with Mctrl. However, the further histopathological amelioration of Mpam might take more time to develop.[5]

Using [^{3}H]-thymidine incorporation assays, we subsequently demonstrated that the TLR2 ligation–enhanced suppressive function in MSCs was reversed with a STAT3 inhibitor, S3I-201 (S3I-201 and Mpam [Ms&p]), in the cell-cell contact-dependent, but not in the transwell system (Fig 2, *A*). Therefore, compared with Mctrl, despite the fact that the expression of both inducible nitric oxide synthase *(iNOS)* and interleukin-1 receptor antagonist *(il-1ra)* was increased in Mpam and diminished in Ms&p, nitric oxide (NO), which works in a cell-proximity manner, was more likely to be the plausible Pam_3CSK_4-induced regulatory factor in MSCs (Fig 2, *B-D*). Moreover, because Mpam compressed more proliferative $CD4^+$ T cells than did Mctrl, whereas Ms&p and iNOS inhibitor, L-NMMA, -treated Mpam (L-NMMA and Mpam [Ml&p]) did not (Fig 2, *E*), we suggested the TLR2/STAT3/iNOS signaling by which Mpam advanced its immunoregulatory function.

Next, other reports have shown that more regulatory T cells are induced through enhanced NO secretion.[6] Using Griess assays, higher NO was detected only in the supernatant of Mpam (Fig 2, *F*). It possibly explained how the $CD4^+CD25^+$ T cells cocultured with Mpam inhibited the responder $CD4^+$ T-cell proliferation more effectively than those cocultured with Mctrl, Ms&p, and Ml&p (Fig 2, *G*). Furthermore, compared with the $CD4^+CD25^+$ T cells alone (Tnaive), Foxp3 was upregulated only in the Mpam-cocultured $CD4^+CD25^+$ T cells (Tpam), but not in the Mctrl-, Ms&p-, and Ml&p-cocultured $CD4^+CD25^+$ T cells (Tctrl, Ts&p, and Tl&p) both at mRNA and protein levels (Fig 2, *H* and *I*). Mononuclear cells were also examined in lungs of asthmatic murine model. With the Mpam treatment, less mononuclear cells, however, a higher percentage of $CD4^+CD25^+Foxp3^+$ cells was observed in the lung homogenate (Fig 1, *I*). Therefore, we suggested that $CD4^+CD25^+Foxp3^+$ regulatory T cells were possibly induced to exert the better suppressive functions of Pam_3CSK_4-modified MSCs through TLR2/STAT3/iNOS signaling.

Finally, the mediation of the seemingly independent mechanisms between TLR2 and STAT3 was examined. The TLR2 ligation is known to lead to inhibitor of κB kinase degradation and then nuclear factor (NF)-κB transcriptional activation. As proven by our results established using Western blotting, compared with Mctrl, the sequential degradation of inhibitor of κB and activation of NF-κB/STAT3/iNOS in Mpam suggested its TLR2 ligation–induced signaling pathway. In these data, TLR2 ligation failed to phosphorylate STAT3 and consequently produced less iNOS in Ms&p. The diminished expression of iNOS in Ml&p, otherwise, was mainly attributed to the translation of mRNA, which was regulated by the availability of its substrate, L-arginine (Fig 2, *J*). Despite the fact that the possibility of the interaction between NF-κB and STAT3 pathways was not excluded,[7] upregulated IL-6 production of Mpam irrespective of the inhibition by neither STAT3 nor iNOS inhibitor suggested the possible mediating role of IL-6 between TLR2 and STAT3 (Fig 2, *K*). TLR2 ligation no longer stimulated iNOS production in MSCs after treatment with IL-6 neutralizing antibody (IL-6 neutralizing antibody and Mpam [M6&p]) (Fig 2, *L*). The augmented immunosuppressive function of Mpam was then abolished by IL-6, but not by IgG_1 κ isotype control neutralization (Fig 2, *M*). We thus suggested that IκB/NF-κB/IL-6 might play the critical roles in mediating TLR2/STAT3 signaling.

