Evidence of microbiota dysbiosis in chronic rhinosinusitis

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Background: **Despite considerable research, the pathogenesis of chronic rhinosinusitis (CRS) remains poorly understood. Potential microbial roles in the etiology or progression of CRS have long been hypothesized, yet few specific associations have been identified. In this study we investigate associations between patterns in resident bacterial communities and clinical variants of CRS.**

Methods: **Bacterial communities were assessed in 94 patients with extensive bilateral CRS undergoing endoscopic sinus surgery (ESS) and 29 controls undergoing ESS for indications other than CRS. Patients were grouped on the basis of phenotypic variants (with or without polyposis) and clinical parameters, including asthma and cystic fibrosis. Bacterial communities were characterized via 16S rRNA gene amplicon sequencing, and quantified by quantitative polymerase chain reaction.**

Results: **Controls and idiopathic CRS subjects tended to be dominated by members of the genera *Corynebacterium* and *Staphylococcus*, together with lower abundances of several other genera, including *Streptococcus*, *Moraxella*, and *Haemophilus*. Aberrant (dysbiotic) bacterial assemblages (with changes in community membership and structure, reduced diversity, and increased bacterial load) and increased inter- and intrasubject variability were more common in subjects with comorbidities such as asthma and cystic fibrosis. Dysbiotic communities were variably dominated by members of the genera *Staphylococcus*, *Streptococcus*, *Haemophilus*, *Pseudomonas*, *Moraxella*, or *Fusobacterium*.**

Conclusion: **Bacterial community dysbiosis was more apparent than specific associations with examined phenotypes or endotypes, and may play a role in the pathogenesis or influence the severity of CRS. Reductions in several common core bacterial taxa, increased inter- and intrasubject variability, reduced bacterial diversity, and increased bacterial load characterized aberrant bacterial communities in CRS.** © 2016 ARS-AAOA, LLC.

Key Words:
bacteria; chronic rhinosinusitis; dysbiosis; inflammatory mucosal disease; microbiota

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Chronic rhinosinusitis (CRS) is a debilitating chronic inflammatory upper respiratory condition, affecting approximately 5% to 15% of the general population,[1] and imposing a significant burden on both patients and the healthcare system.[2–4] The potential for a microbial role in either the initial etiology of CRS, or in exacerbations and progression of the inflammatory process, has long been considered. As yet, however, few clear associations between resident microbes and CRS have been identified.

Previous culture-based studies have investigated potential roles for putative pathogens such as *Haemophilus influenzae*, *Moraxella catarrhalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[5,6] More recently, culture-independent (molecular) approaches for characterizing microbial communities have transformed our understanding of the complexity of the bacterial communities of the sinonasal tract in both healthy subjects and CRS patients,[7–11] and highlight that the picture is much more complicated than previously thought.

Despite the improved understanding of the microbiota in CRS, there remain few certainties about the role of

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microbes in the disease process. Most recently, however, asthma has been identified as one potential mediator of the observed changes in the bacterial communities associated with CRS.[10]

The aim of this study was to build on the current understanding of the sinonasal bacterial communities in CRS patients and healthy controls in a large study cohort, and to further examine associations with potential variants of CRS on the basis of clinical parameters and patients' demographics. We investigate sinonasal bacterial community assemblages via next-generation sequencing and quantitative polymerase chain reaction (qPCR) in 123 subjects (94 CRS and 29 controls) undergoing endoscopic sinus surgery (ESS). In light of recent findings,[10] we focus particularly on the extent to which comorbidities, such as asthma and cystic fibrosis, influence the microbiota in CRS.

Patients and methods

Patient recruitment and sample collection

Ninety-four subjects undergoing ESS for extensive bilateral CRS and 29 controls undergoing ESS for indications other than CRS (including pituitary adenoma surgery and dacrocystorhinostomy) were recruited for this study. CRS subjects were selected in accordance with the 2012 European Position Paper (EPOS) guidelines for CRS.[1] Exclusion criteria included immunodeficiency, sinonasal vasculitis, and age <18 years. Patients on oral antibiotics or corticosteroids in the weeks prior to surgery were not excluded to investigate the effects of these medications on the observed patterns in bacterial community profiles. Twenty-three subjects from this cohort were also sampled at an additional postoperative timepoint for inclusion in a separate time-series study.[12]

Lund-Mackay score, symptom severity scores (rating 5 nasal symptoms on a scale of 0 to 5, including nasal obstruction, anterior and posterior rhinorrhea, sinus pain or pressure, and anosmia[13,14]), and patient demographics (including age, gender, ethnicity, revision surgery for CRS, asthma status, aspirin sensitivity, and antibiotic and corticosteroid administration in the 4 weeks prior to surgery) were collected immediately before surgery (Table 1). Samples were collected intraoperatively before administration of topical mucosal vasoconstrictors and anesthetics or intravenous antibiotics. Pairs of endoscopically guided swab samples were taken from each of the left and right middle meatus using sterile rayon-tipped swabs (Copan Diagnostics, Inc., Murrieta, CA). Samples were transported on ice to the laboratory within 2 hours of collection, and stored at −20°C until the day of processing.

This study was approved by the New Zealand Health and Disability Ethics Committee (NTX/08/12/126), and written informed consent was obtained from all participants.

