

Multiscale study of the oral and gut environments in children with high- and low-threshold peanut allergy



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Background: The oral and gut microbiomes have each been associated with food allergy status. Within food allergy, they may also influence reaction thresholds.

Objective: Our aim was to identify oral and gut microbiota associated with reaction thresholds in peanut allergy.

Methods: A total of 59 children aged 4 to 14 years with suspected peanut allergy underwent double-blind, placebo-controlled food challenge to peanut. Those children who reacted at the 300-mg or higher dose of peanut were classified as high-threshold (HT), those who reacted to lower doses were classified as low-threshold (LT), and those children who did not react were classified as not peanut allergic (NPA). Saliva and stool samples collected before challenge underwent DNA isolation followed by 16S rRNA sequencing and short-chain fatty acid measurement.

Results: The 59 participants included 38 HT children and 13 LT children. Saliva microbiome α -diversity (Shannon index) was higher in LT children ($P = .017$). We identified saliva and stool microbiota that distinguished HT children from LT children, including oral *Veillonella nakazawae* (amplicon sequence variant 1979), which was more abundant in the HT group than in the LT group (false discovery rate [FDR] = 0.025), and gut *Bacteroides thetaiotaomicron* (amplicon sequence variant 6829), which was less abundant in HT children than in LT children (FDR = 0.039). Comparison with NPA children revealed consistent ordinal trends between these discriminating species and reaction thresholds. Importantly, many of these threshold-associated species were also correlated with short-chain fatty

acid levels at the respective body sites, including between oral *V. nakazawae* and oral butyrate ($r = 0.57$; FDR = 0.049).

Conclusion: Findings from this multiscale study raise the possibility of microbial therapeutics to increase reaction thresholds in children with food allergy. (J Allergy Clin Immunol 2022;150:714-20.)

Key words: Peanut allergy, threshold, microbiome, short-chain fatty acid, oral, saliva, stool, gut, metabolomics

INTRODUCTION

Peanut allergy is a clinical and public health problem that affects 2% to 5% of US children¹ and approximately 1% of the overall US population.² The etiology of peanut and other food allergy involves deviation from a default state of immune tolerance that is likely driven by antigen exposure, commensal microbiota, and their interactions.³

Growing evidence supports a role for microbiota in the pathogenesis and course of food allergy.³⁻¹¹ Variations in gut microbiota have been associated with allergen sensitization,⁸ milk allergy,⁵ egg allergy,⁶ and food allergy in general.⁷ Interestingly, murine models demonstrate that food allergy phenotypes can be induced by fecal transfer,^{9,11} suggesting causal roles for gut microbiota in food allergy. Our group identified differential gut microbiota in children with egg allergy versus in the controls,⁶ and we also identified gut microbiota associated with the later resolution of milk allergy.⁵

A modality by which microbiota may influence host immune systems and the course of food allergy is via metabolites. Metabolites produced by gut bacteria such as short-chain fatty acids (SCFAs) induce regulatory T-cell generation, migration, and accumulation^{12,13} and affect the generation of tolerance-promoting dendritic cells and mucosal surface integrity.¹⁴

Because the oral mucosa is the beginning of a continuous gastrointestinal mucosal system rich with immune cells and microbiota, oral microbiota and metabolites produced by them could influence food allergy. Successful desensitization from sublingual immunotherapies for food allergy supports the importance of the oral environment,¹⁵ as do the results from a multidimensional analysis of saliva from children with peanut allergy and controls without food allergy in whom microbial and metabolic profiles associated with mucosal immune disturbances in peanut allergy were identified.¹⁶

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Abbreviations used

ASV:	Amplicon sequence variant
FDR:	False discovery rate
HT:	High-threshold
LT:	Low-threshold
NPA:	Not peanut allergic
SCFA:	Short-chain fatty acid
SPLSDA:	Sparse partial least squares discriminant analysis

This study addresses the hypothesis that microbiota and metabolites in the oral and gut environments are associated with reaction thresholds in challenge-proven peanut allergy. We rigorously identified reaction thresholds in children via double-blind, placebo-controlled food challenges to peanut and examined pre-challenge saliva and stool samples from these children. Here we report our findings about relationships between oral and gut microbiome diversity, composition, SCFA levels, and reaction thresholds.

RESULTS AND DISCUSSION

Fig 1 provides an overview of the study flow.