In summary, we used Pam_3CSK_4 to enhance the immunomodulatory functions of MSCs, and high levels of iNOS and NO were noted in Pam_3CSK_4-stimulated MSCs. In addition, involvement of NO-stimulated $Foxp3^+$ regulatory cell was proposed (Fig 2, *N*). However, iNOS-derived NO might suppress T-cell function through versatile mechanisms.[8,9] It possibly explained the discrepancy between the strikingly NO level and the significant but less amount of $Foxp3^+$ cell induction with Mpam *in vitro* and *in vivo*. More experiments might be needed to clarify the molecular mechanism involved in the immunomodulatory functions induced by MSCs stimulated by Pam_3CSK_4.

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The nasal microbiome in patients with chronic rhinosinusitis: Analyzing the effects of atopy and bacterial functional pathways in 111 patients

To the Editor:

Chronic rhinosinusitis (CRS) is a chronic disease characterized by sinonasal mucosal inflammation. The inflammatory milieu in patients with CRS is thought to be affected by or even possibly initiated by commensal microbes, pathogens, and their products.[1,2]

To date, only a few studies have evaluated the nasal microbiome in patients with CRS. There has been a lack of

TABLE I. RA of selected sequences derived from individual taxa in the sinus cavities of 111 patients with CRS and 21 control subjects

	Patients with CRS			Sex			Age				Race/ethnic groups			
Taxonomic level	FDR *P* value	RA mean in patients with CRS (n = 111)	RA mean in control subjects (n = 21)	FDR *P* value	RA mean in male subjects (n = 58)	RA mean in female subjects (n = 53)	FDR *P* value	RA mean in <30 y (n = 21)	RA mean in 31-60 y (n = 25)	RA mean in >61 y (n = 64)	FDR *P* value	RA mean in white subjects (n = 75)	RA mean in African American subjects (n = 22)	RA mean in Hispanic subjects (n = 11)
Phyla														
Actinobacteria	.006*	1884.06	3548.19	.14	1432.79	2377.91	.14	1624.19	1546.36	2130.23	.52	1710.64	2314.27	2069.09
Bacteroidetes	.895	194.32	161.90	.46	167.72	223.42	.46	294.05	202.24	153.52	.99	195.23	208.73	193.55
Firmicutes	.863	3630.49	3548.43	.82	3575.71	3690.43	.82	3246.71	3331.64	3864.73	.91	3742.24	3433.41	3068.09
Proteobacteria	.281	2223.83	1126.86	.29	2459.52	1965.91	.29	2846.43	2088.08	2061.19	.94	2311.97	2168.64	1988.36
Genera														
Corynebacterium	.012*	1766.97	3471.86	.08	1275.67	2304.62	.33	1560.00	2045.73	1296.96	.45	1574.17	2242.68	1969.55
Prevotella	.870	53.95	63.62	.19	34.67	75.06	.94	69.43	54.25	42.32	.99	55.28	51.86	63.00
Staphylococcus	.593	2174.83	1403.24	.69	2085.22	2272.89	.28	1360.52	2453.70	2067.24	.88	2211.03	2150.23	1990.09
Alloiococcus	.194	123.56	587.19	.23	63.33	189.47	.89	91.76	158.98	64.52	.00	90.61	93.36	13.36
Lactobacillus	.780	5.50	1.24	.19	8.36	2.36	.36	14.00	3.64	3.28	.56	3.21	13.27	6.18
Streptococcus	.560	616.49	77.33	.16	822.40	391.15	.87	829.48	530.63	681.92	.83	724.63	265.23	638.55
Ruminococcus	.780	21.10	10.10	.25	13.55	29.36	.91	12.67	21.67	27.48	.68	17.41	41.18	11.09
Anaerococcus	.478	219.45	461.90	.47	191.05	250.53	.26	328.14	238.89	87.08	.74	212.48	306.82	101.91
Finegoldia	.104	94.16	281.71	.96	93.03	95.40	.13	203.19	67.56	74.32	.40	124.47	47.77	5.36
Peptoniphilus	.007*	144.41	490.90	.54	162.26	124.89	.42	223.05	149.98	69.80	.96	145.92	158.09	145.91
Burkholderia	.880	308.02	282.90	.15	392.43	215.64	.75	366.57	312.06	256.84	.56	281.23	362.41	438.82
Enterobacteriaceae species	.890	370.20	187.81	.85	393.38	344.83	.72	86.57	460.42	392.20	.67	259.96	580.36	553.91
Haemophilus	.881	198.51	41.10	.23	85.07	322.66	.85	179.24	145.59	358.08	.80	286.49	9.73	28.91
Moraxella	.890	156.12	32.33	.43	219.55	86.70	.46	1.05	117.33	391.92	.81	229.11	3.18	4.91
Pseudomonas	.672	507.42	44.14	.97	512.76	501.58	**.02***	1565.62	339.27	69.20	.96	537.40	622.73	209.36