Bacterial community assessment: 16S rRNA gene-targeted sequencing and quantitative PCR

DNA extraction

DNA was extracted from the pairs of swabs using sterile Lysing Matrix E bead tubes (MP Biomedicals, Seven Hills, NSW, Australia) and the AllPrep DNA/RNA Isolation Kit (Qiagen, Hilden, Germany), as described elsewhere.[9] A negative DNA extraction containing 200 μL of sterile water was carried out simultaneously.

Bacterial community sequencing

Bacterial communities were evaluated via gene-targeted amplicon sequencing of the V3-V4 region of the 16S rRNA gene, using the primers 341F and 806R,[15] together with Nextera DNA Library Prep Kit adapters. PCR reactions were carried out in duplicate, and included genomic DNA template (≈100 ng), 0.5 U HotStar DNA polymerase (Qiagen), HotStar PCR buffer (×1), $MgCl_2$ (2 mmol/L), equimolar concentrations of each primer and dinitrophenols (0.2 μmol/L), and PCR-grade water to a final volume of 25 μL. PCR amplification took place under the following conditions: initial enzyme activation and DNA denaturation at 95°C for 15 minutes; 35 cycles of denaturation (95°C for 30 seconds); annealing (55°C for 30 seconds); and extension (70°C for 40 seconds), and a final extension step of 3 minutes at 70°C. Negative PCR controls were included in all PCR reactions and had no detectable amplified DNA. In addition, eluate from the negative DNA extractions was subjected to bacterial PCR amplification and had no detectable DNA product. Duplicate PCR products for each sample were pooled and then purified using Agencourt AMPure magnetic beads (Beckman-Coulter, Inc., Brea, CA) according to the manufacturer's instructions. Purified products were checked for quantity and quality using Qubit dsDNA (Life Technologies, Auckland, New Zealand) and Bioanalyzer DNA (Agilent Technologies, La Jolla, CA) high-sensitivity assay kits. Standardized concentrations of DNA for each sample were submitted to the sequencing provider (New Zealand Genomics, Ltd.) for library preparation and sequencing on the Illumina MiSeq platform. Raw sequences have been uploaded onto the SRA-NCBI database (SRA accession: SRP092370).

Bioinformatics

Sequences were merged and quality filtered in USEARCH[16] with the default settings. Merged reads shorter than 350 basepairs (bp) and singletons were removed. Open reference operational taxonomic unit (OTU) clustering at 97% sequence similarity threshold was performed using the "uclust" command within the USEARCH pipeline at the default settings. An additional chimera checking and filtering step was conducted against the SILVA gold chimera reference database. OTUs were taxonomically assigned in QIIME[17] using the RDP classifier 2.2[18] against the SILVA 16S rRNA gene database (version 111). Eukaryotic OTUs identified as mapping to human mitochondria were

TABLE 1. Subjects' demographics

Variables[a]	Subject groups				Unadjusted test *p* value[c]
	Controls (n = 29)[b]	CRSsAsthma (n = 39)[b]	CRSwAsthma (n = 47)[b]	CRSwCF (n = 8)[b]	
Age	46 (19 to 84)	47 (20 to 67)	48 (18 to 71)	31 (21 to 50)	**0.0451**
European	18 of 28 (64%)	33 of 38 (87%)	36 of 46 (78%)	7 of 8 (88%)	0.1637
Gender	19 of 29 (66%)	14 of 39 (36%)	24 of 46 (52%)	6 of 8 (75%)	0.0503
Polyposis	NA	13 of 39 (33%)	30 of 47 (64%)	8 of 8 (100%)	**<0.0001**
Aspirin sensitivity	1 of 29 (3%)	3 of 39 (8%)	15 of 47 (32%)	0 of 8 (0%)	**0.0016**
AERD	0 of 29 (0%)	0 of 39 (0%)	15 of 47 (32%)	0 of 8 (0%)	**<0.0001**
Preoperative antibiotics[d]	1 of 29 (3%)	5 of 39 (13%)	7 of 46 (15%)	5 of 8 (63%)	**0.0027**
Preoperative steroids[d]	0 of 29 (0%)	3 of 39 (8%)	8 of 46 (17%)	3 of 8 (38%)	**0.0083**
Revision surgery	NA	11 of 39 (28%)	23 of 46 (50%)	7 of 7 (100%)	**0.0006**
Total symptom score	NA	14 (2 to 25)	15 (2 to 25)	17.5 (7 to 21)	0.6623
Lund-Mackay score	NA	14 (5 to 21)	16 (7 to 24)	19 (12 to 24)	0.0698

[a]Categorical variables are summarized as proportion yes/total (%), except for gender, which is given as proportion female. Continuous variables are summarized as median (range).
[b]Total cohort numbers for each group are given. The differences in total numbers for each variable reflect missing data for some subjects.
[c]Difference between groups tested using Fisher's exact test for categorical variables and the Kruskal-Wallis test for continuous variables. Significant *p* values ($\alpha = 0.05$) are expressed in bold.
[d]Antibiotics and/or steroids in the 4 weeks prior to surgery.
AERD = aspirin-exacerbated respiratory disease; CRS = chronic rhinosinusitis; CRSsAsthma = CRS without asthma; CRSwCF = CRS with cystic fibrosis; CRSsNP = CRS without nasal polyps; NA = not applicable.

removed. Samples were rarefied to an even sequencing depth of 1828 reads. Data from left and right middle meatus samples for each subject were pooled for all subsequent analyses (excluding intrasubject analysis) to accommodate intrasubject variability. Alpha diversity was calculated for richness (observed OTUs) and Shannon and Simpson diversity indices within QIIME (version 1.8). Beta diversity (Bray-Curtis dissimilarity) was calculated within R (version 3.3.0)[19] using the "vegdist" command from the Vegan package.[20] The Bray-Curtis dissimilarity index quantifies the relative similarity of the composition of the bacterial communities, incorporating both presence/absence and the relative abundance of taxa within the community.