Study population and design

A total of 59 children aged 4 to 14 years with suspected peanut allergy underwent double-blind, placebo-controlled food challenge as part of the Challenging to Food with Escalating Thresholds for Reduced Food Allergy (CAFETERIA) trial ([Clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03907397) identifier NCT03907397).¹⁷ In all, 38 participants demonstrated allergy symptoms after eating 300 mg or more of peanut protein (443 mg cumulatively eaten) and were classified as high-threshold (HT) (Fig 1, A). As reference, 1 peanut contains 300 mg of peanut protein. Thirteen children who reacted to less than 300 mg were classified as low-threshold (LT), and 8 subjects who did not experience any symptoms in response to peanut were deemed not peanut allergic (NPA). The LT children had higher peanut-specific IgE levels and larger peanut skin test wheal sizes than the other children (Table I). There were no significant differences in demographic characteristics, other atopic disease, or other food allergy between the groups (Table I). Because this study's hypothesis was about reaction thresholds in children with peanut allergy, our analyses focused on comparing children with HT peanut allergy with children with LT peanut allergy. However, threshold-associated microbiota identified from the analyses of the HT children versus the LT children were also later examined in the NPA group for further comparison.

Saliva and stool samples were collected from all children before challenge. DNA was isolated from the samples for 16S rRNA sequencing, yielding high-quality data (the mean sequencing depth for saliva 16S rRNA was 372,886 reads (SD = 34,049 reads); for stool 16S rRNA, it was 383,139 reads (SD = 50,069 reads). In parallel, SCFA levels of the saliva and stool samples were measured by liquid chromatography mass spectrometry. The respective mean (SD) levels of acetate, butyrate, and propionate were 4,977.3 (2,765.2), 33.6 (52.5), and 602.0 (349.0) in saliva

and 425,249.8 (242,990.6), 158,865.5 (118,878.8), and 243,907.6 (128,934.9) in stool.

Distinct saliva and stool microbiomes in children with HT versus LT peanut allergy

We first tested for differences in oral microbiome diversity between HT children and LT children (Fig 1, B), finding that α -diversity (Shannon index) of the oral microbiome was higher in LT children (Wilcoxon $P = .017$) (Fig 2, A). To identify possible differences in oral microbiome composition between HT children and LT children, we next applied sparse partial least squares discriminant analysis (SPLSDA).¹⁸ SPLSDA is a data-driven method that applies dimension reduction to identify features distinguishing between groups. The first 2 variates from SPLSDA, reflecting 20 and 59 oral microbial amplicon sequence variants (ASVs), respectively, distinguished HT children from LT children (Fig 2, B). ASVs are microbial species identifiers based on DNA sequence similarity.

To gain further insight into the oral species that distinguished HT children from LT children, we next tested for differential abundance of ASVs that contributed to SPLSDA variate 1. Six of the variate 1 oral ASVs were differentially abundant between HT children and LT children according to the Wilcoxon test after permutation-based correction for multiple comparisons (false discovery rate [FDR] ≤ 0.05) (Fig 2, C). These genera included known commensals and pathobionts¹⁹ such as *Haemophilus* spp (ASV 588) and *Veillonella nakazawae* (ASV 1979), which were more abundant in HT children than in LT children (Fig 2, C). *V. nakazawae* strains have been previously detected in children's oral cavities,²⁰ and lower *Veillonella* abundance was previously found to be correlated with lower butyrate level in individuals with peanut allergy.¹⁶

We next implemented a similar analytic flow to test for differences in the gut microbiome of children with HT peanut allergy versus in that of LT children with peanut allergy (Fig 1, C). Although there were no significant differences in stool α -diversity (Fig 2, D), SPLSDA identified stool ASVs that distinguished children with HT versus LT peanut allergy (Fig 2, E). Testing for differential abundance of stool ASVs that contributed to SPLSDA variate 1 revealed that 3 ASVs were differentially abundant between HT peanut allergy and LT peanut allergy (Fig 2, F). *Bacteroides thetaiotaomicron* (ASV 6829) showed higher abundance in LT peanut allergy (Fig 2, F). Higher abundance of species within the genus *Bacteroides* have been previously associated with children with non-IgE cow's milk allergy,²¹ but these findings were not specific to the species *B. thetaiotaomicron*.

Correlations between microbiota and SCFA levels in saliva and stool in children with HT and LT peanut allergy

Given that microbiota are known sources of SCFAs, we next examined correlations between microbial taxa and SCFAs (Fig 1, D and E). Specifically, we tested for correlations between the reaction threshold-associated ASVs in the oral (Fig 2, C) and gut (Fig 2, F) environments and SCFA levels from the same samples.