The nasal microbiome was analyzed in patients with CRS compared with control subjects and in subgroups of patients with CRS in relation to demographic factors, including sex, age, and race/ethnicity.
*FDR *P* value of less than .05.

consistency in these studies in terms of both abundance and the α-diversity indices of bacteria.[2] Thus far, all previous studies have evaluated the microbiome in terms of its α-diversity (ie, richness) and relative abundance (RA) in each operational taxonomic unit (OTU) that alone do not provide any in-depth information on the potential functional effect of the nasal microbiota. To our knowledge, the present study is the first to evaluate sinonasal bacterial communities by using predictive functional profiling.

As detailed in the Methods section in this article's Online Repository at www.jacionline.org, a consecutive series of patients with CRS and healthy control subjects were recruited from January 2015 to July 2016. This study was approved by the Institutional Review Board of Rush University, and all participants provided written informed consent. Samples were collected by means of slow application of a sterile small nasal cotton swab to the middle meatus region under endoscopic guidance. Total DNA was extracted from nasal cotton swabs and processed by using high-throughput Illumina amplicon sequencing of the V4 variable region of the microbial 16S rRNA gene. Data were then clustered into OTUs at 97% similarity. Differences in the RA of individual taxa in a tiered fashion from the taxonomic levels of phylum to species were determined for significance by using Kruskal-Wallis nonparametric ANOVA corrected for false discovery rate (FDR) and accepted at a FDR *P* value of less than .05. Additionally, a subgroup analysis was conducted among patients with CRS to find potential factors that are linked to variations in the CRS microbiome.

The nasal microbiome was analyzed in association with 3 main groups of factors, including (1) demographics, (2) allergic comorbidities, and (3) CRS-related factors, including nasal polyps, number of past functional endoscopic sinus surgeries (FESSs), duration of CRS, and severity scores (Sinonasal Outcome Test [SNOT-22] score and Lund-Mackay score [LMS]).

We applied both conventional statistical bioinformatics analyses and an *in silico* approach called Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt) to infer microbiota functional pathways.[3] PICRUSt allows identification and measurement of the RA of each sample's metagenome and potential involvement in different metabolic and functional pathways needed for invasion and metabolism of bacteria, including epithelial invasion, antibacterial resistance properties, and LPS production.

One hundred eleven patients with CRS and 21 control subjects were enrolled and completed the study. The information, including patients' demographics; prevalence of allergic rhinitis, asthma, eczema, and food allergy; and CRS-related factors, including history of nasal polyps, number of FESSs, duration of CRS, SNOT-22 scores, and LMSs are detailed in Table E1 in this article's Online Repository at www.jacionline.org.

The nasal microbiome analysis indicated that there were no significant differences in α-diversity between patients with CRS and control subjects. However, at the phylum level, patients with CRS had significantly lower Actinobacteria levels, which translated to lower *Corynebacterium* species levels compared with those in control subjects. Additionally, the RA of the

TABLE II. RA of selected sequences derived from individual taxa in the sinus cavities of 111 patients with CRS in relation to allergic rhinitis, eczema, asthma, and nasal polyps

