Dermatitis index). Samples were taken for determination of allergen-specific serum IgE (cases) and urinary/fecal eosinophilic protein X. Gastrointestinal permeability was measured. The compositions of fecal bacterial communities were analyzed (culture-independent, nucleic acid-based analyses).

Results: There was no difference in overall profiles of fecal bacterial communities between cases and controls. Family history of allergy increased likelihood of bifidobacteria detection (history, 86%; no history, 56%; $P = .047$); breast-fed infants were more likely to harbor *Bifidobacterium bifidum* (odds ratio, 5.19; 95% CI, 1.47-18.36; $P = .01$). *Bifidobacterium pseudocatenulatum* was detected more commonly in feces of non-breast-fed children (odds ratio, 5.6; 95% CI, 1.3-24.3; $P = .02$) and children with eczema (eczema, 26%; no eczema,

4%; $P = .04$). There were no significant associations between clinical measurements and detection of *B pseudocatenulatum*.

Conclusion: Presence of *B pseudocatenulatum* in feces was associated with eczema and with exclusive formula-feeding; *B bifidum* was associated with breast-feeding.

Clinical implications: *B pseudocatenulatum* provides a biomarker, provisional until larger studies are conducted, of susceptibility to atopic eczema in a subset of patients. Considering its previously reported effects on immune cells, this bacterial species may be shown in the future to have an etiologic role in atopic eczema. (J Allergy Clin Immunol 2007;119:1016-1024.)

Key words: Eczema, atopic dermatitis, atopy, allergy, gastrointestinal, microbiota, microflora, bifidobacteria, fecal bacterial community

Many affluent countries have experienced an increase in the prevalence of allergic diseases in recent decades, including atopic dermatitis, asthma, and atopic rhinitis.¹ Colonization of the infant bowel by specific species of bacteria may be important in the initial regulation of the developing immune system.² Alterations to neonatal exposure to particular bacteria may have occurred because of the effects of hospital deliveries, cesarian sections, special care baby unit admissions, smaller family size, widespread use of antibiotics, good hygiene, and differences in maternal diet in modern times. Changes in the composition of the bowel microbiota might result in exposure, or the lack of exposure, of infants to specific bacteria. This would alter the exposure of children to important bacterial antigens at a critical time in the maturation of the immune system.³ Differences in the composition of the bacterial community in the feces (bowel) of children sampled in countries of high or low prevalence of atopic diseases have been reported,^{4,5} although there is little agreement in bacterial species between studies. Members of the genus *Bifidobacterium* have been considered to be important bacteria for investigation, however, because they form a major portion of the fecal bacterial community in early life.⁶

Previous work⁷ that we have conducted demonstrated that certain bifidobacterial species were characteristic of

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Abbreviations used

Cy5: Indodicarbocyanine
 EPX: Eosinophilic protein X
 F-EPX: Fecal eosinophilic protein X
 OR: Odds ratio
 SCORAD: Severity Scoring of Atopic Dermatitis
 TTGE: Temporal temperature gel electrophoresis
 U-EPX: Urinary eosinophilic protein X

the fecal community of infants born in countries (United Kingdom, New Zealand) with a high prevalence of atopic diseases compared with babies born in a low-prevalence country (Ghana). We report now the results of a study in which the fecal microbiota of infants with or without atopic eczema were compared and associations with clinical parameters related to allergy were sought.

