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Characterization of the facial microbiome in twins discordant for rosacea

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Abstract

Previously, we determined that genetic and environmental factors contributed equally towards rosacea in twins. To assess an environmental factor, we characterized the malar cheek bacterial microbiome from twins discordant for rosacea. We found no significant difference in facial microbiome alpha and beta diversity between related twins discordant for rosacea. However, the relative percentage abundance of *Gordonia* and *Geobacillus*, low-abundant genera, was positively and negatively associated with rosacea severity, respectively. Our data demonstrate a significant correlation between facial microbiome and severity of rosacea in genetically matched twins and importantly that overall microbiome composition is largely unchanged.

Keywords

16S rRNA; alpha and beta diversity; microbiome; NRS score; relative percentage abundance

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AUTHOR CONTRIBUTIONS

Drs. AZ, KS, MM, DS, MT and DP had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: MT and P. Analysis and interpretation of data: AZ, DS, MT and DP. Drafting of the manuscript: AZ and DP. Critical revision of the manuscript for important intellectual content: AZ, KS, MM, DS, MT and DP. Statistical analysis: PF and AZ. Obtained funding: MT, MG and DP. Administrative, technical or material support: MT, MM, CA, YT and DP. Study supervision: MT and DP.

CONFLICT OF INTEREST

The authors have no conflict of interests.

SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article.

1 | BACKGROUND

Rosacea is a chronic inflammatory disease of the facial skin and eyes that affects ~16 million individuals in the United States alone. Although its aetiology is unknown, we previously determined that both genetic and environmental factors contribute equally towards disease using identical (monozygotic) and fraternal (dizygotic) twins.[1] Previous studies have implicated microbial colonization and the cognate immune response as the critical driver of rosacea.[2,3] Increased levels of *Demodex* mites and associated bacteria (bacillus), antimicrobial peptides (eg cathelicidin/LL37), host immune variables (eg Toll-like receptor (TLR)-2) and nutrients (eg vitamin D3) are reported to alter the innate immune response and cause chronic inflammation and the facial skin sensitivity in rosacea.[2,3] In addition, the recent studies suggest significant associations between the severity of rosacea and systemic comorbidities (eg cardiovascular, allergic, respiratory, metabolic and gastrointestinal diseases) that are linked with chronic inflammation.[4,5] Rosacea and its reported comorbidities share involvement with barrier tissues that are colonized with a wide variety of micro-flora that constitute the microbiome.

2 | QUESTION ADDRESSED

The beneficial use of therapeutic drugs for rosacea including oral and topical antibiotics, sulphur compounds and ivermectin that alter the facial microbiome,[6] suggests that altering the resident skin microbiome may play a role in the disease. However, no conclusive evidence has been established.

Microbial dysbiosis could be one of the factors associated with the pathogenesis of rosacea as well as its comorbidities. Harnessing the genetic and environmental control inherent in a twin study, we performed next-generation sequencing of the 16S rRNA gene to evaluate the hypothesis that facial bacterial microbiome dysbiosis will associate with the severity of rosacea in twins discordant for rosacea.

3 | EXPERIMENTAL DESIGN

See Appendix S1.

4 | RESULTS

4.1 | Comparable overall facial skin microbiome composition across subjects

We estimated the relative percentage abundance (RPA) of facial bacterial microbiome at the phylum level in twins with and without rosacea. The top four abundant phyla were Firmicutes (mean ± SD; 42.98% ± 1.05%), Proteobacteria (39.29% ± 1.11%), Actinobacteria (15.88% ± 0.78%) and Bacteroidetes (1.04% ± 1.85%) in all subjects (Figure 1A and Table S1), and our data were generally consistent with the previous reports of other facial skin sites.[7,8] We found that Firmicutes were present in greater abundance than Actinobacteria. However, Proteobacteria were represented by a greater number of genera than Firmicutes and Actinobacteria (Table S1). There was no significant difference in mean abundance for all phyla when compared between different sites (left versus right cheek) or

disease (no rosacea versus rosacea) groups. Thus, both inter- and intrapersonal variability in mean abundance of microbiome was observed. The principal coordinates analysis confirmed both inter- and intrapersonal variability and revealed no distinct difference between individuals with and without rosacea (Figure 1B).

4.2 | Comparable alpha and beta diversity of twin pairs discordant for rosacea

The assessment of facial bacterial microbiome alpha diversity (S1) by Shannon index showed comparable alpha diversity between monozygotic twin pairs with and without rosacea (Figure S1A).