	Asthma			AR			Eczema			Polyps		
Taxonomic level	FDR *P* value	Asthmatic patients (n = 46)	Nonasthmatic subjects (n = 65)	FDR *P* value	Patients with AR (n = 45)	Subjects without AR (n = 51)	FDR *P* value	Patient with eczema (n = 12)	Subjects with without eczema (n = 99)	FDR *P* value	Patients with CRSwNP (n = 39)	Patients with CRSsNP (n = 72)
Phyla												
Actinobacteria	.22	1602.59	2083.26	.01*	1179.51	2450.90	.19	1146.67	1973.44	.90	1902.14	1850.69
Bacteroidetes	.93	190.50	197.02	.43	137.98	210.00	.39	101.50	205.57	.48	213.85	158.26
Firmicutes	.59	3467.02	3746.17	.20	3789.33	3167.61	.01	5497.42	3404.19	.57	3526.38	3822.69
Proteobacteria	.81	2291.85	2175.69	.57	2401.20	2308.08	.42	1678.08	2289.98	.65	2145.35	2368.72
Genera												
Corynebacterium	.29	1521.13	1940.95	.01*	1089.82	2298.43	.24	1100.58	1847.75	.72	1818.00	1672.77
Prevotella	.22	31.83	69.62	.33	32.29	55.25	.29	7.00	59.65	.72	57.97	46.54
Staphylococcus	.20	1819.52	2426.28	.69	2366.00	1885.33	.08	3343.50	2033.17	.12	1911.29	2661.36
Alloiococcus	.34	63.87	165.80	.37	40.09	225.61	.42	1.67	138.33	.70	138.42	96.13
Lactobacillus	.41	3.24	7.09	.69	3.29	8.49	.75	3.42	5.75	.48	6.69	3.28
Streptococcus	.02*	1037.24	318.72	.09	753.98	310.80	.01*	1770.08	476.66	.35	722.26	421.21
Ruminococcus	.14	32.93	12.72	.05	14.18	13.86	.83	17.00	21.60	.39	25.43	13.10
Anaerococcus	.09	138.89	276.46	.98	223.78	212.96	.27	89.83	235.16	.65	233.29	193.90
Finegoldia	.22	61.20	117.49	.71	101.87	107.10	.33	31.50	101.76	.46	81.82	116.95
Peptoniphilus	.23	101.17	175.02	.87	170.04	135.37	.48	81.83	152.00	.40	125.42	179.49
Burkholderia	.05	383.04	254.92	.16	396.29	273.61	.85	327.75	305.63	.24	339.81	249.33
Enterobacteriaceae	.80	331.72	397.43	.70	221.56	428.35	.33	17.33	412.97	.75	399.07	316.90
Haemophilus	.41	102.24	266.65	.94	271.58	161.22	.54	23.08	219.78	.13	89.94	398.95
Moraxella	.71	119.30	182.17	.99	123.09	180.24	.52	2.42	174.75	.79	139.46	186.87
Pseudomonas	.49	649.96	406.55	.74	528.18	629.22	.57	787.42	473.48	.46	412.44	682.77

AR, Allergic rhinitis; *CRSsNP*, chronic rhinosinusitis without nasal polyps; *CRSwNP*, chronic sinusitis with nasal polyps.
*FDR *P* value less than .05.

genus *Peptoniphilus* was significantly lower in patients with CRS (Table I). Among demographic factors, age was associated with significant bacterial RA changes. Patients with CRS younger than 30 years had significantly increased *Pseudomonas* species levels compared with patients with CRS aged between 31 and 60 years and greater than 60 years of age (Table I).

The presence of nasal polyps was not associated with changes in the nasal microbiome. Among patients with CRS, allergic rhinitis was associated with decreased Actinobacteria levels at the phylum level and *Corynebacterium* species at the genus level. Asthma and atopic dermatitis were associated with increased RA of *Streptococcus* species (Table II). There was a significant but weak positive correlation between higher LMSs and higher RA of Enterobacteriaceae ($P = .04$, $R = +0.19$) and a negative correlation between the RA of *Prevotella* species and total SNOT-22 scores ($P = .01$, $R = -0.24$; see Table E2 in this article's Online Repository at www.jacionline.org). There was no association between the number of previous FESSs and the microbiome.

PICRUSt analysis showed that LPS biosynthesis proteins and bacterial invasion of epithelial cell pathways were significantly greater in patients with CRS. Additionally, allergic rhinitis was associated with an increased abundance of the LPS biosynthesis protein pathway (see Table E3 in this article's Online Repository at www.jacionline.org).

To date, this is the largest study analyzing the nasal microbiome in patients with CRS. The large number of patients with CRS enabled us to test the association of multiple CRS-related variables with the nasal microbiome. Furthermore, we used meticulous methods in subject recruitment, sample collection, and specimen handling to avoid the common biases seen in some previous studies. Our study of 111 patients with CRS and one previous study with more than 50 patients with CRS[4] have shown that the bacterial diversity and richness of the nasal cavity does not significantly change in patients with CRS.

