2021 UCB® Resident Research Competition – 1st Place

ORIGINAL RESEARCH

Association between Rosacea, Environmental Factors, and Facial Cutaneous Dysbiosis: A Pilot Study from the Largest National Festival of Twins

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ABSTRACT

Background: To investigate the microbiome composition in individuals with and without rosacea and correlate findings to individual factors that may affect facial cutaneous and enteric microbiome composition.

Methods: Participants with and without rosacea (as determined by a board-certified dermatologist) were surveyed regarding factors that may affect the facial cutaneous/enteric microbiome. Microbiome samples were collected, analyzed for 16S sequences, and mapped to an optimized version of existing databases. R was used to perform Mann-Whitney/Kruskal-Wallis test for categorical comparisons. Correlation between two continuous variables was determined with linear regression models. Primary Component Analysis (PCoA) plots employed Monte Carlo permutation test to estimate p-values. All p-values are adjusted for multiple comparisons with the false discovery rate (FDR algorithm) using Benjamini-Hochberg.

Results: 84 individuals with rosacea and 44 controls were evaluated. Individuals with rosacea were more likely to currently own pets ($p = 0.029$) and consume more alcohol ($p = 0.006$). Absolute bacteria abundance were similar in facial cutaneous ($p = 0.36$) and enteral microbiome ($p = 0.29$). Facial cutaneous microbiome showed significantly decreased richness and evenness (OTU: $p = 0.019$; Shannon: $p = 0.049$) and a three to four-fold decrease in abundance of 8 distinct cutaneous bacterial genera in rosacea. Enteral microbiome analysis showed significant reduction in abundance of Ruminococcaceae (FDR = 0.002) and Blautia (FDR < 0.001) and increase in Prevotellaceae (FDR = 0.024) in rosacea.

Conclusion: Environmental factors may alter relative abundances of specific microbial genera and lead to microbiome diversity. Further studies with increased sample sizes and higher severity cases may further elucidate the role of dysbiosis in rosacea.

INTRODUCTION

Rosacea is a chronic inflammatory disorder of the central face, characterized by transient or persistent erythema and telangiectasias, papules and/or pustules, phymotic changes and/or rare ocular manifestations.[1] It is estimated that upwards of 10% of individuals are afflicted by rosacea, with over 16 million affected in the United States alone.[2] Current

pathophysiology incorporates both environmental and genetic components that stimulate an overactive innate immune system and inflammatory reactions to the skin microbiome.[1-3] More recent studies also suggest that this inflammation may have a systemic component given rosacea's association with various inflammatory conditions, including Crohn's disease, ulcerative colitis, small intestinal bacterial overgrowth (SIBO), and *Helicobacter pylori*.[4-8]

The microbiome is a vast and varied collection of bacteria, viruses, and fungi whose composition has increasingly been demonstrated to have significant influence on whole-body health as well as development and maintenance of immunological activity.[1,9] Early exposure to commensal skin microbes and environmental factors affect the developing microbiome's richness (i.e., diversity of organisms) and evenness (i.e., relative quantity of organisms present) and may influence the function of the immune system and inflammatory response.[9-11] Studies have found a correlation between the relative abundances of several cutaneous microbes, including notably *Demodex folliculorum* (and its native microbe *Bacillus oleronius*), virulent strains of *Staphylococcus epidermidis*, cytotoxin-associated gene A positive ($CagA^+$) *Helicobacter pylori* and *Chlamydophila pneumoniae,* and rosacea.[1,12-15]

Because of the multifactorial nature of the pathophysiology of rosacea, twin studies offer ways to control for genetic causes and isolate environmental factors. [3,16,17] While only 50% of genes are identical between fraternal twins, identical twins share all of their genes. As a result, they offer a unique opportunity to study not only heritability of diseases but also isolate and analyze the impact of environmental factors.

The purpose of this study was to analyze the cutaneous and enteral microbiome and its role in rosacea and correlate findings to demographic/environmental information.

METHODS

This study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practices and local regulatory requirements. The studies were reviewed and approved by institutional review boards. All subjects provided their written informed consent prior to entering the studies.

Participants were recruited from attendees of the annual Twinsburg Festival in Twinsburg, Ohio during August 5-6 2017. Participants ≥18 years-old were evaluated for rosacea by a board-certified dermatologist prior to completing a survey (with the aid of trained staff members as needed). Information on demographics and factors that could affect the microbiome were obtained. Facial swabs and fecal samples were collected for microbiome genomic data (additional information in supplement) and compared to existing databases to identify bacterial taxa abundance and differences within (alpha-diversity) and between (beta-diversity) groups.