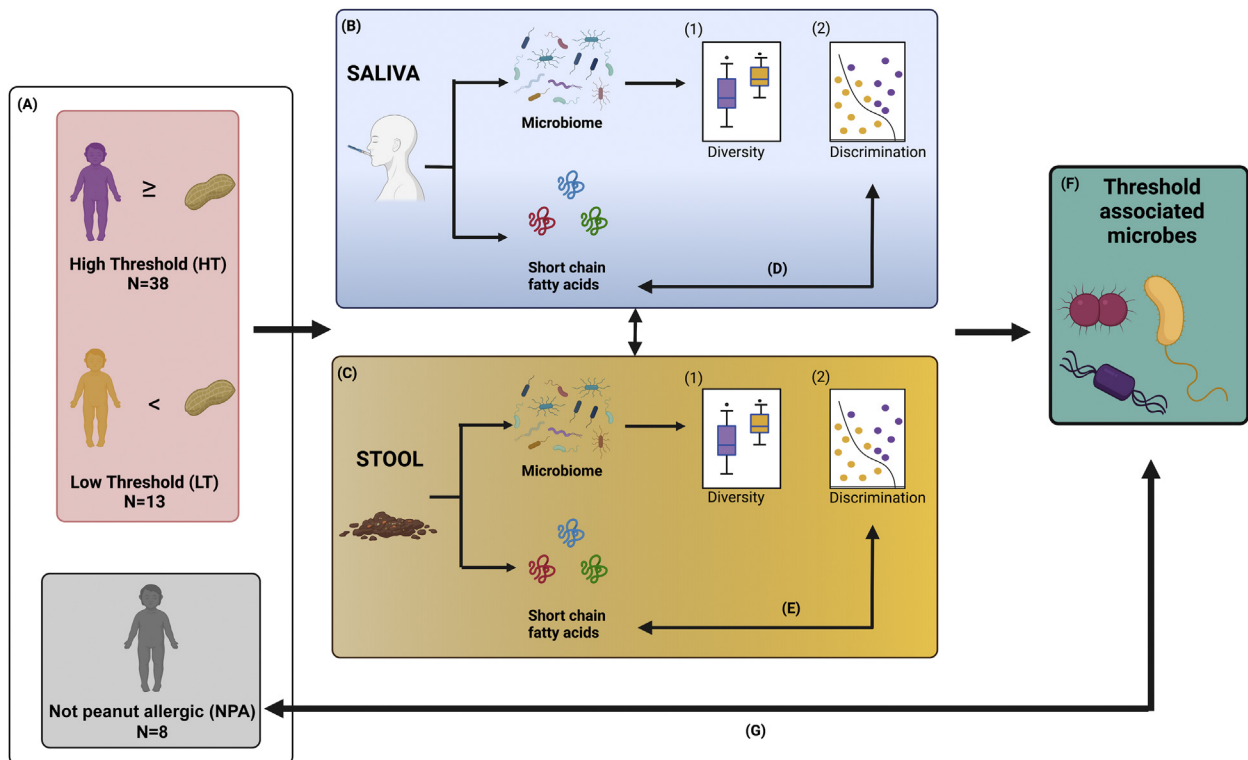


FIG 1. Study flow. **A**, In all, 59 children aged 4 to 14 years with suspected peanut allergy underwent double-blind, placebo-controlled food challenge. Participants who experienced allergy symptoms after eating the 300 mg dose (equivalent to 1 peanut) or more of peanut protein were classified as HT ($n = 38$). Children who reacted to smaller doses were classified as LT ($n = 13$), and subjects who did not experience any symptoms to peanut were deemed NPA ($n = 8$). Saliva and stool samples were collected from all children before challenge. DNA was isolated from saliva (**B**) and stool (**C**) for 16s rRNA sequencing, and SCFA levels were measured in the samples in parallel. Analyses for saliva and stool microbial diversity, discrimination, and their associations with HT versus LT peanut allergy were performed. Next, correlations between microbiota and SCFA levels in both saliva (**D**) and stool (**E**) were characterized. The relative abundances of the threshold-associated saliva and stool taxa identified (**F**), were also assessed among NPA children (**G**). Figure created with Biorender.com.

The correlations showed different patterns in HT and LT peanut allergy (Fig 3, A). For example, whereas oral *V. nakazawae* (ASV 1979) abundance was positively correlated with oral butyrate level in LT peanut allergy ($r = 0.57$; FDR = 0.049), it was correlated with oral propionate level in HT peanut allergy ($r = 0.39$; FDR = 0.019). Both stool *B. thetaiotaomicron* (ASV 6829) ($r = -0.65$; FDR = 0.018) and *Faecalibacterium prausnitzii* (ASV 5521) ($r = -0.67$; FDR = 0.015) were negatively correlated with butyrate level only in LT peanut allergy (Fig 3, A). SCFAs are thought to mediate communications between microbiota and host immunity,¹² and our results show variation in how threshold-associated microbiota from different anatomic sites are associated with SCFAs.

