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Atopic dermatitis and food sensitization in South African toddlers: Role of fiber and gut microbiota

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The pathogenesis of atopic dermatitis (AD) is complex and related to allergic responses and defects in skin barrier function. In common with many atopic diseases, the prevalence of AD has been increasing

across the world.[1] One of the theories for this increase is increased hygiene and urbanization-related changes in the environment, which can affect the human microbiome.[2] Previous studies have found associations between the composition of the early gut microbiome and development of atopic conditions, including AD.[3] Although the rate of atopic conditions, including AD and food allergy, is increasing on all continents, the prevalence of these diseases is still lower in African countries.[1] This is especially interesting because individuals of African origin who live in Western countries, such as African Americans, are at a higher risk for severe AD.[4] This variation places Africa in a special position; studying African populations is necessary not only to find ways to prevent increases of allergy conditions in African countries but also to provide important clues to the causes of this global increasing of allergic conditions.

Young children who have developed AD in African communities with a low incidence of atopic disease might be the transitional group. In the current study, we have, for the first time to our knowledge, analyzed the fecal microbiota composition of a group of young black African children aged 12 to 36 months old with and without AD living in the same community in Cape Town, South Africa. Our primary goal was to examine whether toddlers with AD and control toddlers from Cape Town have different microbiomes in terms of bacterial richness and diversity. We also aimed to investigate the differences in the relative abundance for different operational taxonomic units between these 2 groups. In our subgroup analyses, we further tested the effect of multiple environmental factors on the gut microbiome in these children.

The study participants and methods are detailed in Appendix 1. Briefly, children (12 to 36 months old) with AD and a group of nonallergic control children were recruited from the pediatric dermatology department of the Red Cross War Memorial Children's Hospital and Child Care Facilities in the Cape Town Metropole. Detailed dietary questionnaires were used to obtain data on feeding patterns, including breastfeeding history and duration, total energy intake, total fat composition, and fiber intake. All children were evaluated by skin prick test (SPT) for sensitivity to egg white, peanut, and cow's milk. Early morning feces from the children's diaper were collected and used for microbiome composition assessments using bacterial 16S ribosomal RNA. All data were exported to a STATA database (StataCorp Inc.,

College Station, Texas) for analysis. Tests were performed using the nonparametric Kruskal-Wallis 1-way analysis of variance (ANOVA). A false-discovery rate (FDR)e adjusted P value was calculated for each analysis to adjust for multiple comparisons. Statistical significance was set at $P \leq .05$.

This study was approved by the institutional review boards of the University of Cape Town's Faculty of Health Sciences and of Rush University Medical Center. Written informed consent was obtained from the parent or guardian for all participants.

The study included 29 children with AD and 9 control children. The AD and control groups had no significant differences with regard to age, body mass index, and sex distribution. Among the children with AD, 17 were sensitized to at least 1 food, and 11 were not sensitized to any of the foods tested. One child could not be skin tested because of extensive eczema. All control children had negative skin test results to foods (Table 1).

There was no difference between patients with AD and controls in terms of being breastfed at all or duration of breastfeeding. Children with and without AD took a mean of 12.7 g and 14.1 g of fiber per day. No significance differences were found between the 2 groups in terms of nutrient-related variables measured in their 1- day diet recall (Table A1).

Table 1. Demographic and food sensitivity information in 38 children with and without atopic dermatitis from Cape Town, South Africa

Variable	Children with atopic dermatitis (n = 29)	Controls (n = 9)	χ^2 or *t* test *P* value
Age, mean (SD), y	2.08 (0.57)	2.27 (0.49)	.78
Male sex, No. (%)	17 (58.6)	5 (55.5)	.87
BMI, mean (SD)	17.90 (1.53)	17.17 (1.28)	.19
Food sensitization, No. (%)[a]			
At least 1 tested food	17 (60.7)	0	.002
Egg	12 (42.8)	0	.02
Milk	1 (3.5)	0	.56
Peanut	7 (25.0)	0	.10
Polysensitized	3 (10.7)	0	.30

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by the square of height in meters).

a. Of the 29 children with AD, only 28 could be skin tested because 1 child had severe generalized eczema with no intact skin.

No significant differences were observed in microbial a diversity between the children with AD and the control children, and there were no differences in the relative abundance for any taxa between these 2 groups after adjusting for multiple comparisons. At the taxonomic level of phylum, the relative abundance of Actinobacteria was higher in food sensitized cases compared with patients with AD without food sensitization or controls, although this difference was not statistically significant (P = .02; FDR P = .08). This trend continued at the taxonomic level of genus, where the relative abundance of *Blautia* (P = .04; FDR P = .27) and *Bifidobacterium* (P = .01; FDR P = .20) were higher in food sensitized than nonsensitized children but after FDR correction was not significant.