Genera that were decreased significantly in patients with CRS compared with control subjects were *Corynebacterium* and *Peptoniphilus*. Our findings are in agreement with previous studies reporting a significant decrease in *Peptoniphilus* species in patients with CRS[4] and *Corynebacterium* species in patients with CRS without nasal polyps.[5] *Peptoniphilus* is an anaerobic genus of bacteria from the Clostridia class that has been found in the nasal cavity from the first studies that evaluated the nasal microbiome by using non–culture-based methods.[6] Clostridia-containing microbiota have been shown to downregulate innate lymphoid cell function and hence to decrease the allergic response and subsequent T_H2 inflammation, effects that might diminish the likelihood of development of a type 2 disease.[7] Decreased colonization with certain bacteria from the Clostridia family might remove a protective element and promote T_H2 inflammation and progression of upper respiratory tract inflammation, potentially contributing to the pathogenesis, progression, or both of patients with CRS, especially in those with atopy.

We found that among patients with CRS, those with allergic rhinitis had lower RA of *Corynebacterium* species compared with nonallergic patients with CR. More notably, patients with CRS without allergic rhinitis had similar levels of this organism compared with control subjects, suggesting that the diminution

of *Corynebacterium* species might more reflect the type 2 milieu than CRS disease. In a previous study increased abundance of *Corynebacterium* species at the time of endoscopic sinus surgery was predictive of better surgical outcomes.[4] This indicates a potential protective role for *Corynebacterium* species in maintaining the health of the sinus mucosa. It is noteworthy that the genus *Corynebacterium* in the nasal cavity is believed to be important for maintaining a sustained and stable microbial pattern in healthy infants.[8]

In our study atopic dermatitis was associated with a higher RA of *Streptococcus* species. Colonization of the skin with a microbiome enriched for *Streptococcus* species is seen in atopic dermatitis–prone skin[9] and associated with disease severity. This higher RA of *Streptococcus* species in patients with atopic CRS along with further decreased RA of *Corynebacterium* species in patients with allergic rhinitis suggests that the imbalance between these 2 bacterial genera might be an important factor in defying a certain atopic endotype in patients with CRS.

PICRUSt analyses inferred that the resident bacterial community modifies its functional patterns in patients with CRS, and bacteria with the ability to invade the epithelium and increase production of LPS have overcome the microbial community in patients with CRS. The lack of increase of abundance of any single known LPS-producing bacterium suggests that there might be different cohorts of diverse LPS producers that are increased in individual patients with CRS. This suggests that future efforts should target discovery of the bacterial groups with functional capacities that enable them to overcome the community in disease or in a certain phenotype of disease, which could provide us with clues of how bacteria are contributing or even initiating a disease process.

The results above showing a link between specific genera and CRS-related factors suggest that the nasal microbiome could be used as a tool to characterize and identify endotypes of patients with CRS.

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Pharmacological inhibition of caspase-8 suppresses inflammation-induced lymphangiogenesis and allograft rejection in the cornea

To the Editor:

Graft rejection (GR) remains a major hurdle in transplantation medicine, and lymphangiogenesis plays a critical role in this process. An improved understanding of the mechanisms involved in GR as well as new strategies to manage it are greatly needed. Caspases, which play critical roles in cell death, have received an increasing attention for their immune properties. Pharmacological inhibition of caspases has been proposed as a promising therapeutic strategy for the treatment of various diseases. However, the potential roles of caspase inhibitors and the molecular mechanisms involved in lymphangiogenesis and GR remain elusive. Cornea is accessible for experimental manipulation readily and the normal cornea lacks lymphatic vessels. Therefore, we used a mouse model of corneal transplantation (CT; Fig 1, *A-D*) to explore the clinical application of caspase inhibition and the molecular mechanisms involved in inflammation-induced lymphangiogenesis (ILA) and GR (see the Methods sections in this article's Online Repository at www.jacionline. org).

Topical application of a pan-caspase inhibitor (Z-VAD-fmk) at 60 μM had a maximum inhibitory effect on ILA on day 9 (the experimental end point for lymphangiogenesis) after CT (Fig 1, *E*).