There are earlier reports[9] of skin diseases that showed less bacterial diversity in disease states. Given the paired nature of data, we plotted the National Rosacea Society (NRS) scores differences versus alpha diversity differences between related twin pairs (Figure S1B). We found a negative association between NRS score differences and alpha diversity differences. These data suggest that rosacea negatively impacting bacterial diversity. The non-significance might be due to a low number of samples at high NRS score.

Next, we determined whether facial microbiome beta diversity (S1) is distinct between subjects with rosacea and healthy subjects. Principal component analysis for unweighted, weighted UniFrac (WUF) or Bray-Curtis beta diversity measures (S2) showed no distinct segregation between healthy versus rosacea subjects (data not shown). However, we found that WUF distance between siblings in which only one individual has rosacea was greater than WUF distance between siblings with rosacea and WUF distance between siblings without rosacea (Figure S2). These data indicate the trend that the facial microbiome is more diverse between siblings if rosacea is present, although it did not meet the statistical significance.

Consistent with a previous study,[8] we also found that the related monozygotic twins have more similar (lowest mean WUF distance) facial bacterial microbiome than related dizygotic twins and significantly dissimilar than unrelated twins (Figure S3).

4.3 | Facial skin bacterial microbiome correlates with NRS score

We tested the hypothesis that the changes in the facial microbial community might be associated with rosacea severity. We found a significant ($p < .05$) correlation between NRS score and the RPA of 9 genera (Table S3).

To evaluate the association between NRS score and RPA of bacterial genera and demographics, we performed random effect Poisson regression (REPR) analysis (see Section 3 Experimental Design for rationale and details). Univariate REPR analysis revealed a significant ($P < .05$) association between NRS scores and six genera: *Gordonia*, *Blautia*, *Chryseobacterium*, *Wautersiella*, *Geobacillus* and unknown genus (phylum Proteobacteria) but not with demographics listed in Table S1. Multivariate REPR analyses found that only two genera (*Gordonia* and *Geobacillus*), age group 30–60 years versus 0–30 years, and birth order B versus A remained significantly predictive of NRS score. REPR model suggests that a 10% increase in RPA of *Gordonia* or a 10% decrease in RPA of *Geobacillus* will increase a NRS score by a factor of 1.76 or 2.16, respectively. These data support that bacterial

microbiome (*Gordonia* and *Geobacillus*) is the dominant predictor of NRS score in this cohort (Table S4). Concordantly, *Gordonia* and *Geobacillus* RPA correlated with NRS score (Figure 2).

5 | CONCLUSIONS

This is the first study to characterize the facial bacterial microbiome of twins. We took advantage of the relatively controlled nature of our study cohort and found that changes in the facial microbiome are associated with the changes in the NRS score (severity of rosacea) more so than previously identified demographic correlates. Specifically, we uncovered a positive and a negative association for *Gordonia* and *Geobacillus* with rosacea, respectively. Importantly, this was in the background of a largely unchanged microbiome landscape. This twin study can serve as a reference for future studies to confirm and pursue specific microbial associations with rosacea.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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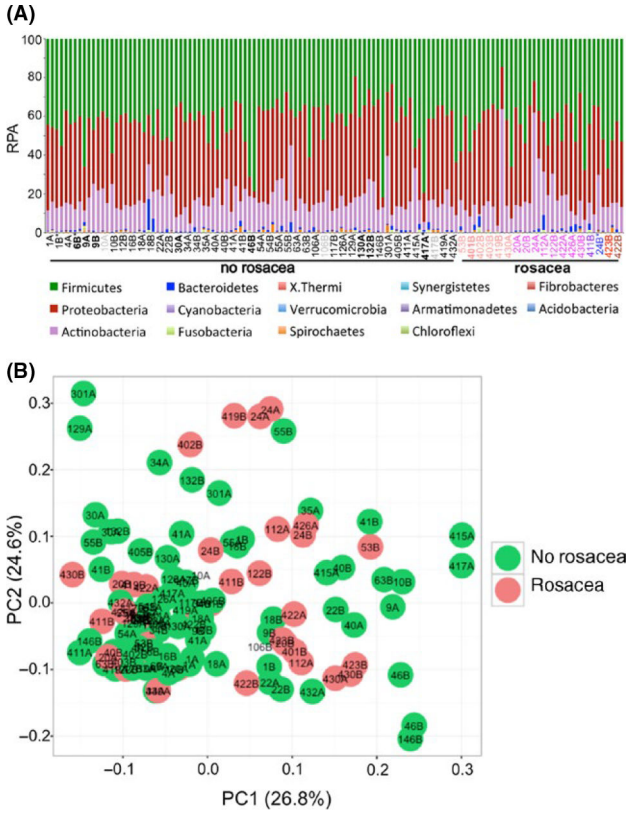

FIGURE 1.