Bacterial community quantification

Bacterial load per sample was assessed via real-time qPCR, as described elsewhere.[9] In brief, quantification of human β-actin gene copies was used to first establish the proportion of extracted DNA that was of human origin. 16S rRNA gene-targeted qPCR was then applied to quantify bacterial 16S rRNA gene copy numbers relative to the remaining proportion of the extracted DNA.

Data analyses

Statistical comparisons of demographic data and bacterial community data (including the 30 most abundant genera, alpha diversity, and bacterial load) were conducted comparing subgroups of CRS subjects on the following bases: (a) healthy controls, idiopathic CRS, CRS with cystic fibrosis (CF) (CRSwCF); (b) healthy controls, CRS without nasal polyps (CRSsNP), CRS with nasal polyps (CRSwNP), CRSwCF; and (c) healthy controls, CRS without asthma (CRSsAsthma), CRS with asthma (CRSwAsthma), CRSwCF. Differences between groups were first tested using Fisher's exact test for comparisons between categorical variables, and the Kruskal-Wallis test for continuous variables. Variables with significant differences were further tested in pairwise comparisons using Fisher's exact test for categorical variables, and Dunn's test of multiple comparisons[21] for continuous variables, with Bonferroni adjustment for multiple comparisons[22] to control for familywise error rate. Significance tests were 2-sided with $\alpha = 0.05$.

Permutational multivariate analyses of variance (PERMANOVA) and dispersion (PERMDISP) were conducted using "Adonis" and "betadisper" functions in the Vegan package in R to analyze the proportion of variation in the data explained by the variables and significant differences in dispersion within the data set, with testing against 999 permutations. Samples were visualized in non-metric multidimensional scaling (nMDS) plots generated within R based on Bray-Curtis dissimilarity. Linear discriminant analysis (LDA) of effect size (LEfSe)[23] was conducted using a logarithmic LDA score threshold of 3.0 for discriminative features, and multiclass analyses of one-against-all with $\alpha = 0.01$. Intrasubject comparisons were conducted using Bray-Curtis dissimilarity indices by comparing of the 2 samples

for each subject. Groups were compared using Dunn's test of multiple comparisons with the Bonferroni adjustment for multiple comparisons.

Results

Cohort demographics

CRSwCF subjects were significantly younger compared with the other groups ($p < 0.05$ for all), had significantly higher rates of antibiotics usage in the 4 weeks prior to surgery compared with healthy subjects and CRSsAsthma patients ($p = 0.004$ and $p = 0.04$, respectively), and were significantly more often undergoing a revision surgical procedure compared with other CRS subjects. There were no significant differences between the other subject groups in terms of age, revision surgery, or antibiotics and corticosteroids use in the 4 weeks prior to surgery.

Approximately two thirds of asthmatic CRS subjects had polyposis compared with one third of non-asthmatics ($p = 0.054$). CRSsNP and CRSwNP subjects were asthmatic significantly more often than healthy controls ($p = 0.014$ and $p < 0.001$, respectively).

There were no significant differences between the subject groups on the basis of gender, ethnicity, or total symptom severity score. Pairwise comparisons for variables with significant differences are shown in Table 2.

Bacterial community profiles

After merging and filtering, 6,121,998 reads were retained. A total of 1214 bacterial OTUs were identified across the entire cohort, and classified into 326 distinct bacterial genera.

Bacterial communities were typically dominated by members of the phyla *Actinobacteria*, *Firmicutes*, and *Proteobacteria*. *Fusobacteria* and *Bacteroidetes* made up the remainder of the communities, comprising a comparatively small relative abundance (Figure 1A). Bacterial community diversity was highly variable, ranging from 4 to 351 distinct OTUs present per subject (within 2 to 128 different bacterial genera). Bacterial community membership and structure, diversity, and overall bacterial load, as well as subjects' Lund-Mackay and total symptom severity scores, were highly variable between subjects (Figure 1B-E).

Bacterial communities from control subjects were polymicrobial and variable, but consistently dominated by the genera *Corynebacterium* and *Staphylococcus*. A diverse array of other genera occurred at lower abundances, including *Streptococcus*, *Haemophilus*, *Moraxella*, *Anaerococcus*, *Peptoniphilus*, *Propionibacterium*, *Dolosigranulum*, and *Finegoldia* (Figure 1B). A shift in community structure was observed, with aberrant (dysbiotic) bacterial assemblages involving depletion of the dominant genus *Corynebacterium*, accompanied by changes in the rest of the community structure, including elevations in relative abundance of genera of the phyla *Firmicutes* (including *Staphylococcus* and *Streptococcus*), *Proteobacteria* (including *Haemophilus*, *Pseudomonas*, and *Moraxella*), or *Fusobacteria*. This trend was particularly evident in asthmatic and CF subjects, with significantly reduced *Corynebacterium* in these groups compared with controls ($p = 0.009$ and $p < 0.001$, respectively).