Closer examination of the threshold-associated saliva and stool microbiota that were also associated with SCFA levels (Fig 1, F and G) revealed that the relative abundance of oral *V. nakazawae* (ASV 1979) followed a pattern corresponding to peanut reaction threshold category, with the highest relative abundance in NPA, less relative abundance among the group with HT peanut allergy, and least abundance in the group with LT peanut allergy (Fig 3, B). Given this pattern and the

positive correlation of oral *V. nakazawae* with butyrate and propionate levels, these results suggest that it may be a salutary oral commensal with regard to threshold. In contrast, the relative abundance of stool *B. thetaiotaomicron* (ASV 6829) followed an opposite pattern, with the highest median abundance in the group with LT peanut allergy, followed by in the group with HT peanut allergy, and then in the NPA group (Fig 3, C). There were no associations between these microbiota and other atopic diseases, nor with other food allergies.

Analyses of SCFA levels without consideration of microbiota showed that salivary SCFAs trended highest in the NPA group compared to the HT and LT groups, but the differences were not significant, and there were no significant differences between groups for stool SCFA.

Correlations between saliva and stool microbiomes in children with peanut allergy

As prior studies have suggested interactions between oral and gut microbiota,²² we were also interested in characterizing correlations between the saliva and stool microbiomes, which has not previously been done in individuals with food allergy. Block

TABLE I. Characteristics of the children

Characteristic	Children with LT peanut allergy (n = 13)	Children with HT peanut allergy (n = 38)	NPA children (n = 8)	P value
Age (y), mean (SD)	8.5 (4.1)	7.8 (3.5)	7.5 (3.0)	.80
Female sex, no. (%)	4 (31%)	12 (32%)	2 (25%)	1
Race/ethnicity, no. (%)				.70
Asian	3 (23%)	4 (11%)	0 (0%)	
Black	1 (8%)	1 (3%)	1 (13%)	
Latino	1 (8%)	4 (11%)	0 (0%)	
Multiple races or unknown	2 (15%)	7 (18%)	2 (25%)	
White	6 (46%)	22 (58%)	5 (63%)	
Cumulative peanut dose at reaction (mg), mean (SD)	117 (50.1)	2448 (2855.6)	NA	.005
Peanut-specific IgE level (kU _A /L), mean (SD)	17.1 (11.7)	6.6 (7.3)	5.3 (7.3)	.0007
Peanut skin prick test (wheal size in mm), mean (SD)	9.5 (2.7)	8.6 (2.7)	6.3 (2.1)	.03
Other atopic disease, no. (%)				
Atopic dermatitis	7 (54%)	22 (58%)	5 (63%)	1
Asthma	4 (31%)	11 (29%)	1 (13%)	.76
Allergic rhinoconjunctivitis	9 (69%)	22 (58%)	3 (38%)	.37
Other food allergy, no. (%)				
Cow's milk	3 (23%)	6 (16%)	1 (13%)	.87
Egg	7 (54%)	11 (29%)	3 (38%)	.27
Sesame	6 (46%)	15 (39%)	2 (25%)	.63
Wheat	3 (23%)	3 (8%)	2 (25%)	.18
Tree nut*	8 (62%)	29 (76%)	4 (50%)	.20

P values were calculated by using the Fisher exact test for categorical variables and ANOVA for continuous variables.

*Tree nut includes almond, hazelnut, cashew, and/or walnut.