Comparable overall facial skin bacterial microbiomein twin subjects. (A) The relative percentage abundance (RPA) atthe phylum level displayed. Phylum ordered from greatest to least abundant from top to bottom in each bar. NRS score of individual twins with rosacea: NRS score (Twin ID): 1 (53B, 401B, 402B, 403B, 419B, 430A (orange)), 2 (20A, 20B, 24A, 112A, 122B, 422A, 426A, 430B (pink)), 3 (411B (violet)), 4 (24B (blue)), 6 (423B (red)) and 9 (422B (brown)). Twin numbers assigned in the order of recruitment with “A” being used per convention for the firstborn of the twin pair versus “B” for the second born twin. Two bars are displayed for each twin representing the composition of left and right cheeks, respectively. Twin IDs in bold font are fraternal (dizygotic) twins and in grey (10A, 106B, and 417B) did not have a validated NRS score (disease status) and thus were excluded from all further analysis. *Individuals used antibiotic within last 3 months. (B) Principal coordinates analysis (PCoA) based on weighted UniFrac distance of twin subjects with (n = 18) and without (n = 42) rosacea. Twin IDs (10A, 106B, and 417B) without a validated NRS score (disease status) shown without a filled circle

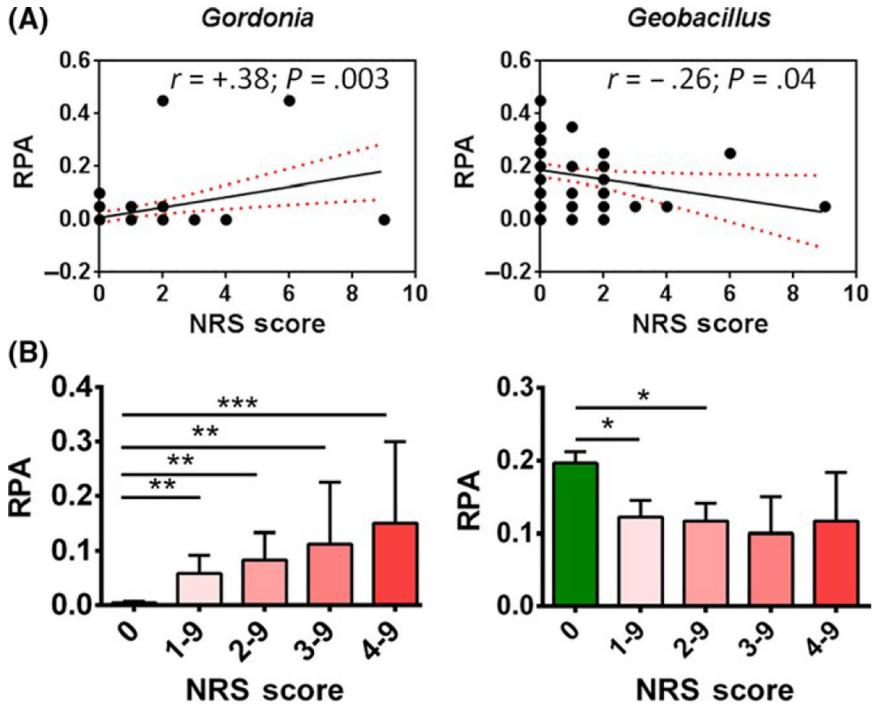

FIGURE 2.

Facial skin bacterial microbiome correlates with NRS score. (A) The positive and negative correlation of relative percentage abundance (RPA; closed dots) of bacterial microbiome genera (*Gordonia* and *Geobacillus*) with NRS score displayed. The Pearson correlation coefficient (*r*), and *p*-values, and linear regression line fitting the data (black solid line) with 95% confidence interval (red dotted line) are shown on each graph. n = 60 (n = 42 (no rosacea); n = 18 (rosacea)). There are several closed dots that overlap with each other. Therefore, the number of total dots appears less than total number of sample. (B) Relative percentage abundance (RPA) of *Gordonia* and *Geobacillus* for subjects with "No Rosacea (subjects with NRS score = 0)" and "Rosacea (subjects with score NRS score = 1–9)" was plotted with increasing NRS score. The difference in mean of RPA between no rosacea and rosacea is greater with increasing NRS score. Data are shown as mean ± SEM of n = 42 (NRS score = 0), 18 (NRS score = 1–9), 12 (NRS score=2–9), 4 (NRS score=3–9) and 3 (NRS score = 4–9). *p*-values for no rosacea vs. rosacea are shown as *<.05; **<.001; ***<. 0001. NRS, National Rosacea Society measured for severity of rosacea