Analysis was performed using R. Categorical comparisons were performed using the non-parametric Mann-Whitney test for two-category comparisons or the Kruskal-Wallis test for ≥3 categories. Correlation between two continuous variables was determined with linear regression models, where p-values indicate the probability that the slope of the

regression line is zero. Principle Component Analysis (PCoA) plots employed the Monte Carlo permutation test to estimate p-values. All p-values were adjusted for multiple comparisons with the false discovery rate (FDR algorithm) using Benjamini-Hochberg.

RESULTS

136 participants (Rosacea, n = 88; Control, n = 48) were included in the final analysis. Participants were with rosacea were predominately female (70.5%), mean age 50.3 years (Standard Deviation ± 12) with mild to moderate rosacea (97.4%). Notably, control counterparts were proportionally more likely to be female (97.9%) (Table 1). Fitzpatrick score between participants with and without rosacea was significantly different (p<.001) with a skew towards lower phototype in individuals with rosacea.

Participants with rosacea reported consuming more alcoholic beverages/week than controls (2.42 vs 0.78, p=.006) and were more likely to currently own pets (72.4% vs 52.1%, p = .029) (Table 2). Regular use of over the counter skin care products did not significantly differ between groups.

There was no significant difference between absolute microbial counts between rosacea and control in either facial (30,880 vs 29,533, p=.36) or enteric (14,198 vs 13,566, p = .29) microbiomes (Figure 1 A&B).

Intra-sample (alpha-diversity) richness and evenness was significantly less in the facial cutaneous microbiome of participants with rosacea versus control (Operational Taxonomic Units (OTU): p = 0.019; Shannon: p = 0.049)(Figure 2A&B). No significant difference was found when comparing the enteric microbiome between groups (OTU: p = 0.96; Shannon: p = 0.49)(Figure 2 C&D). Between groups (beta-diversity) there was a significant difference within respective facial cutaneous microbiome (p= .024, R^2 = 0.037, F-statistic = 3.21) but not in the enteric microbiome (p = .256, R^2 = .0152, F-statistic = 1.27) (Figure 2 E&F).

Table 1. Participant Demographics. There was a significant difference in phototype distribution between participants with and without rosacea. Participants with rosacea reported consuming more alcoholic beverages per week than their control counterparts.

	Rosacea (N = 88)	Control (N = 48)	p-value
IGA – n (%)			
Mild	43 (55.1)	-	-
Moderate	33 (42.3)	-	-
Severe	2 (2.6)	-	-
Age – Mean (SD)	50.3 (12)	46.75 (13.4)	0.116
Female Gender – n (%)	62 (70.5%)	47 (97.9%)	< 0.001
Fitzpatrick Score – n (%)			<0.001
2	54 (61.4)	26 (54.2)	
3	31 (35.2)	8 (16.7)	
4	3 (3.4)	8 (16.7)	
5	0 (0.0)	6 (12.5)	
Drinks/Week – Mean (SD)	2.42 (4.0)	0.78 (1.1)	0.006

Table 2. Potential Microbiome-altering Factors. Participants with rosacea were significantly more likely to report currently owning a pet than their control counterparts

	Rosacea (N = 88)	Control (N = 48)	p-value
Pet Ownership – n (%)			
Pets in Childhood	79 (89.8)	45 (93.8)	0.642
Pets Now	63 (72.4)	25 (52.1)	0.029
Caesarean Section – n (%)	17 (19.3)	8 (17.4)	0.969
Breast Fed – n (%)	20 (23.3)	7 (28.0)	0.824
Skin Care – n (%)			
Moisturizer	51 (58.0)	30 (62.5)	0.739
Facial Cleanser	62 (70.5)	35 (72.9)	0.916
Sunscreen	64 (72.7)	33 (68.8)	0.770

