THE CINCO DE MAYO STUDY

Temporal Changes in the Facial Skin Microbiome -

A One-Year Longitudinal Study in Normal Healthy Men and Women

Kausar Malik
Amway Corporation, Ada, Michigan
October 29, 2018

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PRESENTATION OUTLINE

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- Background and Objectives
- Study Design and Methods
- Findings
- Conclusion

BACKGROUND -

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RESIDENT MICROFLORA

Predominant Microflora		Skin Physiology	Microbial Diversity and Stability
Phylum	Genus		
Actinobacteria	*Propionibacteria*	Sebaceous	Low Microbial Diversity High Stability
Actinobacteria	*Corynebacteria*	Moist	High Microbial Diversity Low stability
Firmicutes	*Staphylococci*		
Proteobacteria	*Pseudomonas*	Dry	

Depending on the skin conditions (sebaceous, moist or dry) - Different bacterial species harbor different parts of a human body

HUMAN MICROBIOTA FUNCTION

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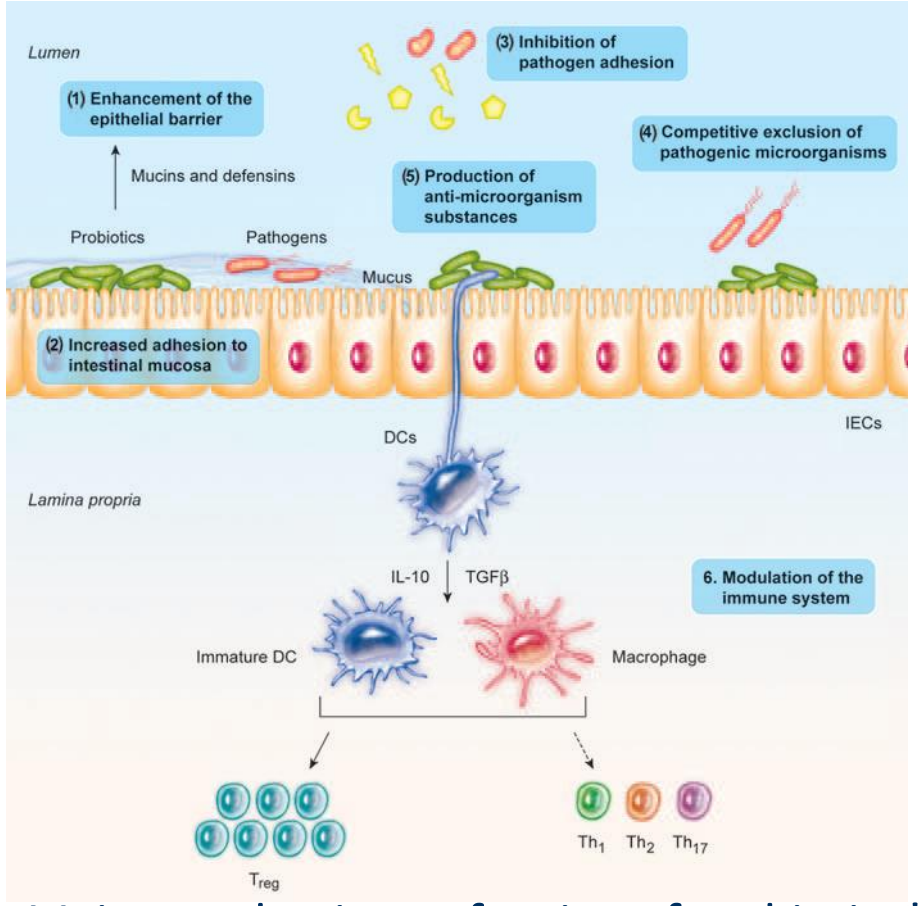
Skin Commensals act as Immunomodulators

- **Educate Prime Adaptive Immunity –**
  - Tune local cytokine production
  - Educate adaptive immunity
  - Influence regulatory T-cells
- **Enhance host innate immunity –**
  - Increase AMP production
  - Reduce inflammation after injury
  - Strengthen epidermal barrier
- **Directly inhibit pathogen growth –**
  - Competition -Occupy space and nutrients
  - Inhibition- Produce AMPs
  - Antagonism - Prevent *S. aureus* biofilm formation