There were no significant differences between CRSsNP and CRSwNP groups with regard to bacterial taxa examined. However, several differences were evident when CRS subjects were instead delineated on the basis of asthma. Asthmatic subjects had reduced relative abundance of *Anaerococcus*, *Peptoniphilus*, *Finegoldia*, and unclassified *Corynebacterium* ($p < 0.02$) compared with their non-asthmatic counterparts (Table 2). Asthmatic subjects also trended toward reduced relative abundance of *Propionibacterium*, but this result was not significant ($p = 0.058$). LEfSe comparison of asthmatic and non-asthmatic CRS subjects further highlighted the associations of OTUs of *Corynebacterium*, *Peptoniphilus*, *Anaerococcus*, and *Finegoldia*, together with *Prevotella* and *Campylobacter*, as differentiating non-asthmatic subjects' bacterial communities from those with asthma (data not shown). Numerous differences in bacterial communities were observed between CF subjects and all other groups. Significant differences in all pairwise comparisons between groups delineated on the basis of CRSsAsthma, CRSwAsthma, CRSwCF and healthy controls are presented in Table 2.

Grouping subjects on the basis of polyposis and CF (healthy, CRSsNP, CRSwNP, and CRSwCF) explained 7% of the variability seen in the bacterial communities of subjects ($p = 0.001$). Grouping by asthma rather than polyps explained 7.8% ($p = 0.001$). Entering both sequentially into the model, asthma still explained 2.5% ($p = 0.001$) of the variability in the model after polyposis, whereas polyposis explained 1.6% ($p = 0.022$) after asthma had been taken into account.

In addition to changes in membership and structure of the bacterial communities, both non-CF CRS and CRSwCF subjects had significantly reduced community diversity and increased overall bacterial load compared with healthy controls ($p < 0.05$ for all). Among the non-CF CRS subjects, asthmatics had significantly reduced diversity in all 3 alpha diversity metrics ($p < 0.03$ for all) and elevated bacterial load compared with controls (Figure 1C and D).

Inter- and intrasubject variability

The nMDS plot revealed that, with the exception of CRSwCF subjects, the centroids for each group remained comparable with findings from the healthy cohort (Figure 2A). However, subjects within each of the CRS groups showed greater spread across the range of different bacterial community types observed. This was reflected in PERMDISP testing: CRSwAsthma subjects' bacterial communities were significantly more dispersed compared with both healthy subjects ($p = 0.008$) and their non-asthmatic counterparts ($p = 0.038$) (Figure 2B). The difference between CRSwCF and healthy subjects trended toward

TABLE 2. Pairwise comparisons for significant variables*

Variables[a]	Nonparametric pairwise comparison (Bonferroni-adjusted *p*-value)					
	Controls vs CRSsAsthma	Controls vs CRSwAsthma	Controls vs CRSwCF	CRSsAsthma vs CRSwAsthma	CRSwCF vs CRSsAsthma	CRSwCF vs CRSwAsthma
Subject demographics						
Age	1.0000	1.0000	**0.0309**	1.0000	**0.0179**	**0.0255**
Polyposis	**0.0020**	**0.0000**	1.0000	0.0536	0.5129	**0.0053**
Asthma	1.0000	**0.0000**	1.0000	**0.0000**	1.0000	**0.0000**
Aspirin sensitivity	1.0000	**0.0187**	1.0000	**0.0442**	1.0000	0.5457
AERD	1.0000	**0.0018**	1.0000	**0.0004**	1.0000	0.5457
Preoperative antibiotics[b]	1.0000	0.8467	**0.0043**	1.0000	**0.0401**	0.0572
Preoperative steroids[b]	1.0000	0.1199	**0.0432**	1.0000	0.3162	1.0000
Revision surgery	—	—	—	0.1447	**0.0018**	**0.0444**
Bacterial genera[c]						
Corynebacterium	0.6182	**0.0093**	**0.0000**	0.2183	**0.0000**	**0.0016**
Streptococcus	0.3377	1.0000	**0.0344**	0.1373	0.3298	**0.0177**
Anaerococcus	1.0000	0.1337	**0.0002**	**0.0080**	**0.0000**	**0.0103**
Pseudomonas	1.0000	0.9049	0.0817	1.0000	**0.0138**	**0.0097**
Peptoniphilus	0.8403	0.3554	**0.0036**	**0.0103**	**0.0002**	**0.0464**
Propionibacterium	0.7711	**0.0026**	**0.0000**	0.0575	**0.0000**	**0.0048**
Unclassified *Neisseriaceae*	0.2116	**0.0228**	**0.0007**	1.0000	**0.0256**	0.0877
Unclassified *Enterobacteriaceae*	1.0000	0.0638	0.0964	0.2158	0.2120	1.0000
Finegoldia	1.0000	**0.0469**	**0.0017**	**0.0088**	**0.0006**	0.1051
Unclassified *Corynebacteriaceae*	1.0000	0.0721	**0.0112**	**0.0130**	**0.0041**	0.3065
Prevotella	0.1517	**0.0024**	**0.0015**	0.4533	0.0581	0.3565
Escherichia/Shigella	0.9722	0.0912	1.0000	0.6409	0.4452	0.0898
Neisseria	0.1099	**0.0164**	**0.0067**	1.0000	0.2040	0.4192
Unclassified *Bradyrhizobiaceae*	0.2068	0.2201	0.2451	1.0000	**0.0098**	**0.0104**
Porphyromonas	1.0000	**0.0359**	**0.0190**	0.0858	**0.0371**	0.5819
Acinetobacter	1.0000	1.0000	**0.0071**	1.0000	**0.0138**	**0.0229**
Rothia	0.2404	0.0745	**0.0007**	1.0000	**0.0215**	**0.0410**
Bacterial diversity and abundance						
Richness (observed OTUs)	0.3730	**0.0288**	**0.0000**	0.8359	**0.0001**	**0.0012**
Shannon diversity	0.5943	**0.0101**	**0.0000**	0.2460	**0.0002**	**0.0077**
Simpson diversity	0.5548	**0.0116**	**0.0001**	0.2975	**0.0013**	**0.0245**
Bacterial load	0.0878	**0.0021**	**0.0000**	0.6464	**0.0037**	**0.0303**