Breastfeeding or no breastfeeding at all had no effect on the gut microbiota at ages 12 to36months.We grouped the children based on their total daily fiber intake to 2 groups (high vs low based on median fiber intake). Total fiber intake had no effect on the gut microbiota.

To assess the association between fecal microbiota and age, we pooled data for all individuals (total of 38 African black children with and without AD) and categorized the children based on their age to those children aged 12 to 24 months (n = 15) and 24 to 36 months (n=23), which were found to have similar gut microbiota (Table A2).

This pilot study found no major differences in the composition of the gut microbiota of 12- to 36-month-old children with and without AD. This lack of association of changes in the gut microbiota with AD could be attributable to our small sample size. However, post hoc power analysis for our primary end point, the difference in the species richness between children with AD and control toddlers revealed that we had a power of 78.7% to find a difference under P = .05. Another possible explanation for this lack of association between AD and microbiota composition in our cohort is that in this African population AD is mainly influenced by other factors, such as skin barrier integrity or other predisposing genetic factors. Our negative finding is in agreement with a previous study[5] in this age group. It is possible that the gut microbiota differences reported in association with AD and food allergy[3,6,7] are limited to the first year of life as the immune system is shaping. Furthermore, it is possible that some previous studies that found large changes in the microbiota in association with AD and food sensitization in small sample sizes are reporting crude

differences driven by ANOVA without adjusting for multiple comparisons, which can tremendously affect the results.

We found that Black South African children aged 12 to 36 months consume approximately 13 g/d of fiber, which is higher than the consumption for US children in the same age group who consume approximately 8.2 g/d of fiber.[8] The Prevotella genus comprised 28.8% of the gut bacteria in South African children in our study. Similar to our results, De Filippo et al[9] found that African children had a high daily intake of fiber and their gut microbiota was enriched in the taxonomic levels of phylum Bacteroidetes and genus *Prevotella*, which was significantly higher than in European children. That study also found significantly more short chain fatty acids in the feces of African than in European children and hypothesized that the intestinal microbiota coevolved with diets rich in polysaccharides in these African children. The total fiber intake among the South African children in our study did not vary between children with AD and control children, which is most likely attributable to the homogeneity of this population in terms of their cultural and diet habits. The lack of association of AD or food sensitization with changes in the gut microbiota at 12 to 36 months indicates that the underlying mechanism of AD in this population might be related to other factors or bacterial microbiota composition during the first 12 months of life only.

The main limitation of our study is the small number of participants, which might have affected our results, limited the ability to conduct subgroup analyses, and affected the power of the analyses. Whether our negative results are attributable to our small sample size or a true lack of association needs to be investigated in a larger study.

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Appendix 1

Study Participants

Children (12 to 36 months old) with AD were recruited from the pediatric dermatology department of the Red Cross War Memorial Children's Hospital in Cape Town. AD was diagnosed by a dermatologist based on diagnostic criteria proposed by American Academy of Dermatology, including essential features of pruritus and eczematous rash with typical morphologic and age-specific patterns and chronic or relapsing history. Nonallergic non-food-sensitized children with no history of skin problems living in the same area were recruited from Child Care Facilities in the Cape Town Metropole as control children.

Diet Assessments

Detailed questionnaires were used to obtain data. Food consumption was assessed with one 24-hour dietary recall, which was verbally administered by a trained study staff using portion size illustrations to obtain accurate quantities. The 24-hour food consumption data were entered into the Food Processor Nutrition software program (created by Elizabeth Stewart, Hands and Associates, Salem, OR) and was analyzed for key nutrients of interest, including total energy intake, fat composition, and fiber intake. Fiber intake was dichotomized based on median intake.

Allergic Sensitization Assessments

All children enrolled in the study were evaluated by skin prick test for sensitivity to foods. Skin prick tests were performed using standardized solutions from ALK Abello (Thermo Fisher Scientific) for sensitivity to egg white and to peanut and cow's milk extracts. Children were considered food sensitized if they had a wheal larger than 3 mm to at least one allergen.

Fecal Collection

Parents were asked to collect the early morning feces from the children's diaper. Study staff scooped 2 to 3 walnut-sized pieces of feces with a sterile spatula into a clear Gaspack bag and transferred them to a –20°C freezer within 2 hours of being passed.