METHODS

Study population

Infants age 3 to 6 months were recruited to an intervention study to investigate the effect of probiotic administration on the prevalence of atopic dermatitis. A subset of these infants formed the basis of a nested case-control study that we report here. The infants were divided into 2 groups: those with physician-diagnosed eczema (cases)⁸ and healthy infants without eczema (controls). The infants were recruited at community clinics, were in good general health, and had normal growth. Detailed information was obtained on the history of maternal exposure to antibiotics during pregnancy, exposure of the infant to antibiotics, feeding, gastrointestinal symptoms (colic, vomiting, stool characteristics), and family history of allergic disease. Exclusion criteria were gestation of 34 weeks or less, congenital abnormality, chronic disease, current administration of oral antibiotics, and current consumption of soya or extensively hydrolyzed formula. The infants were examined by a pediatrician (C. G.) who evaluated skin changes (Severity Scoring of Atopic Dermatitis [SCORAD] index)⁹ at 3 to 6 months of age. Urine was collected for determination of urinary eosinophilic protein X (U-EPX), and feces was collected for fecal eosinophilic protein X (F-EPX) assay and analysis of the fecal bacterial community. Gastrointestinal permeability was also measured. Blood was collected for measurement of allergen-specific serum IgE (cases only). Samples used in this study were collected before probiotic or placebo interventions. Infants with eczema (cases) were matched with infants without eczema (controls) according to the following variables: sex, age (days/weeks), feeding (breast/bottle/mixed), weaned to solids, and ethnicity (which might affect maternal diet). All aspects of the study were approved by the South Manchester Local Research Ethics committee (approval number 01/320), and informed consent was obtained from the parents.

U-EPX estimation

Urinary EPX was measured by immunofluorometric assay using a kit (Phadia, Uppsala, Sweden; lower detection limit <0.5 $\mu\text{g/L}$). Total urine volumes were not known; therefore, urinary creatinine was measured (Jaffé reaction; HiCo Creatinine; Boehringer Mannheim GmbH, Mannheim, Germany), and the results were expressed as the ratio U-EPX/creatinine ($\mu\text{g}/\text{mmol}$).

F-EPX estimation

Fecal extracts were prepared and F-EPX was measured by using a kit as described. EPX concentrations in feces were adjusted for water and were expressed as nanograms of EPX per gram of dried feces.¹⁰

Allergen-specific IgE measurements

Determination of specific serum IgE (cow's milk, egg, peanut, dust mite, cat, dog) was achieved using a kit (ImmunoCAP; Phadia). We defined sensitization as specific IgE >0.35 kU/L.

Gastrointestinal permeability

The lactulose/mannitol dual sugar test was used as described previously.^{11,12} Briefly, infants were administered the test solution (5 g lactulose and 1 g mannitol in 50 mL water) after a 3-hour fasting period. Then water was allowed for 2 hours after the test drink. Urine was collected from at least 60 minutes after administration of the test solution. Lactulose and mannitol were quantified in the urine by using HPLC with pulsed amperometric detection, and the lactulose/mannitol excretion ratio was calculated.

Analysis of the fecal bacterial community by temporal temperature gel electrophoresis

Fecal samples were collected before the clinic visit (held refrigerated in the home) or at the clinic, then stored at -80°C until analysis. The majority of samples were frozen within 8 hours of collection. Bacterial DNA was extracted from feces by using a previously described protocol.¹³ 16S rRNA gene sequences (V3 region) obtained by PCR¹⁴ from the DNA were used to generate profiles of the fecal bacterial community with the DCode universal mutation detection system apparatus (Bio-Rad, Hercules, Calif) on a 6% polyacrylamide gel prepared in 1.25 $\times$ TAE buffer (50 mmol/L TRIS, 25 mmol/L acetic acid, 1.25 mmol/L EDTA), and 7 mol/L urea (Merck, Darmstadt, Germany). Forty microliters of tetramethylethylenediamine (Sigma, St Louis, Mo) and 400 μL 10% ammonium persulfate (Bio-Rad) were added to 40 mL of the polyacrylamide/urea solution before pouring the gels. Thirty-five-microliter samples of PCR product were loaded into each well of the gel. Voltage was fixed at 20 V for 15 minutes at the beginning of electrophoretic period and then run at a fixed voltage of 40 V for 16 hours with an initial temperature of 63°C and a final temperature of 70°C (ramp rate, $0.4^{\circ}\text{C}/\text{h}$). A reference sample with fragments distributed throughout the whole gel was included in gel runs to permit normalization and valid comparison of profiles. After electrophoresis, the gels were stained with ethidium bromide solution (5 $\mu\text{g}/\text{mL}$) for 15 minutes, washed with deionized water for 30 minutes, and viewed by UV transillumination. The temporal temperature gel electrophoresis (TTGE) profiles were compared by using the Dice similarity coefficient with the Bionumerics software package (version 3.0; Applied Maths, Austin, Tex) at a sensitivity of 2% to 3%.