*Significant *p* values ($\alpha = 0.05$) are expressed in bold.
[a]Categorical variables are tested as binary (yes/no) using Fisher's exact test. Continuous variables are tested using Dunn's test of multiple comparisons, with the Bonferroni adjustment for multiple comparisons.
[b]Antibiotics and/or steroids in the 4 weeks prior to surgery.
[c]Bacterial genera data are based on relative sequence abundances.
AERD = aspirin-exacerbated respiratory disease; CRS = chronic rhinosinusitis; CRSsAsthma = CRS without asthma; CRSwCF = CRS with cystic fibrosis; CRSsNP = CRS without nasal polyps; OTU = operational taxonomic unit.

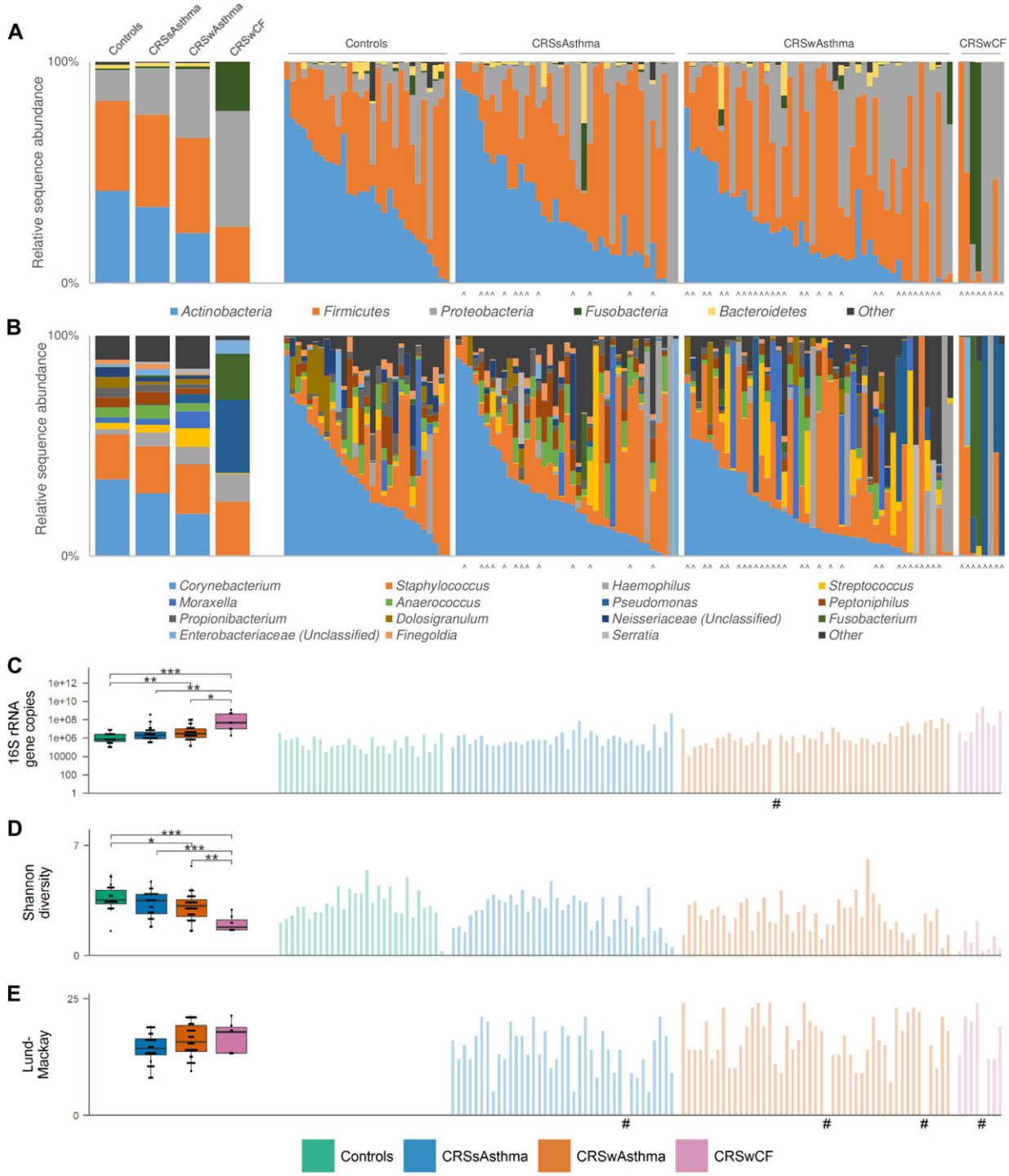

FIGURE 1. Group- and patient-level bacterial community profiles and disease severity. Group comparisons show increased bacterial load and reduced bacterial community diversity in CRS, whereas grouped taxonomic profiles of communities mask high degrees of intersubject variability within each group. Bacterial community profile summaries for (A) phyla, (B) genera, (C) bacterial load (16S rRNA gene copy numbers/sample/subject, graphed on a log scale), and (D) alpha diversity (Shannon diversity index). Disease severity index for (E) Lund-Mackay computed tomography staging score. Broad bars and box-and-whisker plots represent group summaries; individual bars represent individual subjects. Subjects are grouped by controls, CRSsAsthma, CRSwAsthma, and CRSwCF, and ordered in each group by descending relative abundance of the bacterial genus *Corynebacterium*. Asterisks denote significant group differences (Bonferroni-adjusted, $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$, respectively). (^) CRS with nasal polyps; (#) no data. CRS = chronic rhinosinusitis.