Major mechanisms of action of probiotics*

***Probiotic Mechanisms of Action** - Miriam Bermudez-Brito et al. Ann Nutr Metab 2012; 61:160–174

BACKGROUND

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Flores *et al. Genome Biology* 2014, **15**:531
http://genomebiology.com/2014/15/12/531

Genome Biology

RESEARCH Open Access

Temporal variability is a personalized feature of the human microbiome

Gilberto E Flores[1*†], J Gregory Caporaso[2,3†], Jessica B Henley[4], Jai Ram Rideout[3,5], Daniel Domogala[2], John Chase[2], Jonathan W Leff[4,6], Yoshiki Vázquez-Baeza[7], Antonio Gonzalez[8], Rob Knight[8,9,10], Robert R Dunn[11] and Noah Fierer[4,6*]

Cell

Article

Temporal Stability of the Human Skin Microbiome

Julia Oh,[1,5,7] Allyson L. Byrd,[1,2,5] Morgan Park,[3] NISC Comparative Sequencing Program,[3] Heidi H. Kong,[4,6,*] and Julia A. Segre[1,6,*]
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http://dx.doi.org/10.1016/j.cell.2016.04.008

Variability in community composition among healthy individuals exceeds the variability within individuals over time:

- "Inter-individual" variability is higher than "Intra-Individual"

Despite the skin's exposure to the external environment,

- Microbial communities are largely stable over time

MICROBIOME STUDY OBECTIVES

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1. Measure the “Stability of Skin Microbiome” over several years in a large population of healthy adults.

2. Measure the association of “Skin Microbiome Diversity and Composition” with facial skin condition using biophysical and imaging methods.

3. Determine if the skin microbiome composition co-varies with important signs of skin health and beauty to develop microbiome-based treatment interventions.

Goal – Target Scientifically Sound Technology in Amway Products

CINCO DE MAYO - LONGITUDINAL COHORT

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Cinco de Mayo Cohort

- ~150 Amway employee volunteers
- Skin measurements annually in May since 2014
- Apples vs apples: Same staff, instruments, etc..
- Added skin microbiome in 2017 and 2018

Note: Most women used make-up between two sampling points for microbiome

CINCO DE MAYO – LONGITUDINAL STUDY

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Conducted in 2017 and 2018 -

- On 150 Normal Healthy Men and Women

Measured -

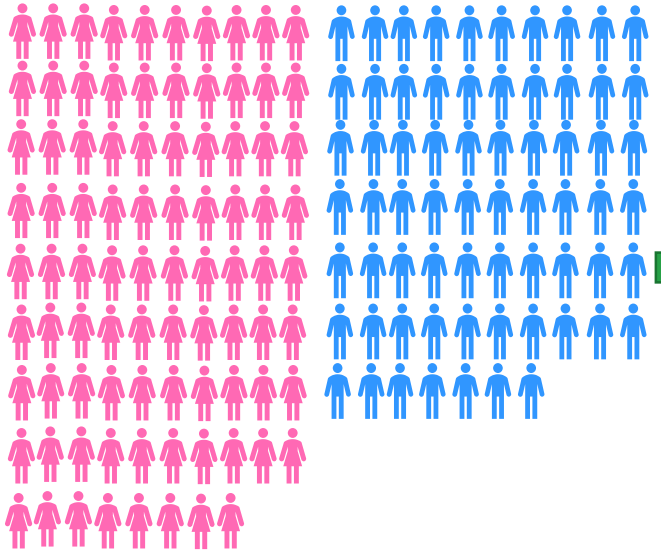
- Biophysical Skin Properties -
  - Barrier Function, Skin Surface pH and Elasticity,
- Visible Facial Features -
  - Wrinkles, Hyperpigmentation, Porphyrins, Changes in Skin Health
- Microbiome -
  - Changes in Skin Microbiome Composition and Diversity

The study is designed to collect data over a 10-year period to identify the visual signs of skin aging, and wrinkles and shift in natural skin microflora composition.