Quantification of bifidobacterial populations in feces by fluorescence *in situ* hybridization

Approximately 0.5 g (wet weight) stool sample was suspended in 5 mL PBS (130 mmol/L NaCl, 3 mmol/L NaH_2PO_4 , 7 mmol/L Na_2HPO_4 , pH 7.2) and vortexed with glass beads for 3 minutes. The fecal suspension was subsequently centrifuged at 300g for 1 minute, and a volume of 300 μL of the supernatant was removed and added to 900 μL 4% paraformaldehyde. After overnight storage at 4°C , the fecal suspension was centrifuged at 9000g for 5 minutes at 4°C . The pellet was suspended in 1 mL PBS and centrifuged at 9000g for 5 minutes at 4°C . Finally, the pellet was suspended in 400 μL PBS and mixed with 400 μL ice-cold ethanol and stored at -20°C until analysis.

The EUB 338 probe, which targets a conserved 16S rRNA gene sequence within the domain *Eubacteria*,¹⁵ was used as a positive hybridization control. The NON 338 probe¹⁶ was used as the negative control. Both control probes were covalently linked at their 5' ends either to fluorescein isothiocyanate or to the sulfoindocyanine dye indodicarbocyanine (Cy5). The bifidobacterial probe Bif164¹⁷ was labeled with Cy5. The probes were purchased from MWG Biotech (Bangalore, India).

As described previously,^{18,19} fixed cells were washed once in PBS and then resuspended in 1 mL TRIS-EDTA buffer (100 mmol/L TRIS-HCl, pH 8.0, 50 mmol/L EDTA). After centrifugation, pellets were resuspended in TRIS-EDTA buffer containing 1 mg mL⁻¹ lysozyme (Serva, Heidelberg, Germany) and incubated for 10 minutes at room temperature. The cells were then washed in PBS and suspended in 1.0 mL hybridization solution (900 mmol/L NaCl, 20 mmol/L TRIS-HCl, pH 8.0, 0.01% SDS, 30% formamide). Aliquots of 50 μ L were used for fluorescence *in situ* hybridization with control and group-specific probes. Protected from light, hybridizations were performed by using 1-mL 96-well microtiter plates overnight at 35°C in 50 μ L hybridization solution per well, to which was added 4 μ L of the appropriate labeled probe at 50 ng/ μ L (4 ng/ μ L final concentration). After hybridization, 150 μ L hybridization solution was added to each well, and the cells were pelleted at 4000 g for 15 minutes at room temperature. Bacterial cells were resuspended and incubated at 37°C for 20 minutes in 200 μ L washing solution (65 mmol/L NaCl, 20 mmol/L TRIS-HCl, pH 8.0, 5 mmol/L EDTA, pH 8.0, 0.01% SDS) to remove nonspecific binding of the probe. The cells were finally pelleted and resuspended in 150 μ L PBS. Aliquots of 100 μ L were added to 500 μ L PBS for data acquisition by flow cytometry.

Data acquisition was performed with a FACSCalibur flow cytometer (Becton Dickinson, Franklin Lakes, NJ) as described previously^{18,19} equipped with an air-cooled argon ion laser providing 15 mW at 488 nm, combined with a 635-nm red-diode laser. The parameters were collected as logarithmic signals. The 488-nm laser was used to measure the forward angle light scatter (FSC in the 488-nm band pass filter), the side angle light scatter (SSC in the 488-nm band pass), and the green fluorescence intensity conferred by fluorescein isothiocyanate-labeled probes (FL1 in the 530-nm band pass filter). The red-diode laser was used to detect the red fluorescence conferred by Cy5-labeled probes (FL4 in a 660-nm band pass filter). The acquisition threshold was set in the side scatter channel. The rate of events in the flow was generally below 3000 events/s. A total of 100,000 events was stored in list mode files. Subsequent analyses were conducted by using the CellQuest Software (Becton Dickinson). Quantification of bifidobacteria was performed as described in Lay et al,¹⁸ in which the bifidobacterial population was recorded as a percentage of total bacteria (EUB 338 probe).

