

## SUPPLEMENT

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

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### eMethods

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.

#### Study Design and Questionnaire

Participants were recruited from attendees of the annual Twinsburg Festival in Twinsburg Ohio during August 5-6 2017. Individuals ages 18 and older were evaluated for rosacea by a board-certified dermatologist prior to completing a survey with the aid of trained staff members as needed. Survey questions included basic demographic information (e.g. age, sex, weight, height, twin type, and birth order), Fitzpatrick score, information about their rosacea (e.g. duration, symptoms, previously used treatment modalities), average alcohol consumption, as well as other information about factors that could potentially alter the microbiome (e.g.

consumption of probiotics, and past and present pet ownership, use of over the counter skin care products).

#### Microbiome Sample Acquisition and Analysis

Participants were given verbal and visual instructions on how to use the Diversigen/DNA Genentech facial and fecal sample collection kits to obtain facial and fecal samples. To collect facial samples, participants were instructed to apply a provided sterile saline solution to the DNA genetek swab and swab affected, symptomatic areas for 30 seconds before storing the swab in a buffer medium. To collect fecal samples, participants were instructed to use a provided toilet accessory to collect their fecal sample free of urine or toilet water. Sample was collected with a provided spatula and stored in buffer medium. After collection samples were stored at -80.0 °C or on dry ice en route to the DNA genetek laboratory for analysis.

16S sequences are clustered into Operational Taxonomic Units (OTUs) at a similarity cutoff value of 97% using the

UPARSE algorithm. OTUs were mapped to an optimized version of the SILVA and UNITE Databases containing only the 16S region to determine taxonomies. Abundances were recovered by mapping the demultiplexed reads to the UPARSE OTUs. A custom script constructed a rarefied OTU table from the output files generated in the previous two steps for downstream analyses of alpha-diversity, beta-diversity, and phylogenetic trends. An R software suite combining publicly available packages (i.e. APE and VEGAN) and purpose written code is used to import sample data and identify trends in taxa abundance, alpha-diversity, and beta-diversity with sample metadata. Significance of categorical variables are determined using the non-parametric Mann-Whitney test for two category comparisons or the Kruskal-Wallis test when comparing three or more categories. Correlation between two continuous variables is determined with linear regression models, where p-values indicate the probability that the slope of the regression line is zero. PCoA plots employ the 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.

## **Investigator Global Assessment and Lesion Count**

A board-certified dermatologist graded each participant's rosacea, using the standard 5-point Investigator Global Assessment (IGA) scale [(1) clear, 2) near clear, 3) mild, 4) moderate, or 4) severe], performed a lesion count of inflammatory lesions, identified the Clinicians Erythema Assessment (CEA) 4-point scale [none, mild, moderate, severe] and examined for the presence of telangiectasias, rhinophyma, and ocular manifestations. For consistency and reproducibility, the same dermatologist

performed all counts on all patients. Participants were also surveyed about symptoms (e.g. gritty feeling or burning in their eyes; flushing, blushing; stinging, burning; changes in nose size and appearance).