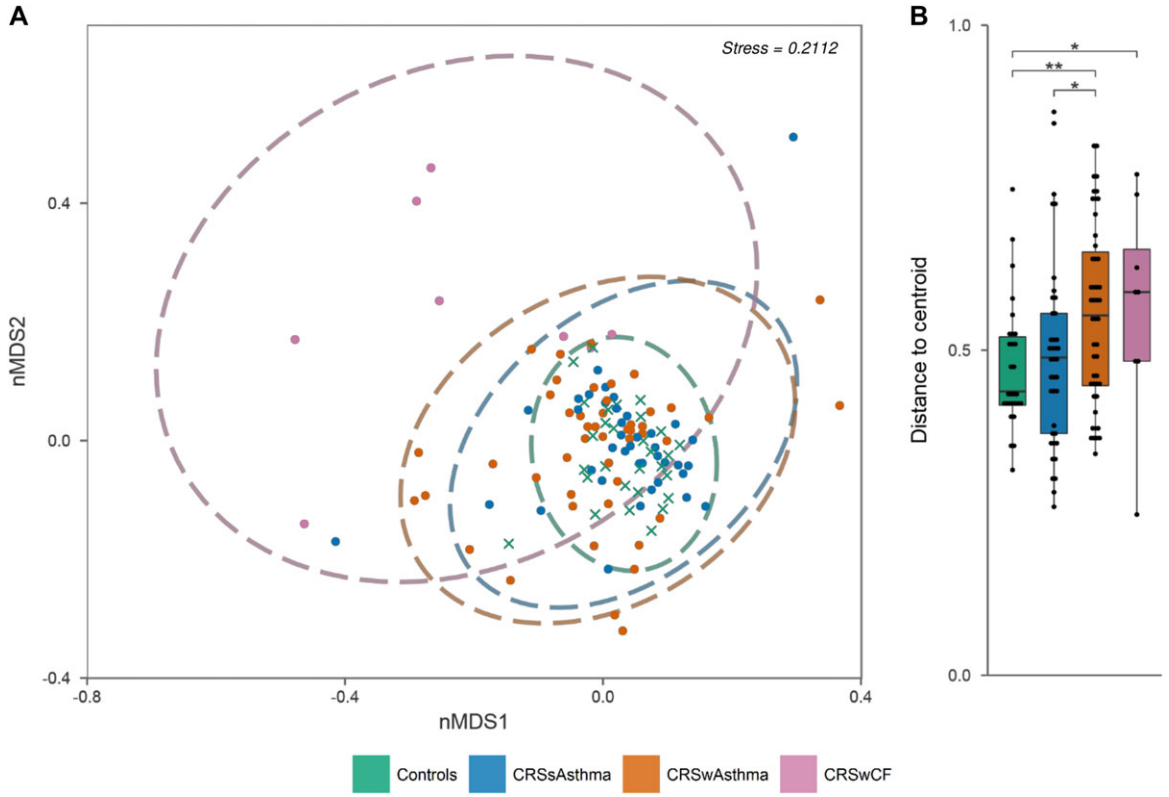

FIGURE 2. Intersubject variability. Subjects in CRS groups show higher degrees of intersubject variability, with increased dispersion across the range of bacterial community types rather than characteristic communities associated with each respective CRS patient group. (A) nMDS plot based on bacterial community Bray-Curtis dissimilarity between each subject. Points represent each individual subject and the relative bacterial community similarity—incorporating both presence/absence and relative abundance of bacterial community members—when compared with all other subjects (closer = more similar, further apart = more dissimilar). Ellipses represent the 95% confidence interval spread from centroids. (B) Box-and-whisker plot of Bray-Curtis dissimilarity measures between subjects and the centroid for their respective group. Asterisks denote significant group differences (unadjusted, *$p < 0.05$ and **$p < 0.01$, respectively). nMDS = non-metric multidimensional scaling.

significance ($p = 0.055$). With adjustment for multiple pairwise comparisons using Tukey multiple comparisons testing, the difference between CRSwAsthma and healthy subjects fell just outside the range of significance ($p = 0.052$).

Subjects with more aberrant bacterial communities also tended to have greater within-subject variation between the left and right side (Figure 3A). Intrasubject Bray-Curtis dissimilarities (comparing between the bacterial communities of the left and right middle meatus within each respective subject) in asthmatic CRS subjects were significantly larger than those of both healthy controls ($p = 0.041$) and non-asthmatic CRS subjects ($p = 0.039$) (Figure 3B).