Total bacterial counts

To determine the total bacterial count in feces, cells hybridized with the EUB 338 probe were used. Unlabeled beads with a diameter of 6 μ m at known concentration (Flow Check Undyed Particle; Polysciences, Inc, Warrington, Pa) were added to each sample at a final concentration of 10⁵ beads/mL and served as an internal standard to calibrate the sample volume. The ratio of EUB 338-stained cells to the number of beads was used to determine the bacterial counts expressed per gram of feces.

Detection of bifidobacterial species in feces

Fecal samples were thawed and diluted 10-fold in PBS (pH 7.5), and 1-mL volumes were used to extract bacterial DNA as described previously.¹³ Bifidobacterial species present in the feces were detected by PCR (amplification of a region of the transaldolase gene

TABLE I. Demographics of study population

Variable	Cases (eczema) (n = 37)	Controls (no eczema) (n = 24)	P value
Sex (male)	21 (57%)	13 (54%)	*
Ethnicity (white)	35 (95%)	23 (96%)	*
Family history of allergic diseases	31 (84%)	18 (75%)	.40
Age in days (SD)	154 (25)	150 (26)	*
Weight in grams (SD)	7267 (804)	7298 (882)	.89
Weaning diet ("solids")	37 (100%)	24 (100%)	*
Exclusively formula-fed	27 (73%)	15 (63%)	*
Exclusively breast-fed	2 (5%)	2 (8%)	*
Breast-fed ever	24 (65%)	19 (79%)	.23
Vaginal delivery	25 (68%)	17 (71%)	.79

*Matching factors.

showing different nucleotide composition in different species) and denaturing gradient gel electrophoresis, as described previously.²⁰ Only DNA from bifidobacteria is amplified from feces using these primers; therefore, every gel fragment represents a bifidobacterial species. Species were identified by reference to an identification ladder or by sequencing and alignment of sequences with those of reference strains.²⁰ The majority of the identifications (39/52) were the result of sequence analysis.

Statistical methods

The χ^2 test and multiple logistic regression were used for dichotomous outcomes. In multiple logistic regression analysis, corrections were made for the major confounders of breast-feeding, family history of allergic diseases, previous antibiotic administration, and mode of delivery. Log-transformation or square root transformation was used where possible to normalize the EPX and SCORAD data. Results were presented as geometric mean with 95% CIs if log-normalized or if square root-transformed as detransformed mean with 95% CI. Analysis of covariance was used to compare data (SPSS version 11.0 statistical package, SPSS Inc, Chicago, Ill).

RESULTS

Study population

Satisfactory matches between 26 of 40 healthy infants and 38 of 208 infants with eczema were achieved in which there was no difference in demographic and matching criteria. Fecal samples were obtained from 61 of these infants, which were therefore included in the study (37 cases, 24 controls; Table I).

Bacteriologic analysis

Similarity analysis of TTGE profiles of the fecal bacterial communities did not reveal clustering of eczema and control groups (Fig 1). The profiles were individually unique as has been reported previously for human feces.²¹ Biodiversity, as judged by counting the number of fragments per fecal profile, was similar in eczema and control infants (average number of fragments, 6; range, 5-9). The total number of bacteria detected in the feces did not differ between the cases and the controls (Fig 2, A; $P = .62$; Fisher exact test, 2-sided). Similarly, the proportion of