Microbial Community Structure Analysis

Bacterial genomic DNA from fecal samples was polymerase chain reaction amplified using primers (515F/806R) targeting the V4 variable region of microbial small subunit (SSU or 16S) ribosomal RNA genes and prepared for next-generation sequencing using a modified 2-step targeted amplicon sequencing approach. Sequencing was performed using an Illumina MiSeq, with a V2 kit and paired-end 250 base reads at the University of Illinois at Chicago. Demultiplexing of sample reads was performed. Raw FASTQ files for each sample

were processed using the software package PEAR (Paired-End Read Merger). The merged FASTQ files were imported into the software package CLC Genomics Workbench 8.0 (CLC Bio, Qiagen, Hilden, Germany). Primer sequences were removed, and sequences without both forward and reverse primers were discarded. Sequences were also trimmed using quality trimming algorithms (quality threshold, Q20) and length trimming (discarded everything <250 bp). The trimmed files were then exported as FASTA files into the software package QIIM for chimera removal using the USEARCH6.1 algorithm. The chimera-free FASTA files were then processed to cluster sequences into operational taxonomic units at a similarity threshold of 97% using the QIIME script. Representative sequences for each operational taxonomic unit were selected, and these sequences were annotated using the Greengenes_13_5 reference (97_otus.fasta) and taxonomy database (97_otu_taxonomy.txt). These data were processed into a multitaxonomic level biological observation matrix (BIOM; McDonald et al, 2012). The BIOM files were subsampled (rarefied) to the same number of sequences (18,000 sequences per sample) to reduce the effect of variable library size on diversity measures. Taxa with a mean abundance of less than 1% across the entire sample set were removed from such analyses. Raw sequence data (FASTQ files) were deposited in the National Center for Biotechnology Information Sequence Read Archive. Differences in the relative abundance of individual taxa among different groups were tested using the group significance algorithm, implemented within QIIME. Tests were performed using the nonparametric Kruskal- Wallis 1-way analysis of variance. To adjust for multiple comparisons, a false-discovery rate adjusted *P* value was calculated for each analysis.

Table A1. Dietary information for children from Cape Town, South Africa with and without AD and food sensitization[a]

Variable	Atopic dermatitis with food sensitivity (n = 17)	Atopic dermatitis without food sensitivity (n = 11)	Controls (n = 9)	χ^2, ANOVA, or Kruskal-Wallis *P* value
Breastfeeding at all, %	76.4	81.8	77.7	.94
Breastfeeding >6 months, %	41.1	63.6	33.3	.34
Duration of breastfeeding, mean (SD), mo	8.47 (9.62)	10 (9.43)	3.16 (4.47)	.19
Energy, kcal	1302.9 (548.4)	1146.8 (904.4)	1143.7 (814.6)	.76
Carbohydrate, % of energy	59.7 (19.1)	66.4 (13.0)	68.7 (16.6)	.55
Total fiber intake, g	12.6 (56)	12.8 (8.5)	14.1 (8.7)	.65
Soluble fiber	0.7 (1.1)	1.0 (1.5)	0.9 (2.1)	.47
Sugar	72.4 (36.6)	57.9 (48.5)	82.9 (36.2)	.25
Fat, % of energy	29.6 (14.4)	23.2 (14.8)	20.3 (11.9)	.50
Saturated fat, g	11.7 (13.2)	16.3 (15.5)	9.4 (6.3)	.53
ω3 fatty acids, mg	315.0 (390.0)	365.0 (370.0)	270.0 (320.0)	.85
Protein, % of energy	12.8 (5.9)	12.1 (6.5)	12.8 (7.4)	.45
Calcium, mg	563.9 (465.0)	593.0 (462.5)	351.2 (617.8)	.89
Vitamin D, IU	32.7 (188.7)	67.1 (105.7)	14.6 (159.6)	.82
Vitamin A, RAE	381.6 (311.2)	282.2 (498.7)	230.0 (706.9)	.70
Vitamin C, mg	35.4 (61.6)	40.8 (70.0)	63.8 (106.1)	.89
Thiamine, mg	0.6 (0.3)	0.6 (1.1)	0.9 (0.2)	.55
Iron, mg	5.1 (7.2)	7.7 (7.4)	9.7 (6.2)	.36
Iodine, mg	16.4 (33.8)	35.3 (33.1)	14.0 (41.3)	.85

Abbreviations: ANOVA, analysis of variance; RAE, retinol activity equivalent.

a. Data expressed as median (calculated interquartile) unless otherwise noted; nutrient data expressed as intake per day.