2017 AND 2018 COHORTS

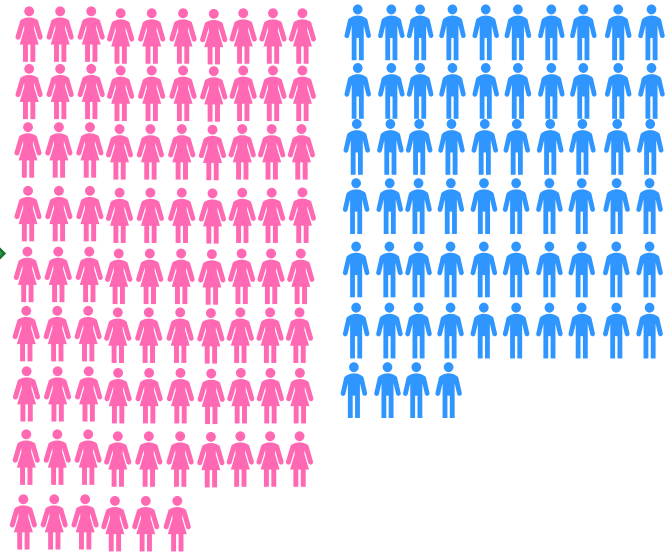
Longitudinal Study Subjects

May, 2017
n=155

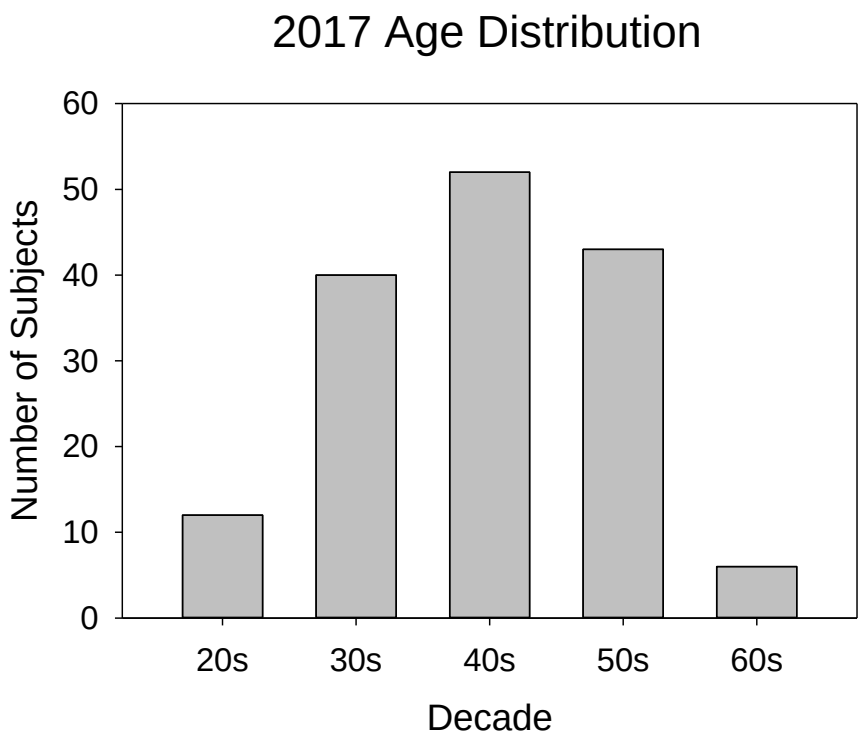


n=88 n=67

May, 2018
n=150



n=86 n=64



90% Caucasian
Age 26-61

90% Caucasian
Age 27-62

90% at Age = 30s - 50s

STUDY FLOW

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Individual Consent → Microbiome → pH → Face Cleansing

Height/Weight → Elasticity → Imaging → Questionnaire Equilibrate 20’

Barrier Function → Exit

Microbiome Samples Requirement
Dirty Face –

- No face washing
- No Make Up

FACIAL MEASUREMENTS - METHODS

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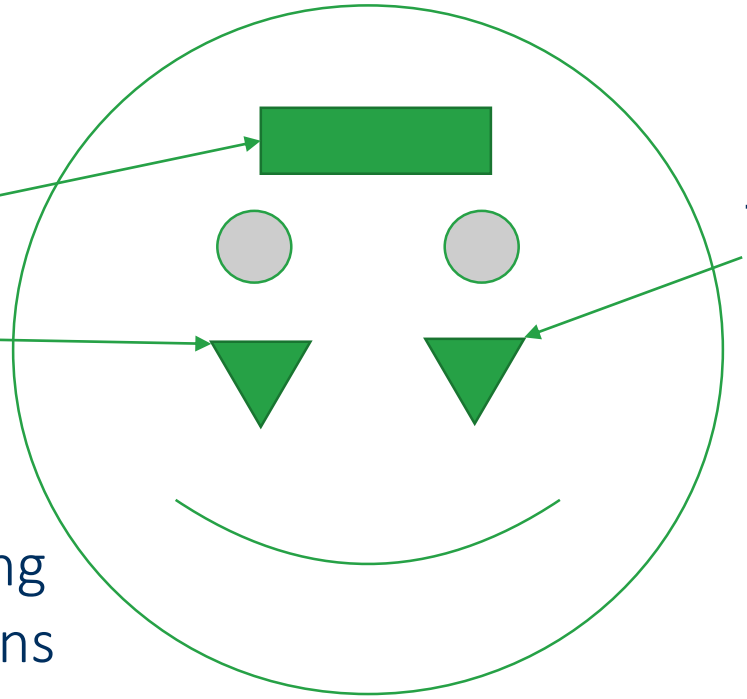
Facial Swabbing