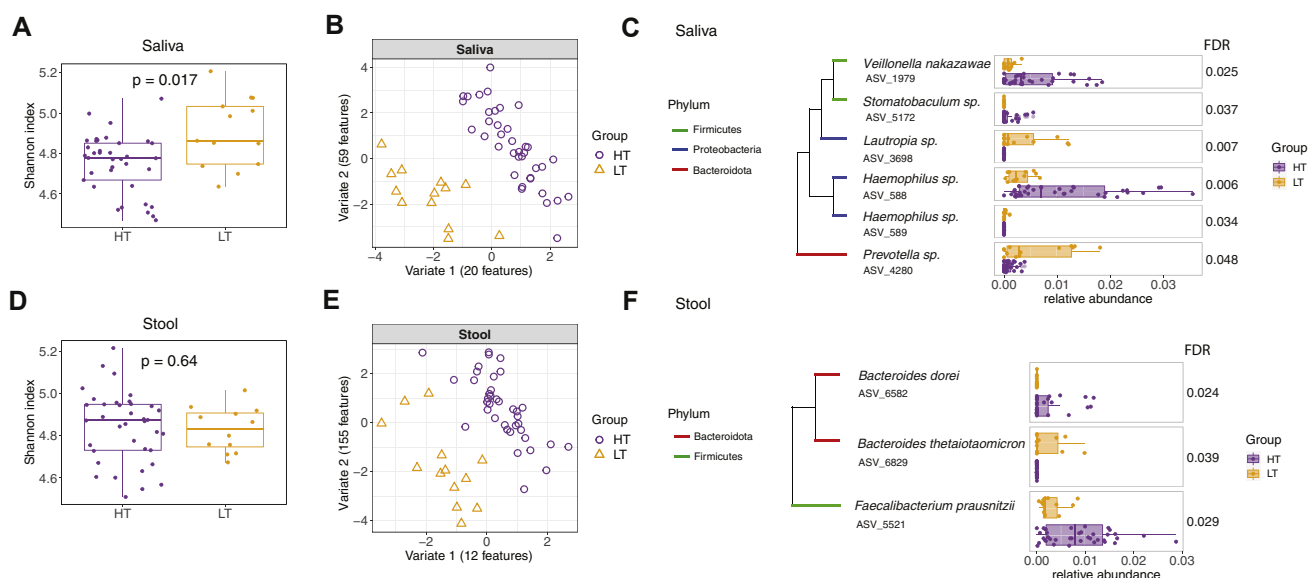


FIG 2. Distinct saliva and stool microbiomes in children with HT versus LT peanut allergy. **A**, The α -diversity (Shannon index) of the saliva microbiome showed significant differences by Wilcoxon test between the HT children and LT children. Higher Shannon index indicates higher α -diversity. **B**, SPLSDA of the saliva microbiome composition showed separations between the HT and LT children. The numbers of ASVs contributing to each of the first 2 variates are shown. **C**, Saliva ASVs from SPLSDA that were differentially abundant between the HT and LT groups ($FDR \leq 0.05$) are shown. The phylogenetic trees show the taxonomic relationships between these taxa. **D-F**, The equivalent findings for the stool microbiome in these children are shown.

SPLSDA²³ identified variates that not only discriminated HT children from LT children, but also demonstrated covariance between saliva and stool microbiota (Fig 4, A). Focused examination of oral and gut microbiota contributing to the variates identified ASVs that were differentially abundant (Wilcoxon

$FDR \leq 0.05$) between HT children and LT children. Spearman correlation testing between these saliva and stool ASVs identified those that were significantly correlated between the anatomic sites, for example, saliva *Haemophilus* (ASV 588) and stool *Bacteroides dorei* (ASV 6583) (Fig 4, B) ($r = 0.39$;