Use of antibiotics and steroid in the 4 weeks prior to surgery

Use of antibiotics in the 4 weeks prior to surgery explained 2% of the variability in the data in this study ($p = 0.01$). When examining the interacting effects of asthma or polyposis status and recent antibiotics use, antibiotics no longer significantly explained any of the variability after the effect of these CRS subtypes had been taken into account. Use of corticosteroids did not explain any variation in the model, and there were no significant differences in dispersions of the bacterial communities of subjects based on use of antibiotics or steroids in the 4 weeks prior to surgery. Similarly, when each group (controls, CRSsAsthma, CRSwAsthma, and CRSwCF) was assessed independently, recent use of antibiotics did not contribute to variation, and nor were there any significant differences in dispersion between those who had taken antibiotics recently and those who had not.

Discussion

In this study we have investigated bacterial community profiles of the middle meatus in 94 CRS subjects and 29 healthy controls via next-generation sequencing and quantitative PCR. Bacterial community profiles were broadly comparable to previous studies,[8–11] and our data add to the growing understanding of the membership and structure of the microbiota in healthy controls and CRS patients.

Aberrant bacterial communities

Previous studies have highlighted potential associations of specific bacterial taxa with CRS,[7] specific symptoms

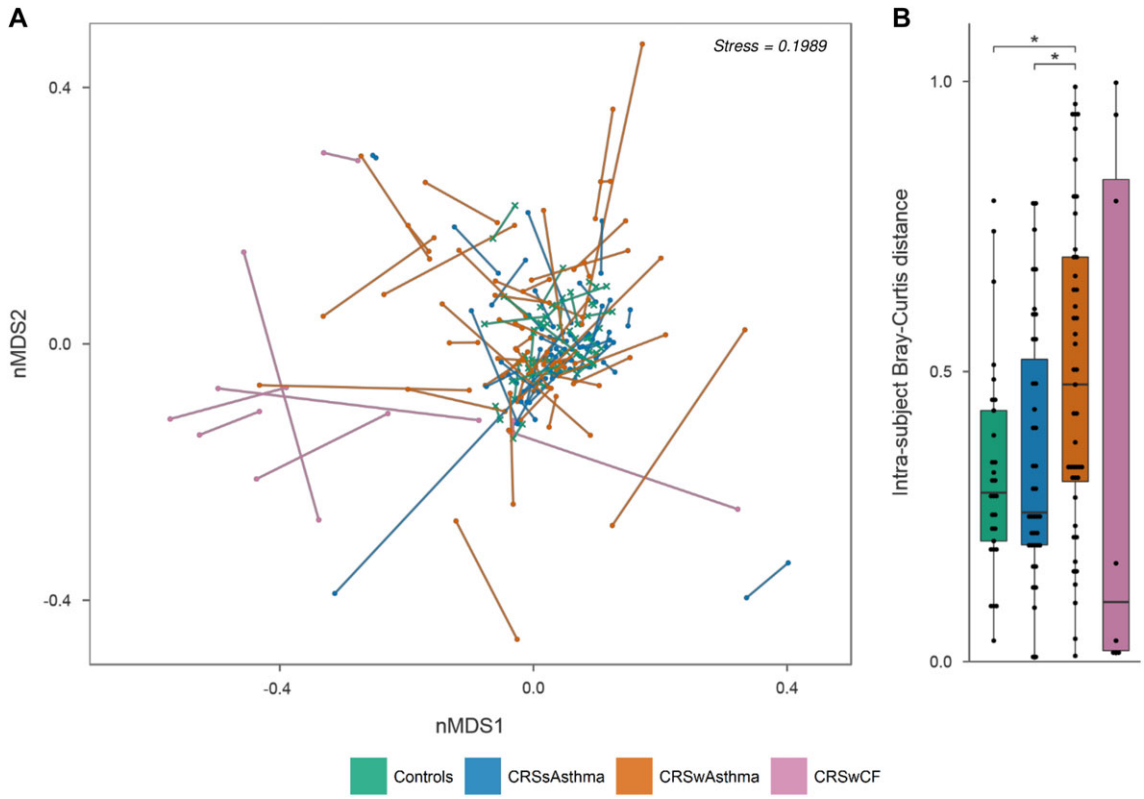

FIGURE 3. Intrasubject variability. Asthmatic CRS subjects show higher degrees of intrasubject variability, with increased differences of bacterial community composition when comparing between samples from the left and right middle meatus within each subject. (A) nMDS plot based on bacterial community Bray-Curtis dissimilarity between all samples. Points represent each sample (one for each of the left and right middle meatus for each subject) and the relative bacterial community similarity—incorporating both presence/absence and relative abundance of bacterial community members—when compared with all other samples (closer = more similar, further apart = more dissimilar). Vector lines join pairs of samples for each subject. (B) Box-and-whisker plot of intrasubject Bray-Curtis dissimilarity measures (between the left and right middle meatus for each subject). Asterisks indicate significant group differences (Bonferroni-adjusted, $p < 0.05$). nMDS = non-metric multidimensional scaling.

of CRS,[10] and microbiota changes and recovery after surgery.[11,12,24–28] However, many of these findings have not been reproduced in subsequent studies. In this study, unpredictable changes in bacterial community structure, together with reduced diversity and increased bacterial load of the community overall, were a more apparent feature of aberrant bacterial profiles than specific associations with putatively pathogenic bacteria or bacterial communities. Overall, a shift away from the healthy microbiota appeared to be more central than any specific new community that this shift occurred toward.