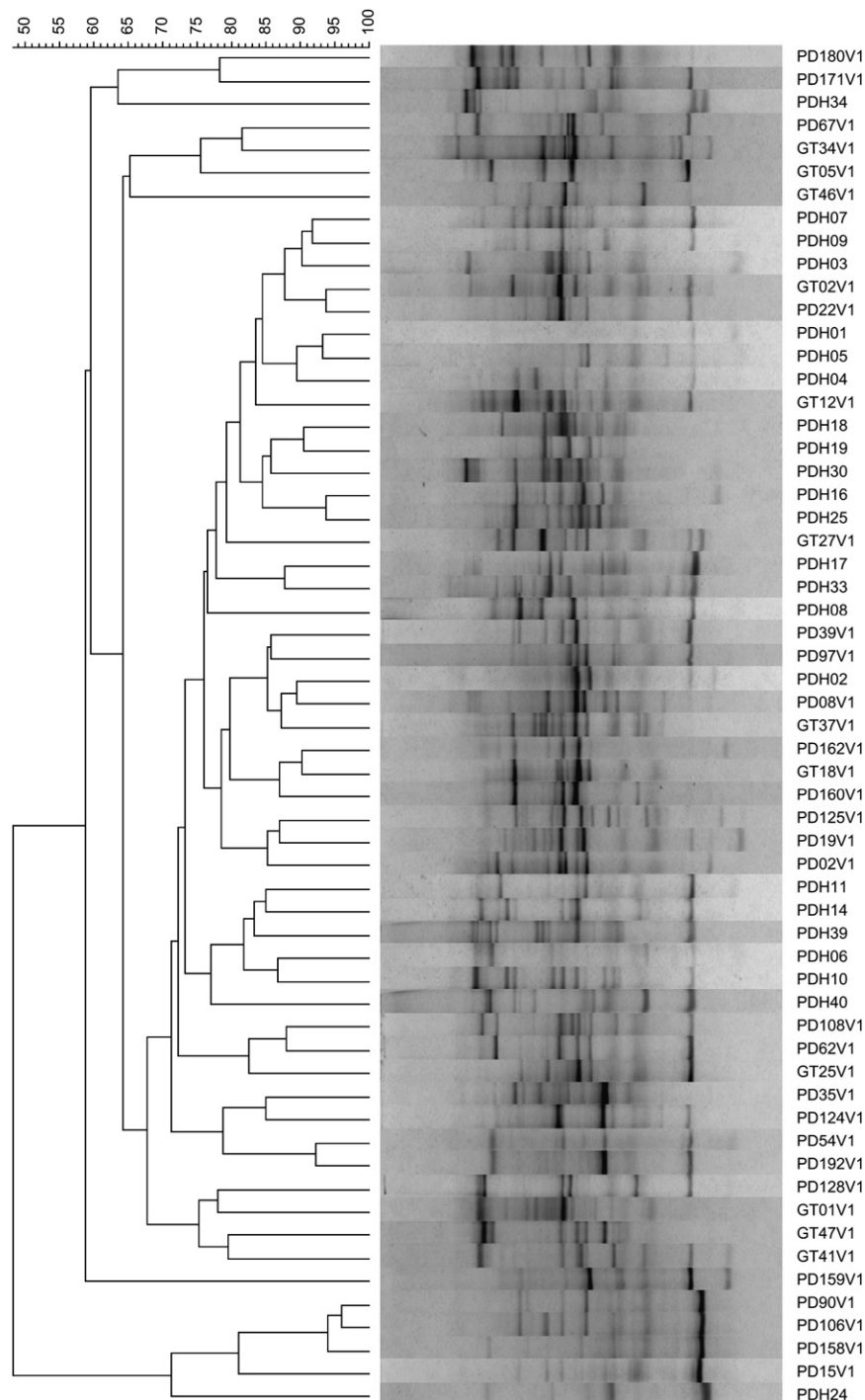


FIG 1. Dendrogram showing that TTGE profiles of fecal communities are randomly distributed and do not cluster as cases (*PD*, *GT*) or controls (*PDH*) on the basis of profile similarities. Profiles of suitable quality for analysis were obtained from 59 of 61 subjects.

the total bacterial community composed of bifidobacteria did not differ between the groups (Fig 2, *B*; $P = .94$).