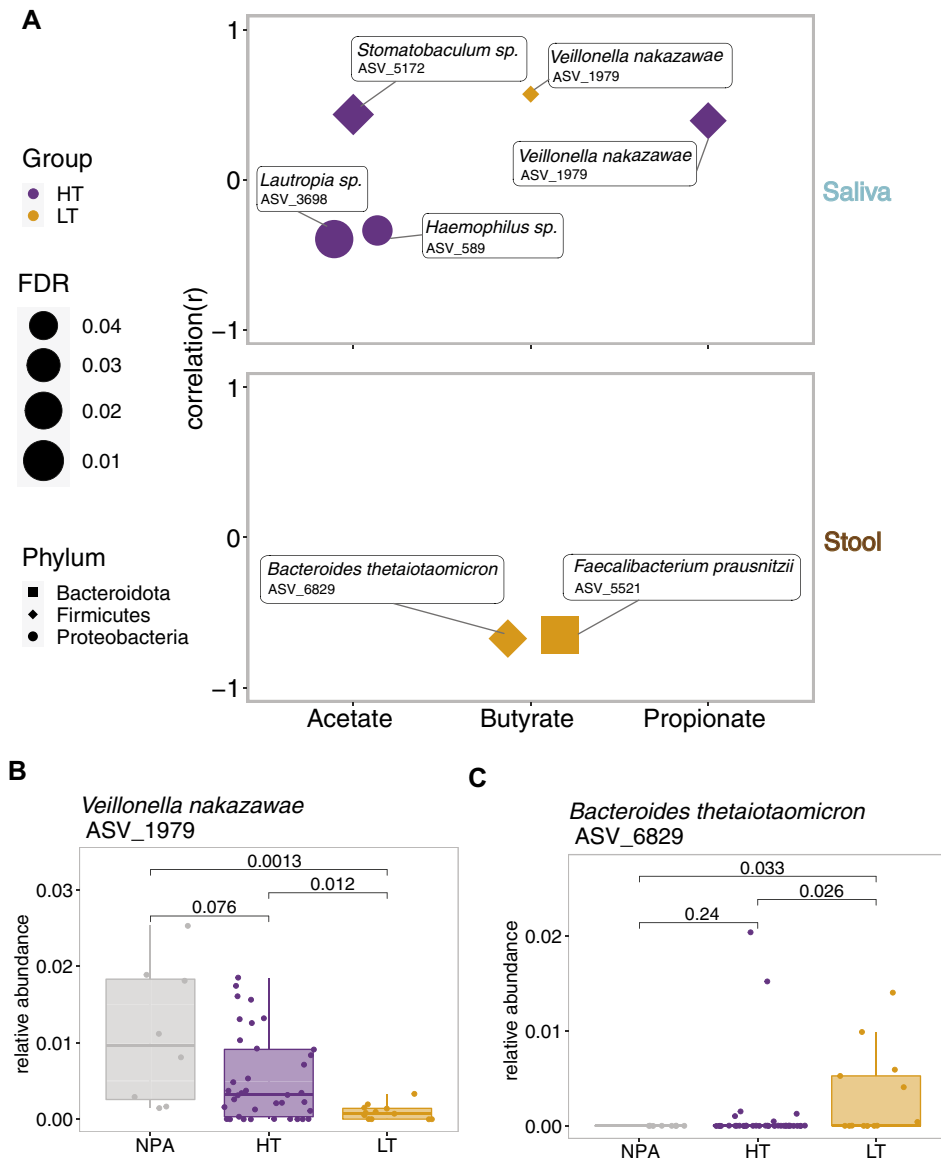


FIG 3. Correlations between microbiota and SCFA levels in saliva and stool in children with HT and LT peanut allergy. **A**, Saliva and stool ASVs from SPLSDA that were differentially abundant between children with HT and LT peanut allergy were correlated with saliva and stool SCFA levels, respectively. Significant correlations ($FDR \leq 0.05$) are shown. **B**, The relative abundance of *V nakazawae* was correlated with butyrate and propionate levels in saliva and was also significantly different between the HT, LT, and NPA groups. Wilcoxon test P values are shown. **C**, The relative abundance of *B thetaiotaomicron* was correlated with butyrate level in stool and was also significantly different between the HT, LT, and NPA groups. Wilcoxon P values are shown.

FDR = 0.005). Although we often think of the oral and gut environments as distinct, our results showing correlations between saliva and stool ASVs suggest possible cross talk between these important environments for host-microbe-immune interactions in food allergy.

Whereas prior studies have examined associations between microbiota and food allergy status,^{3-11,16,24} our multiscale study is, to our knowledge, the first to report oral and gut microbiota associated with reaction thresholds in peanut allergy. We further found that the threshold-associated oral and gut

microbiota identified are correlated with SCFA levels in these anatomic locations important to mucosal tolerance in food allergy. Our multiscale study contributes important mechanistic characterization of the oral and gut microbial and metabolite environments associated with reaction thresholds in peanut allergy. Although this study's limitations include its sample size and uneven racial/ethnic distribution, and its cross-sectional design precludes conclusions about causality, our findings raise the possibility of microbial therapeutics²⁵ to increase reaction threshold for individuals with food allergy.