METHODS

Patients

A consecutive series of patients with CRS who underwent evaluation in the Department of Otorhinolaryngology–Head and Neck Surgery at Rush University Medical Center were recruited from January 2015 to July 2016. Patients with CRS who received oral or topical antibiotics or oral steroids in the 3 months before enrollment were excluded. The CRS diagnosis was based on 12 weeks of persistent sinonasal symptoms, with objective findings based on endoscopy, computed tomographic sinus imaging, or both. A group of healthy control subjects without a history of sinus or allergic disease were also enrolled. All patients were evaluated clinically by an allergist to confirm or properly rule out asthma and allergic conditions. This study was approved by the Institutional Review Board of Rush University Medical Center, and all participants provided written informed consent. Samples were collected by means of slow application of a sterile small nasal cotton swab to the middle meatus region under endoscopic guidance. The cotton swab heads were placed in sterile tubes and frozen at −80°C within 1 hour of sampling until the time of DNA extraction.

Microbiome analyses

Total DNA was extracted from nasal cotton swabs by using a commercially available kit (Fast DNA Spin Kit; MP Biomedicals, Solon, Ohio), according to the manufacturer's recommended protocol. DNA was processed by using high-throughput Illumina amplicon sequencing of the V4 variable region of the microbial 16S rRNA gene[E1] and implementing a modified 2-step targeted amplicon sequencing approach.[E2] Negative controls were used with each set of amplification, which indicated no contamination. Raw sequence data (FASTQ files) were deposited in the National Center for Biotechnology Information Sequence Read Archive under project PRJNA395923. Raw FASTQ files for each sample were processed to merge reads, remove low-quality data and chimeras, and perform annotation with the Greengenes 13_8 reference database, as previously described.[E3,E4] Data were then clustered into OTUs at 97% similarity, and the sample sequence set was rarefied to 4400 sequences.[E4] α-Diversity indices were calculated by using the software package Primer7.[E5] We applied both conventional statistical bioinformatics analyses to interrogate microbiota composition in nasal cavity and also used an *in silico* approach called PICRUSt to infer microbiota functional pathways.[E6] During the process of DNA amplification in nasal microbiome analysis, a significant number of short DNA fragments are generated. PICRUSt allows identification and measurement of the RA of each sample's metagenome and potential involvement in different metabolic and functional pathways needed for invasion and metabolism of bacteria, including epithelial invasion, antibacterial resistance properties, and production of LPS.

Biostatistics

Microbial community analysis was done in a tiered fashion from the taxonomic levels of phylum to species. Differences in the RA of individual taxa (>1% of data set) were determined for significance by using Kruskal-Wallis nonparametric ANOVA. The RA of individual taxa reported was corrected for FDR and accepted at an FDR P value of less than .05. Bioinformatics analyses were used to test differences in nasal microbiota composition and identify key taxa that were most strongly altered when comparing CRS with control samples.

In addition, a subgroup analysis was conducted among patients with CRS to find potential factors linked to variations in the CRS microbiome. The 5 most abundant phyla and 15 most abundant genera were chosen for the subgroup analysis. Associations between the RA of each OTU and nominal and continuous variables were assessed by using ANOVA and Spearman correlation tests, as appropriate. For statistical analyses, SPSS software (version 21.0; SPSS, Chicago, Ill) was used. Significance was accepted at a P value of less than .05 corrected for multiple analyses. Graphs were created by using GraphPad Prism (version 5.00; GraphPad Software, La Jolla, Calif) software.

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TABLE E1. Demographic and clinical characteristics of 111 patients with CRS and 21 control subjects

Characteristic	Patients with CRS	Control subjects	*P* value, χ^2 or *t* test
Sex			
Male	58	13	.28
Female	53	8	
Age (y), mean ± SD	47.34 ± 15.47	52 ± 13.7	.31
BMI (kg/m^2), mean ± SD	29.42 ± 6.22	31.30 ± 7.23	.21
Nasal polyps			
CRSsNP	39	NA	—
CRSwNP	72		
Asthma			
Yes	46	3	.006
No	65	171	
Not known	0	1	
AERD			
Yes	18	NA	—
No	93		
Atopy			
Negative skin test result	52	15	.015
Positive skin test result	45	1	
No skin test*	14	5	
Eczema			
Yes	12	1	.004
No	99	20	
Food allergy			
Yes	12	2	.093
No	99	19	
LMS, mean ± SD	9.46 ± 7.07	NA	—
No. of surgeries, mean ± SD†	1.81 ± 1.82	NA	—
Duration of CRS (y), mean ± SD	12.43 ± 10.28	NA	—
Total SNOT-22 scores	32.57 ± 24.65	13.28 ± 20.9	.001

AERD, Aspirin-exacerbated respiratory disease; *CRSsNP*, Chronic rhinosinusitis without nasal polyps; *CRSwNP*, chronic sinusitis with nasal polyps; *NA*, not applicable.