There was no significant difference in the presence/absence of bifidobacteria between cases and controls (DGGE analysis; bifidobacteria absent in cases $n = 7$ [19%] vs controls $n = 5$ [21%]; $P > .85$). Breast-feeding

(ever/current) was not related to the presence/absence of bifidobacteria. Infants (cases and controls) without a family history of allergic disease were less likely to have bifidobacteria present in their feces (7/12 [58%] vs 42/49 [86%]; $P = .05$; Fisher exact test, 2-sided). Logistic regression analysis, controlling for breast-feeding (ever)

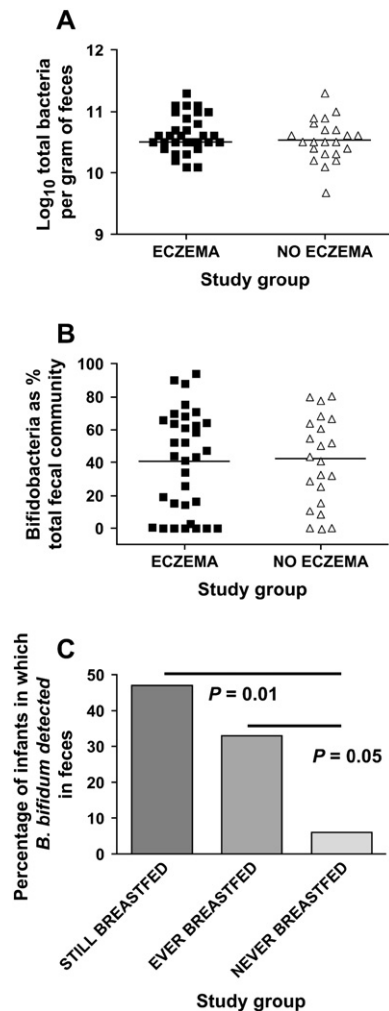


FIG 2. A. Total bacterial numbers in the feces of cases (*eczema*) and controls (*no eczema*). Squares and triangles represent individual infants; median is shown by horizontal line. Sufficient sample was available to assay 33 cases and 22 controls. **B.** Bifidobacterial population as percentage of total fecal community. Other details as per graph A. **C.** Prevalences of *B. bifidum* in feces in relation to breast-feeding.

and presence/absence of eczema, confirmed this finding that the absence of family history reduced the likelihood of infants having detectable fecal bifidobacteria (odds ratio [OR], 0.23; 95% CI, 0.06-0.96; $P = .04$). Infants (cases and controls) who were breast-fed were more likely to harbor *Bifidobacterium bifidum* in their feces compared with children who had never been breast-fed (Fig 2, C). Logistic regression analysis correcting for presence/absence of eczema and family history of allergic diseases confirmed that the odds for having detectable *B. bifidum* in the feces were significantly higher in breast-fed children compared with those who were never breast-fed (OR, 5.19; 95% CI, 1.47-18.36; $P = .01$). Nonbreast-feeding (cases and controls), however, was significantly associated with the presence of *Bifidobacterium pseudocatenulatum* (not breast-fed, 7/18 [39%]; breast-fed, 4/43 [9%]; $P = .01$). Logistic regression analysis confirmed this increased

TABLE II. Prevalences of bifidobacterial species in the feces of cases (*eczema*) and controls (*no eczema*)

Bifidobacterial species detected in feces*	Cases (<i>eczema</i>) [n (%)]	Controls (<i>no eczema</i>) [n (%)]	P value (Fisher exact, 2-sided)
<i>B. adolescentis</i>	8/37 (22%)	6/24 (25%)	.76
<i>B. bifidum</i>	7/37 (19%)	8/24 (33%)	.24
<i>B. catenulatum</i>	2/37 (5%)	2/24 (8%)	.64
<i>B. infantis</i>	4/37 (11%)	2/24 (8%)	1.00
<i>B. pseudocatenulatum</i>	10/37 (27%)	1/24 (4%)	.04
<i>B. longum</i>	2/37 (5%)	None	—
None detected	7/37 (19%)	5/25 (21%)	1.00

*More than 1 bifidobacterial species was detected in the feces of some infants.

risk (OR, 5.6; 95% CI, 1.3-24.3; $P = .02$) for nonbreast-fed infants when family history of allergic diseases and presence/absence of eczema were included as potential confounders.