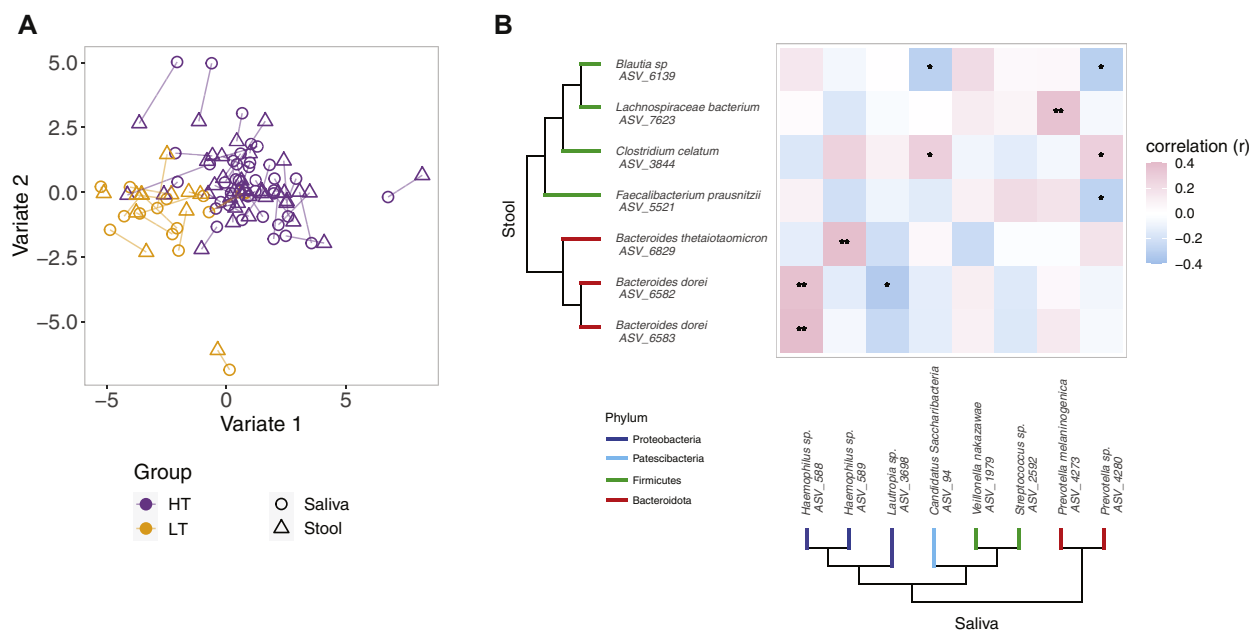


FIG 4. Correlations between saliva and stool microbiomes in peanut allergic children. **A**, Block SPLSDA was used to analyze correlations between saliva and stool microbiomes with respect to the HT and LT groups. The identified variates maximized covariance between the saliva and stool microbiomes as well as separation between the HT and LT groups. Lines connect saliva and stool samples from the same subject. In all, 100 saliva ASVs and 155 stool ASVs contributed to variate 1, and 25 saliva taxa and 5 stool taxa contributed to variate 2. **B**, Saliva and stool taxa from the block SPLSDA that were differentially abundant (FDR ≤ 0.05) between the HT and LT groups were selected for Spearman correlation. *Spearman FDR ≤ 0.05 ; **Spearman FDR ≤ 0.01 . The phylogenetic trees show the taxonomic relationships between the taxa.

Key messages

- This study of children who underwent double-blind, placebo-controlled peanut challenge identified saliva and stool microbiota associated with HT and LT peanut allergy.
- Many threshold-associated microbiota were also correlated with SCFA levels in the oral and gut environments.
- Findings from this study raise the possibility of using microbial therapeutics to increase reaction thresholds in children with food allergy.

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METHODS

Study subjects and sample collection

Study subjects were participants in the Challenging to Food with Escalating Thresholds for Reducing Food Allergy (CAFETERIA) study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03907397) identifier NCT03907397),^{E1} a National Institute of Allergy and Infectious Diseases–sponsored clinical trial to determine whether daily ingestion of sub-threshold amounts of peanut will accelerate recovery in children with HT peanut allergy. Children aged 4 to 14 years with history of peanut allergy and elevated peanut-specific IgE level not exceeding 50 kUA/L were recruited. Participants underwent criterion standard, allergist-supervised, double-blind, placebo-controlled food challenge at enrollment to determine their reaction threshold. The peanut protein doses in the oral food challenge schedule were 3 mg, 10 mg, 30 mg, 100 mg, 300 mg, 600 mg, 1000 mg, 3000 mg, and 4000 mg. Dosing was stopped following allergic reaction assessed by an allergist. LT peanut allergy was defined as an allergic reaction at a dose less than the 100-mg dose (143 mg cumulatively consumed). HT peanut allergy was defined as an allergic reaction at or higher than the 300-mg dose (443 mg cumulatively consumed). Participants who tolerated all doses without reaction were considered NPA. Oat flour was used for the placebo challenge.

Saliva and stool samples were collected before challenge. Participants were required to not have eaten or drunk in the hour before saliva collection. Unstimulated saliva was collected by having subjects hang their head and drip saliva into a collection tube, which was then immediately frozen in -80°C . Stool samples were collected by using a stool hat, with sterile scooping of a fecal sample immediately into a DNA/RNA Shield Fecal Collection Tube (Zymo Research, Irvine, Calif) followed by storage at -80°C .

Saliva and stool DNA isolation, library preparation, and 16S rRNA sequencing

DNA from saliva samples was extracted by using the Qiagen QIAamp DNA Micro kit (Qiagen, Hilden, Germany) with the addition of lysozyme (Sigma Aldrich, St Louis, Mo) incubation at 30°C for 30 minutes before extraction. DNA extraction from stool samples was performed by using the DNeasy PowerLyzer PowerSoil Kit (Qiagen).