*This case with unknown asthma status had breathing symptoms but normal office spirometric results and was lost to follow-up for further advanced testing for asthma.

†Range for number of previous sinus surgeries was 0 to 8.

TABLE E2. Correlation between RA of selected sequences derived from individual taxa and CRS symptom scores measured by using SNOT-22 scores and LMSs in 111 patients with CRS

	SNOT-22 score		LMS	
Taxonomic level	**Correlation coefficient (R)**	**FDR *P* value**	**Correlation coefficient (R)**	**FDR *P* value**
Phyla				
Actinobacteria	−0.043	.651	−0.054	.576
Bacteroidetes	−0.015	.874	−0.002	.984
Firmicutes	0.041	.670	−0.070	.465
Proteobacteria	0.052	.586	0.138	.150
Genera				
Corynebacterium	−0.050	.600	−0.053	.578
Prevotella	−0.233	**.014***	0.064	.507
Staphylococcus	0.039	.684	−0.117	.220
Alloiococcus	0.040	.675	0.019	.840
Lactobacillus	−0.114	.233	0.097	.312
Streptococcus	0.111	.246	0.036	.709
Ruminococcus	0.040	.679	−0.055	.566
Anaerococcus	−0.158	.097	−0.032	.740
Finegoldia	−0.100	.297	−0.119	.213
Peptoniphilus	−0.131	.171	−0.080	.405
Burkholderia	0.070	.463	−0.095	.322
Enterobacteriaceae	−0.043	.657	**0.189**	**.045***
Haemophilus	−0.004	.963	0.065	.496
Moraxella	0.029	.760	0.092	.337
Pseudomonas	−0.112	.243	−0.164	.086

The correlation of higher SNOT-22 scores with low RA of *Prevotella* species is in agreement with studies in lower airways showing that *Prevotella* species are decreased in airways of patients with asthma and chronic obstructive pulmonary disease[E7] and negatively correlated with markers of inflammation.[E8] A higher LMS was correlated with increased *Enterobacteriaceae* species levels, which is in agreement with studies showing a correlation between this genus and airway inflammation in patients with other chronic inflammatory diseases, such as cystic fibrosis.[E8]

*$P < .05$.

TABLE E3. Differences in selected KEGG pathways using PICRUSt analysis of nasal microbiomes

	Patients with CRS vs control subjects				Patients with CRS with allergic rhinitis vs patients with CRS without allergic rhinitis			
Pathway	**Patients with CRS, RA mean**	**Control subjects, RA mean**	**Patients with CRS/control subjects, ratio**	***P* value***	**Patients with CRS with AR, RA mean**	**Patients with CRS without AR, RA mean**	**Patients with CRS with AR/CRS without AR, ratio**	***P* value***
Bacterial invasion of epithelial cells	191,003.76	63,446.76	3.01	.03*	228,467.99	154,631.88	1.47	.11
LPS biosynthesis proteins	74,341.98	32,890.76	2.26	.04*	98,073.42	34,233.58	2.86	.04*
Bacterial toxin production	49,787.94	21,994.04	2.26	.09	42,846.76	53,209.27	1.24	.67
RNA transport	20,471.63	11,123.90	1.84	.26	23,423.63	17,433.20	1.34	.24
β-Lactam resistance	9,167.56	5,300.62	1.72	.23	10,178.52	8,350.42	0.82	.86
Bacterial motility	152,008.65	108,989.38	1.39	.40	142,468.64	154,781.02	1.08	.87
Bacterial chemotaxis	234.18	174.80	1.33	.54	150.31	165.18	1.09	.79
Bacterial secretion system	17,863.96	15,339.47	1.16	.87	17,346.11	17,887.11	1.03	.91

AR, Allergic rhinitis.

*Kruskal-Wallis nonparametric 1-way ANOVA: $P < .05$.