Aberrant community types showed increased dominance by various taxa, including *Staphylococcus*, *Streptococcus*, *Haemophilus*, *Pseudomonas*, *Moraxella*, and *Fusobacterium*. Several members of these genera have been of particular interest in previous culture-based studies,[5,6] as well as in studies of changes after surgery.[12,24,25] However, in our study, dominance by these taxa was unpredictable and no specific patterns emerged with CRS phenotypes or endotypes examined. Of note, these genera were often present within the communities of healthy subjects, albeit at lower relative abundance, suggesting a possible role of opportunistic dominance by these various taxa in response to instability and disturbance of the microbiota.

Asthma as a mediator of change in microbiota

Grouping patients on the standard phenotypic basis of polyposis did not give any clear insights into bacterial community patterns in this study. Analysis by coexistence of asthma, in contrast, identified several key differences. Chief among these was a reduction of several healthy community-associated taxa, including *Anaerococcus*, *Peptoniphilus*, *Finegoldia*, *Corynebacterium*, and *Propionibacterium*. Furthermore, reduced bacterial diversity and increased bacterial load observed in CRS, in general, was also particularly pronounced in asthmatics and in patients with underlying CF.

Asthma has also been reported as a determinant of the patterns of bacterial communities in CRS.[10] Taken together, these findings lend further weight to the argument that microbiologic and immunologic changes associated with asthma may be key influences on the bacterial community assemblages of subjects with CRS. The effect of polyposis on these communities, in contrast, remains less clear.

Inter- and intrasubject variability

Although asthma appears to be a major factor, a substantial amount of the variability in the data (>90%)

remain unexplained. We showed previously that intersubject variability contributes to a considerable proportion of this (up to 37% in another cohort).[9] Herein we have expanded on this view by also showing that the degree of intersubject variability is increased in CRS, and particularly so in those subjects with asthma or CF. Furthermore, this was also evident within individual patients, with increasing incidence of marked differences between the bacterial communities of the left and right middle meatus.

The findings further highlight dysbiosis as a potential key explanatory factor of the microbiota in CRS. Observed changes may represent a gradient of increasing disturbance and instability, in contrast to direct pathogenic associations with specific opportunistically dominant taxa. The role of microbiota dysbiosis has received increasing interest in a diverse range of inflammatory conditions, including diseases of the gut,[29–31] mouth,[32] and skin,[33] as well as autoimmune,[34] and even neuroinflammatory,[35] conditions. Importantly, it remains unclear whether these changes in the microbiota in CRS feed into the heterogeneity or progression of CRS, or whether they are merely a benign reflection of the changing environment in the inflamed tissues of the sinonasal tract. Additional work should address the extent to which shifts away from a normal healthy bacterial community type play significant roles in the inflammatory disease process. Moreover, other members of the microbiota (fungi and viruses) may also influence dysbiosis in CRS, and need to be investigated further in the future.

High natural intersubject variability, even in healthy subjects, represents a major complicating factor in studies of CRS. Large subject numbers are likely required to establish genuine associations. To our knowledge, this study represents the largest study of the microbiota of CRS to date. Nonetheless, it may remain underpowered to detect subtler patterns. Additional large studies and subsequent meta-analyses will be required to further resolve some of the finer-scale aspects of the microbiota in CRS.

In addition, the data also highlight that grouped bacterial community summaries (as seen in Figure 1A and B) should be interpreted with caution. These summaries can mask the high degree of intersubject variability within each group, and do not necessarily represent actual patterns seen at the individual subject level. In particular, if instability and dysbiosis better describe changes in the microbiota than characteristic community types, we speculate that grouped summaries may lead to spurious associations with disease, and likely contribute to the limited reproducibility of studies to date.

Influence of preoperative antibiotics and steroids

Whether or not patients had taken antibiotics or corticosteroids in the 4 weeks prior to surgery had little effect on the observed patterns of bacterial communities in this study. This is a common exclusion criterion in many studies.[9–12] Although it is an intuitive measure, its importance for microbiota studies in CRS has remained unclear.

Considered in isolation, recent use of antibiotics explained only 2% of the variability seen within the data set. Moreover, on incorporating CRS phenotypes into the model, antibiotics no longer explained any of the variability seen. These data provide a cross-sectional snapshot, and suggest that recent antibiotics use does not explain the patterns seen in resident bacterial communities in healthy controls or CRS subjects. Longitudinal studies are required to better determine the effects over time at the individual patient level.

Legacy effect of antibiotics over the course of medical management of CRS

All CRS subjects will likely have undergone multiple courses of antibiotics as part of maximal medical therapy prior to referral for surgery.[36,37] The legacy effect of this on the microbiota in CRS remains poorly understood. It may contribute to a reduced effect of the most recent course in the weeks prior to surgery: the community that remains is likely made up of members that tend to be more resistant to the antibiotics prescribed.[38] Furthermore, in the event that microbiota dysbiosis may play a role in CRS, it is necessary to better understand the extent to which this legacy effect influences the observed microbial instability. Further work is required to tease apart the short- and long-term effects of antibiotics on the microbiota in CRS.

Conclusion

Increased variability and imbalance (dysbiosis) may be a better explanatory model of changes in the microbiota in CRS than specific associations between putative pathogenic bacteria or bacterial communities and clinical phenotypes or endotypes. A depletion of several common core taxa (including *Anaerococcus*, *Peptoniphilus*, *Finegoldia*, *Corynebacterium*, and *Propionibacterium*), coupled with reduced bacterial community diversity and increased overall bacterial load, was more characteristic of aberrant changes in the microbiota than dominance by any specific bacteria. These changes were particularly pronounced in subjects with concomitant asthma or underlying CF.

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