Strikingly, *B. pseudocatenulatum* was more commonly detected in the feces of cases (Table II).

Clinical comparisons

Mode of delivery, maternal antibiotic use during/since pregnancy, and infant antibiotic use (ever) were not related to differences in bifidobacterial populations. Abnormal gastrointestinal permeability, fecal or urinary EPX levels, sensitization (specific IgE >0.35), and SCORAD score at the time of sample collection were not associated with the detection of particular bifidobacterial species (Table III). U-EPX at age 3 to 6 months tended to be higher in the group without detectable bifidobacteria ($P = .06$). Inclusion of potential confounders and interaction terms in the ANOVA model revealed significantly higher U-EPX levels in infants with eczema and no detectable bifidobacteria compared with infants with bifidobacteria as well as controls with/without bifidobacteria ($P = .03$).

DISCUSSION

Temporal temperature gel electrophoresis profiling of the fecal bacterial communities of infants with or without eczema was not predictive of clinical condition in this study. There is huge variation in the composition of the fecal bacterial community in terms of predominant phylotypes between individuals, both children and adults, and this provides a confounding factor in attempts to understand the impact of specific bacteria on physiological functions, and hence on the health of human beings.^{6,18,22}

Investigation of the bifidobacterial populations identified independent, significant associations of environmental (mode of feeding) as well as familial factors (family history of allergic disease) with presence/absence of different bifidobacterial species in cases and controls. Moreover, striking differences in the prevalence of bifidobacterial species between cases and controls were noted. Unlike previous investigators,²³ we did not see a

TABLE III. Clinical measurements and bifidobacteria in feces

	Bifidobacteria present?			P value	<i>B bifidum</i> ?			P value	<i>B pseudocatenulatum</i> ?			P value
	N	Mean* (95% CI)			N	Mean (95% CI)			N	Mean (95% CI)		
SCORAD age	No 7	23.7 (16.4-32.4)		.97	No 30	24.4 (21.3-27.8)		.24	No 27	24.4 (21.1-28.0)		.35
3-6 mo (cases only)	Yes 30	23.6 (20.4-27.0)			Yes 7	20.2 (13.1-26.8)			Yes 10	21.4 (15.5-28.2)		
F-EPX (ng/g)	No 12	1533 (818-2875)		.42	No 44	1723 (1263-2350)		.20	No 48	1759 (1300-2381)		.22
age 3-6 mo	Yes 47	2018 (1475-2762)			Yes 15	2578 (1392-4774)			Yes 11	2722 (1331-5585)		
U-EPX/creatinine (μg/mmol)	No 12	34.8 (13.9-87.3)		.06†	No 42	17.4 (10.4-28.9)		.34	No 45	15.9 (9.7-26.2)		.72
age 3-6 mo	Yes 44	12.2 (7.4-20.4)			Yes 14	10.5 (3.7-29.7)			Yes 11	13.0 (3.8-45.2)		
Lactulose/mannitol	No 4	0.11 (0.01-1.33)		.64	No —	Insufficient numbers		—	No —	Insufficient numbers		—
ratio age 3-6 mo	Yes 9	0.08 (0.05-0.12)			Yes —				Yes —			
Sensitized to any	No 4	Sens: n = 2 (50%)		.38	No —	Insufficient numbers		—	No —	Insufficient numbers		—
allergen (cases only)	Yes 21	Sens: n = 15 (88%)			Yes —				Yes —			
Skin problems age	No 5	Prob: 2 (40%)		.64	No 16	Prob: 8 (50%)		.18	No 23	Insufficient numbers		—
1 year (controls only)	Yes 19	Prob: 7 (37%)			Yes 8	Prob: 1 (12%)			Yes 1			

Sens, Sensitized; Prob, problems.