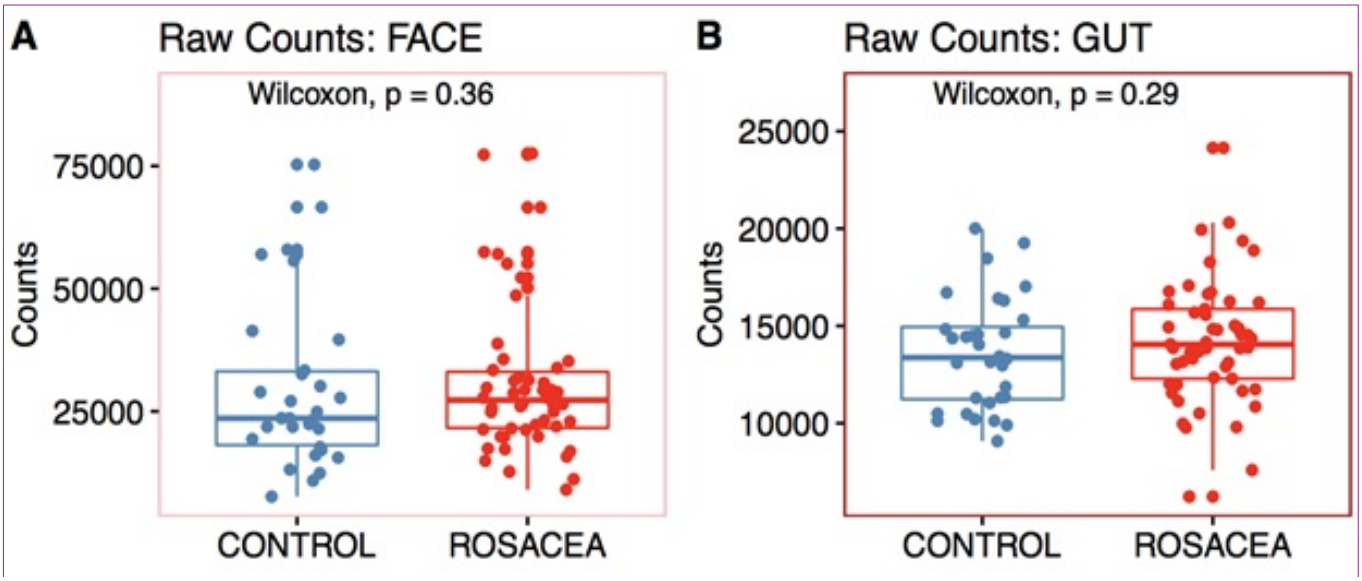

Figure 1. Absolute Bacterial Abundance. Scatterplot with superimposed box-plot demonstrating distribution of absolute abundance of all bacteria within facial cutaneous (A) and enteric microbiome (B). No significant difference was found between the bacterial load within the microbiome between participants with and without rosacea. Wilcoxon signed-rank test used for non-parametric comparison with p<.05 demonstrating significance.

There was a 3-4 fold decrease in abundance of facial cutaneous bacterial genera *Streptococcus* (FDR = 0.015; FDR = 0.004), *Corynebacterium* (FDR = 0.003), *Actinomyces* (FDR = 0.015), *Lactococcus* (FDR = 0.016), *Veillonella* (FDR < 0.001) and *Chloroplast* (FDR = 0.015) in rosacea compared to control (Figure 3A). In the enteric microbiome, there was significant reduction in abundance of *Ruminococcaceae* (8-fold reduction; FDR = 0.002) and *Blautia* (2-fold reduction; FDR < 0.001) and a 6-fold increase in *Prevotellaceae* (FDR = 0.024) in rosacea compared to control (Figure 3B).

DISCUSSION

Data suggests that, although primarily thought of as an inflammatory condition of the central face, rosacea's classically cutaneous dysregulated inflammatory response may extend systemically, especially given associations with multiple gastrointestinal, metabolic, and neurological disorders.[5-8,15,18] Studies have previously implicated species such as *Demodex folliculorum* (and its native microbe *Bacillus oleronius*), virulent strains of *Staphylococcus epidermidis*, cytotoxin-associated gene A positive (CagA+) *Helicobacter pylori, Chlamydophila pneumoniae* and lesser known organisms of the facial (*Gordonia, Geobacilus)* and enteric (*Peptococcaceae*, *Methanobrevibacter, Acidaminococcus* and *Megasphaera)* microbiome in the dysbiosis that may play a role in rosacea's (systemic) inflammatory response.[1,5,12-17]

Our findings demonstrated a correlation between rosacea and decreased diversity (richness) and relative of abundance (evenness) of organisms within the facial cutaneous microbiome of individual's with rosacea. Interestingly, significantly more participants with rosacea reported currently owning pets and consuming more alcohol which may play a role in instigating or propagating the observed dysbiosis.