Table A2. Relative abundance of sequences derived from individual taxa

Taxonomic level	Sample type: AD vs control				Ages of children				Children breastfed				Food sensitivity of children			
	P	FDR *P*	No. of sequences for children with AD	No. of sequences for controls	*P*	FDR *P*	No. of sequences for 12- to 24-month-olds	No. of sequences for 24- to 36 month-olds	*P*	FDR *P*	No. of sequences for breastfed children	No. of sequences for children not breastfed	*P*	FDR *P*	No. of sequences for children with sensitivity ≤6 months	No. of sequences for children with sensitivity >6 months
Phylum																
Actinobacteria	.04[a]	0.17	577.28	177.11	.46	0.86	304.93	598.30	.51	0.65	313.77	1115.25	.02[c]	0.08	772.94	246.95
Bacteroidetes	.07	0.17	6433.62	8607.11	.52	0.86	7497.27	6590.43	1.00	1.00	7030.57	6640.25	.06	0.16	5716.24	7850.25
Firmicutes	.31	0.39	9830.69	8618.89	.69	0.86	9358.47	9664.48	.33	0.65	9747.33	8780.00	.26	0.34	10156.12	9116.55
Proteobacteria	.14	0.24	535.59	240.78	.94	0.94	462.13	468.13	.41	0.65	358.57	867.75	.27	0.34	594.65	377.10
Other	.80	0.80	580.59	339.67	.09	0.43	335.07	646.43	.52	0.65	523.87	522.25	.69	0.69	714.71	379.05
Genus																
Bifidobacterium	.10	0.47	572.62	171.00	.49	0.86	308.00	588.04	.68	0.84	306.90	1117.25	.01[d]	0.20	760.76	248.85
Bacteroides	.31	0.67	1793.34	3235.22	.69	0.86	2059.93	2183.70	.38	0.84	1981.40	2710.25	.34	0.64	1521.76	2759.30
Prevotella	.57	0.77	4297.45	5104.00	.48	0.86	5130.20	4069.96	.67	0.84	4766.33	3446.50	.74	0.78	3931.06	4715.25
Clostridium	.46	0.77	582.69	1111.78	.85	0.89	546.20	813.52	.67	0.84	607.13	1086.25	.30	0.64	485.47	925.70
Blautia	.01[b]	0.19	740.38	260.11	.60	0.86	648.53	612.35	.69	0.84	639.17	579.63	.04[d]	0.27	836.06	450.75
Roseburia	.85	0.85	594.62	587.89	.89	0.89	724.93	507.00	.20	0.84	591.10	600.25	.65	0.75	559.06	573.40
Ruminococcus	.06	0.46	385.55	219.33	.23	0.68	383.00	322.17	.80	0.84	333.93	392.13	.08	0.30	422.18	266.75
Clostridium	.55	0.77	375.34	152.11	.40	0.86	444.07	243.17	.28	0.84	389.90	69.63	.52	0.74	210.47	433.60
Peptostreptococcus	.25	0.64	296.10	108.67	.64	0.86	298.60	221.13	.84	0.84	173.60	544.63	.38	0.64	303.82	211.35
Faecalibacterium	.13	0.47	3224.79	1886.22	.69	0.86	3023.27	2832.43	.33	0.84	3057.90	2344.75	.05	0.27	3656.24	2368.90
Oscillospira	.21	0.63	550.90	940.33	.02[e]	0.33	344.00	838.22	.24	0.84	544.40	1013.38	.25	0.62	620.00	677.40
Ruminococcus	.62	0.77	1400.07	1891.56	.06	0.43	999.67	1853.52	.54	0.84	1634.60	1073.50	.60	0.75	1311.88	1721.30
Dialister	.68	0.78	310.83	515.22	.88	0.89	429.53	313.39	.76	0.84	408.13	175.88	.21	0.62	367.65	334.60
Other	.80	0.85	588.72	338.44	.10	0.49	343.27	650.87	.51	0.84	527.13	538.13	.78	0.78	724.29	381.35

Abbreviations: AD, atopic dermatitis; FDR, false discovery rate.

a. Although there was a trend toward higher relative abundance of bacteria from the phylum Actinobacteria in the children with AD compared with controls ($P = .04$), after adjusting for multiple comparisons, this was not significant (FDR $P = .17$).

b. At the taxonomic level of genus, the relative abundance of *Blautia* was higher in children with AD than in control children ($P = .01$), but after adjusting the analyses with FDR, this was not significant (FDR $P = .19$).

c. At the taxonomic level of phylum, the relative abundance of Actinobacteria was higher in food sensitized cases compared with atopic cases without food sensitization or controls, although this difference was not significant ($P = .02$; FDR $P = .08$).

d. The relative abundance of *Blautia* ($P = .04$; FDR $P = .27$) and *Bifidobacterium* ($P = .01$; FDR $P = .20$) were higher in food sensitized than nonsensitized children, but after FDR correction, was not significant.

e. The 24- to 36-month-old children had higher relative abundance of the genus level taxa *Oscillospira* than the 12- to 24-month-old children, but after FDR correction, this was not significant ($P = .02$; FDR $P = .33$). Analysis of similarity at the taxonomic level of genus was not significant when comparing patients with AD and controls (global $R = 0.124$, $P = .096$), food sensitized vs nonsensitized (global $R = 0.002$, $P = .43$), breastfed vs nonbreastfed (global $R = 0.019$, $P = .34$), or higher fiber consumers vs low fiber consumers (global $R = –0.025$, $P = .76$).