Skin Microbiome

- Forehead
- Left+Right Cheeks
- Buffer-wetted Sterile Swabs
- 16S Amplicon Sequencing
  → **V4** and **V1-V3** Regions



Facial Features

- Left/Right Cheeks and Forehead
- Standardized Imaging and Image Analysis
- Canfield Scientific **VISIA CR** and **VAESTRO**

Region of Interest (ROI) – Exact Same

FACIAL MEASUREMENTS

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Skin Feature Detection and Quantitation

Wrinkles
Visible Spots
Brown Spots
Red Features
Porphyrins
Pores

Standard 1 for Wrinkles and Pores –

- Multiple Lighting Modalities

Ghost Repositioning for 2017 and 2018 image analysis –

- Locked image location for each subject to get the image at the same location each year

FACIAL MEASUREMENTS – Contd.

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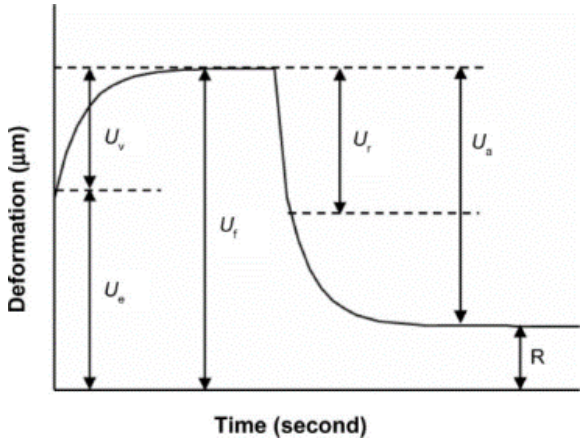
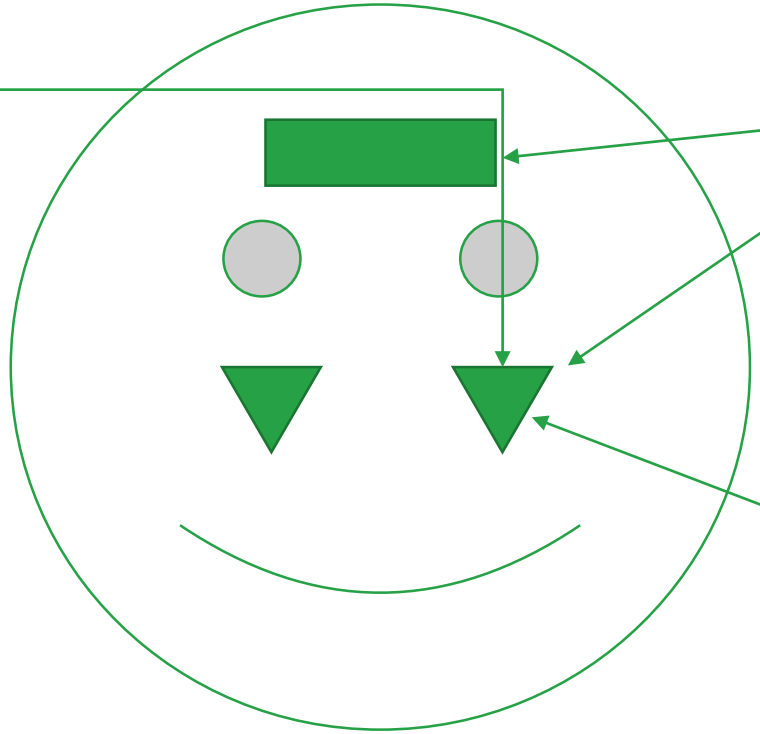
Skin Elasticity

Left Cheek
Suction Method
C&K Cutometer
N=3 per site.