DNA quality and quantity were measured by using Nanodrop (Thermo Fisher Scientific, Waltham, Mass) and Bioanalyzer (Agilent, Santa Clara, Calif). The sequencing libraries were generated from saliva and stool DNA samples by using the MetaVx Library Preparation kit (GENEWIZ, Inc, South Plainfield, NJ). Amplicon covering the V3 and V4 hypervariable regions of prokaryotic 16S rDNA were generated. Indexed adaptors were added to the ends of the DNA amplicons, and the DNA libraries were quantified and validated. The libraries were sequenced on an Illumina MiSeq instrument according to the manufacturer's instructions (Illumina, San Diego, Calif) with a 2×250 paired-end configuration. Image analysis and base calling were conducted by using the Illumina Control Software on the Illumina instrument. The average base Phred quality scores were higher than 30 for each sample at each sequence position. Raw sequence data were demultiplexed by using Illumina's bcl2fastq 2.17 software, and paired-end reads were joined by using Qiime2.^{E2} We applied Qiime2 to classify the sequences into ASVs. Taxonomic assignments were done on ASVs by using the SILVA 138 database.^{E3} The ASV tables were rarefied with read numbers set to the samples with minimal reads (16,000 for saliva data and 20,000 for stool). The sequences of the ASVs of interest were extracted and blasted against the National Center for Biotechnology Information nt^{E4} database for species- or genera-level taxonomic assignments.

Saliva and stool SCFA measurement

Saliva samples were spiked with stable labeled internal standards before protein precipitation in organic solvent. An aliquot supernatant was derivatized after centrifugation, and the reaction mixture was injected onto an Agilent 1290/AB Sciex QTrap 5500 LC-MS/MS system equipped with a C18 reversed-phase ultrahigh-performance liquid chromatograph column. The mass spectrometer was in negative mode using electrospray ionization. The

peak area of the analyte product ions was measured against the peak area of the internal standards product ions. Weighted linear least squares regression analysis generated from fortified calibration standards was used to do the quantification. The liquid chromatography with tandem mass spectrometry data were collected and processed by using Analyst 1.6.2 (SCIEX, Framingham, Mass).

Stool samples were weighed into bead blaster tubes with zircon beads and homogenized in 100% methanol (at a final concentration of 10 mg/mL) containing deuterated SCFA internal standards (CDN Isotopes, Québec City, Québec, Canada). Control samples were created with either 1 mL of pooled sample or 1 mL of methanol (instrument blank). A portion of the pooled control was then subjected to successive rounds of extraction ($n = 3$) to deplete SCFAs but retain the insoluble particles to generate null-matrix material. A standard curve for the cocktail of SCFAs was prepared in null matrix, and these sample were extracted side by side with the study samples. The resulting SCFA extracts were then derivatized as described^{E5} for liquid chromatography–mass spectrometry. Samples were analyzed with a QExactive HF mass spectrometer coupled to a Dionex Ultimate 3000 automated system (Thermo Fisher Scientific). A Waters BEH C18 column (2.1×150 mm; $1.7 \mu\text{m}$) was used with buffer A as 0.1% formic acid in water, and buffer B as 0.1% formic acid in acetonitrile. A gradient elution was used (15% B to 55% B in 9 minutes; $200 \mu\text{L}/\text{min}$), and the mass spectrometer was operated in negative ion mode (heated electrospray ionization, -3.5kV). The order of acquisition was randomized, and several control blocks including blanks and external standards were interspersed throughout the run to assess instrument performance and quality control of the derivatization protocol. All analytes were corrected to their respective internal standard, and the resulting ratio was interpolated against the matrix-controlled standard curve to quantify the level of SCFA in each sample. The limit of detection was determined from null-matrix samples for each analyte, and this limit was imputed for any point falling below the limit of detection. The interpolated values were used for the downstream analyses, and the imputed ratio threshold values were used for the samples below the limit of detection.

Analyses

Because our hypothesis was about reaction thresholds in children with peanut allergy, our analyses focused on comparing children with peanut allergy in the HT group with children with peanut allergy in the LT group. The identified threshold-associated microbiota from the HT versus LT analyses were also later examined in the NPA group for further comparison. Qiime2 was used to calculate Shannon index values from ASV tables. Shannon index values were compared by using the Wilcoxon test. For SPLSDA, we applied the `splsd` and `block.splsd` functions from the `mixOmics`^{E6} R package to the ASV tables. The ASV tables were filtered with a 20% frequency cutoff to remove low-prevalence ASVs. SPLSDA applies dimension reduction and feature selection to select dimensions that maximize covariance between the input data and outcomes or among the input data together with outcomes for `block.splsd`. The number of dimensions and features are selected to minimize cross-validation errors. Wilcoxon tests were used to analyze the differential abundances of the ASVs selected in the `splsd` and `block.splsd` models. Spearman correlation was used to correlate the threshold-associated ASVs with SCFAs or correlate the threshold-associated saliva and stool ASVs. We focused our analyses on the SCFAs acetate, butyrate, and propionate because these particular SCFAs have previously been found to be associated with saliva and stool microbiota in subjects with food allergy.^{E7-E9} To address potential false discovery from multiple comparisons, permutation testing with 5000 permutations was used to calculate FDR values from the Wilcoxon and Spearman correlation tests. All of the ASVs and correlations shown have an FDR not exceeding 0.05.

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