*Mean = geometric mean for log-transformed values (F-EPX, U-EPX), detransformed square-root mean (SCORAD).

†Tested in ANOVA model with relevant confounders. (Estimated marginal means: eczema/no bifidobacteria n = 7, 78.5 [23.5-261.4]; eczema/bifids n = 30, 10.7 [6.0-19.2]; no eczema/no bifids n = 5, 11.2 [2.7-46.6]; no eczema/bifids n = 19, 16.2 [6.9-38.1]; P = .03 for interaction; overall model P = .08). Breast-feeding and family history of allergic disease not significant in the model.

difference between infant groups in terms of overall bifidobacterial counts.

The species *B bifidum* was more prevalent in the feces of breast-fed infants than of infants who were not breast-fed. Almost half of the breast-fed infants had this species in their feces during the period of breast-feeding, indicating that substrates and physiological conditions in the bowel of breast-fed children are conducive to the establishment of *B bifidum*. Finnish investigators have reported, on the basis of the results of culture-based studies involving few infants, that healthy infants were more likely to harbor *B bifidum* than infants with allergy.^{4,24} We observed, however, that the prevalence of *B bifidum* was not significantly different between cases and controls, just as we found with older children in a previous investigation.²²

Breast-feeding affected the prevalence of *B pseudocatenulatum* in the feces of infants; those who had never been breast-fed or were no longer breast-fed at the time of the study had significantly increased odds of harboring this species. *B pseudocatenulatum* was more common in infants with atopic eczema, and 39% of cases that were formula-fed had detectable *B pseudocatenulatum* in the feces and hence in their bowels.

Clinical measurements did not reveal an association between *B pseudocatenulatum* and specific factors of importance in allergic diseases. Therefore, we cannot propose an etiologic role for this bacterial species in atopic eczema. Nevertheless, the association of these bacteria with an atopic condition fits well with previous observations that we have made in relation to bifidobacteria and allergic diseases of children. Comparison of the composition of the bifidobacterial population of infants age 25 to 35 days who had been born in Ghana (32 children), the United Kingdom (17 children), and New Zealand (23 children)

showed that although *Bifidobacterium infantis* was present in the case of all infants from Ghana in whom fecal bifidobacteria were detected, none of the British or New Zealand children harbored this species.⁷ In contrast, the bifidobacterial population of British and New Zealand infants was composed of 1 or more of the species *B bifidum*, *Bifidobacterium longum*, *Bifidobacterium adolescentis*, and *B pseudocatenulatum*. The majority of infants in this previous study were wholly or partially breast-fed (100% of Ghana subjects, 71% United Kingdom, 97% New Zealand) but were younger than those investigated in our current study. Ghana has a low incidence of atopic diseases relative to the United Kingdom and New Zealand, where there is a high prevalence. The bifidobacterial species detected in the infants showed a differential effect in activation of naive dendritic cells and cytokine (specifically IL-10) production.⁷ It was concluded that *B infantis* cells did not trigger the activation of dendritic cells to the degree necessary to initiate an immune response but that *B bifidum*, *B longum*, and *B pseudocatenulatum* induced a T_H2-driven immune response.

The outcome of our study reported in this article focuses further attention on *B pseudocatenulatum* as a species with a potential role in the etiopathogenesis of atopic eczema. More prevalent in the feces of formula-fed infants with eczema, this bacterial species may, at the least, provide a biomarker of susceptibility to atopic eczema in a subset of patients. *B pseudocatenulatum* may yet be shown to have an etiologic role in atopic dermatitis after further investigations of the interplay between bifidobacteria and the immune system have been conducted.

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