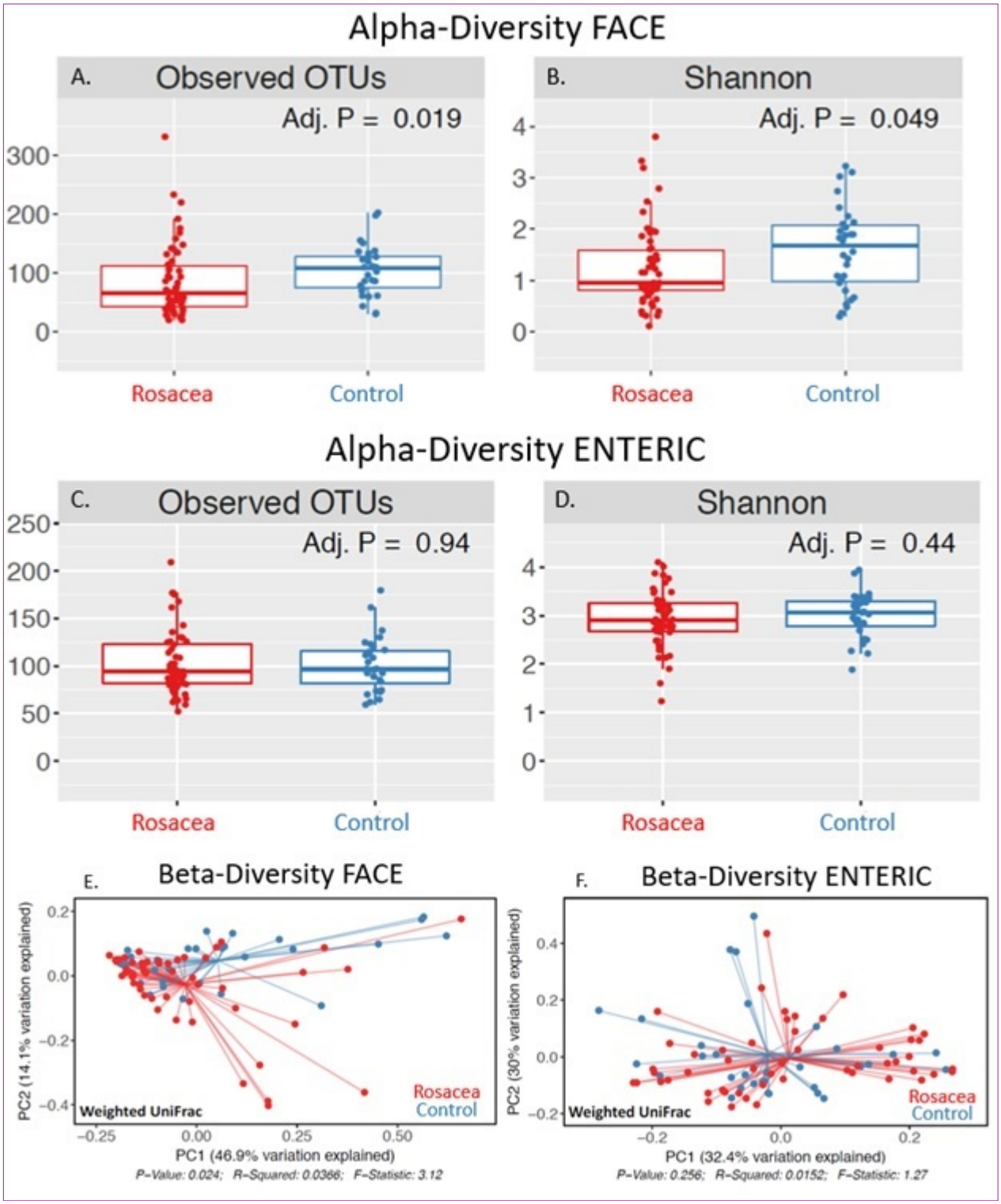

Figure 2. Diversity in the Facial Cutaneous and Enteric Microbiome. Alpha diversity (differences within a sample) showed significantly less diversity/richness in bacteria (operational taxonomic units (OTUs))(A) and evenness (Shannon)(B) in facial cutaneous but not in enteric microbiome (C&D). Beta-diversity (differences between samples across groups) assessed with principal component analysis with Weighted UniFrac (accounting for number of different species and relative abundance) demonstrated clustering of the microbiome samples that were significantly different for facial cutaneous microbiome (E) but not for the enteric microbiome (F). P-value calculated with Mann-Whitney U with false discovery rate corrections using Benjamini-Hochberg.

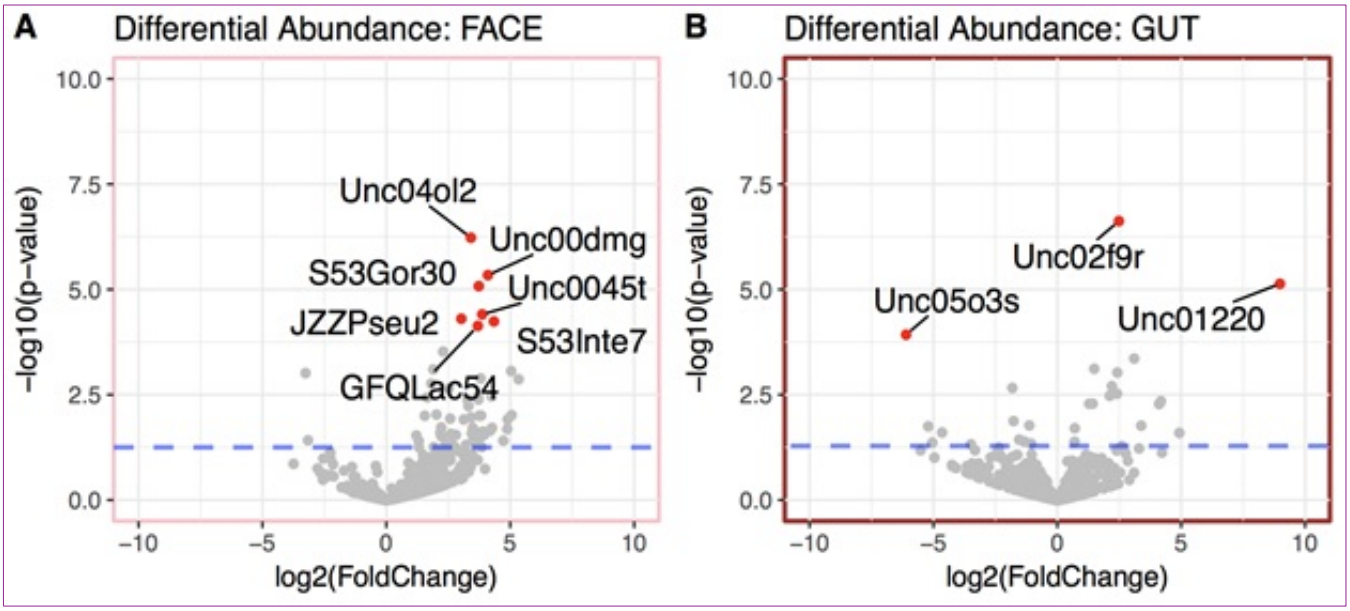

Figure 3. Differential Abundances of Bacterial Genera. Volcano plot demonstrating -$\log_2$ fold changes in abundance of bacterial genera within the facial cutaneous (Streptococcus (S53Inte7, FDR = 0.015; S53Gor30, FDR = 0.004), Corynebacterium (Unc00dmg, FDR = 0.003), Actinomyces (Unc0045t, FDR = 0.015), Lactococcus (GFQLac54, FDR = 0.016), Veillonella (Unc04ol2, FDR < 0.001) and Chloroplast (JZZPseu2, FDR = 0.015)) (A) and enteric (Ruminococcaceae (Unc01220, FDR = 0.002) and Blautia (Unc02f9r, FDR < 0.001) and Prevotellaceae (Unc05o3s, FDR = 0.024))(B) microbiome.

To the authors knowledge, the implicated species within the facial cutaneous (and enteric microbiome) have not yet been widely investigated in rosacea pathogenesis. This may suggest that dysbiosis as a whole, as opposed specific virulent or beneficial species, may play a (more central) role in the inflammation seen in rosacea. The current study did not find alterations within the diversity of the enteric microbiome, which may be due to a limited sampling of participants with (more) severe rosacea who may have a more dysregulated systemic inflammatory response.

There are some reports in the literature that suggest improving epidermal barrier dysfunction in rosacea may improve disease severity.[19,20] Interestingly, our sample reported no significant difference in over the counter skin care usage. This may be due to limited sample size or reporting/recall bias and should be further explored in future studies.

Limitations include relatively small sample size comprised of mostly mild-moderate rosacea that may limit statistical power, detection of deviations in microbiome diversity and composition, and ability to perform intra-twin variability. Participants were recruited from a regional festival which may limit result generalizability to other populations. Retrospective survey results may also be subject to recall bias.

CONCLUSION

Rosacea is an inflammatory condition primarily of the central face that has been associated with systemic inflammatory conditions. Dysbiosis of the facial cutaneous microbiome may be affected by an individual's local environment and contribute to ongoing rosacea pathogenesis. Future studies should investigate the causality between dysbiosis and rosacea pathophysiology and may benefit from more

highly powered twin studies to control for confounding genetic (and environmental) factors.

Conflict of Interest Disclosures: JWM, SB, PM have no relevant disclosures. HEB is a speaker for Galderma, Valeant, Sun, Mayne, and Bayer, and investigator for Galderma and Valeant.

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