Stratum Corneum Barrier Function

Left Cheek and Forehead
Transepidermal Water Loss
Delfin Vapometer
N=3 per site.

Surface pH (2018 only)

Left Cheek
pH meter - (Mettler Toledo flat probe)
N=3 per site

Facial Microbiome Composition and Diversity

DATA -FACIAL COMMUNITY COMPOSITION

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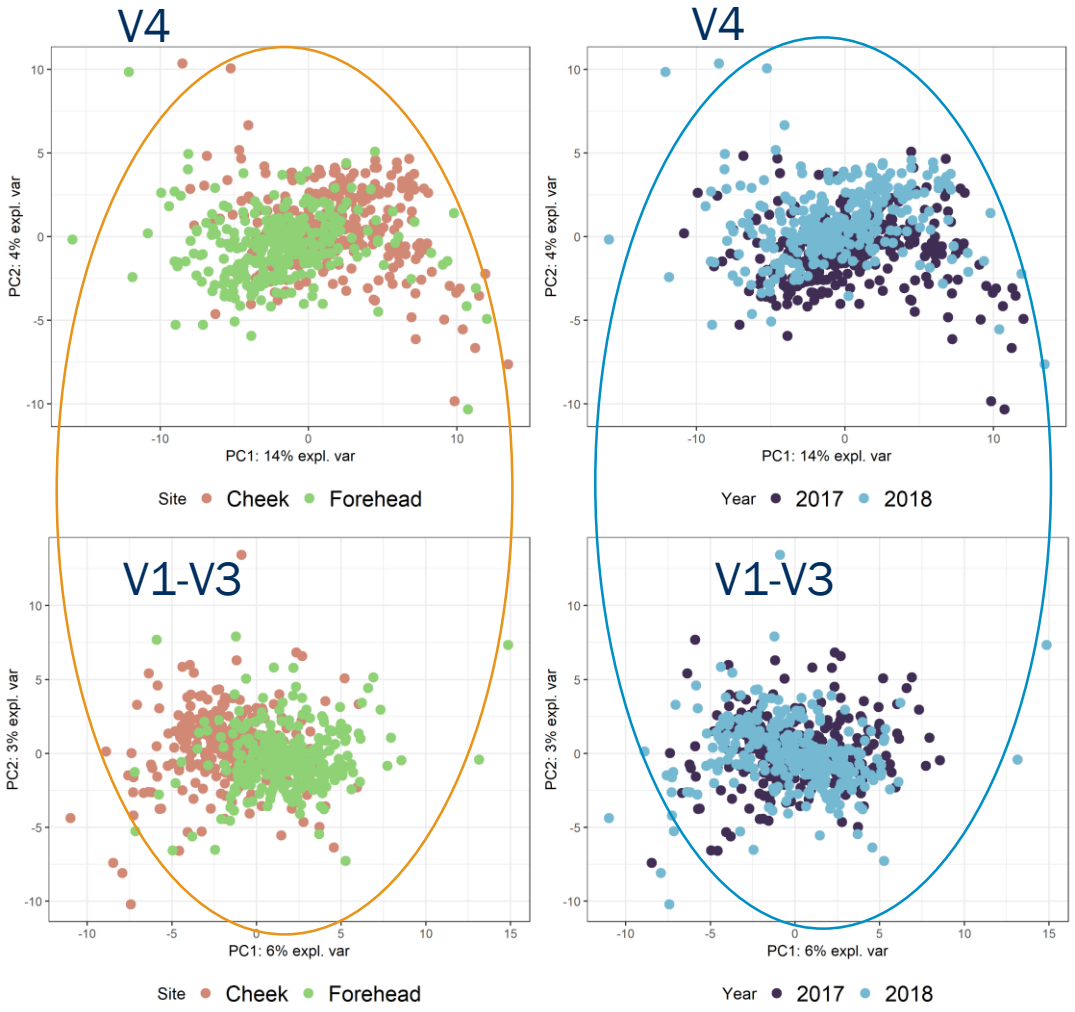
Cheeks and Forehead Microbiome Composition using **V4 and V1-V3 Primers**

Relatively moderate differentiation between the cheek and forehead microbiomes.



2017 vs. 2018
V4 vs. V1-V3 Composition

Insignificant differentiation between 2017 and 2018.

No difference between V4 vs. V1-V3 Primers

CHANGE IN ALPHA DIVERSITY OVER 1 YEAR

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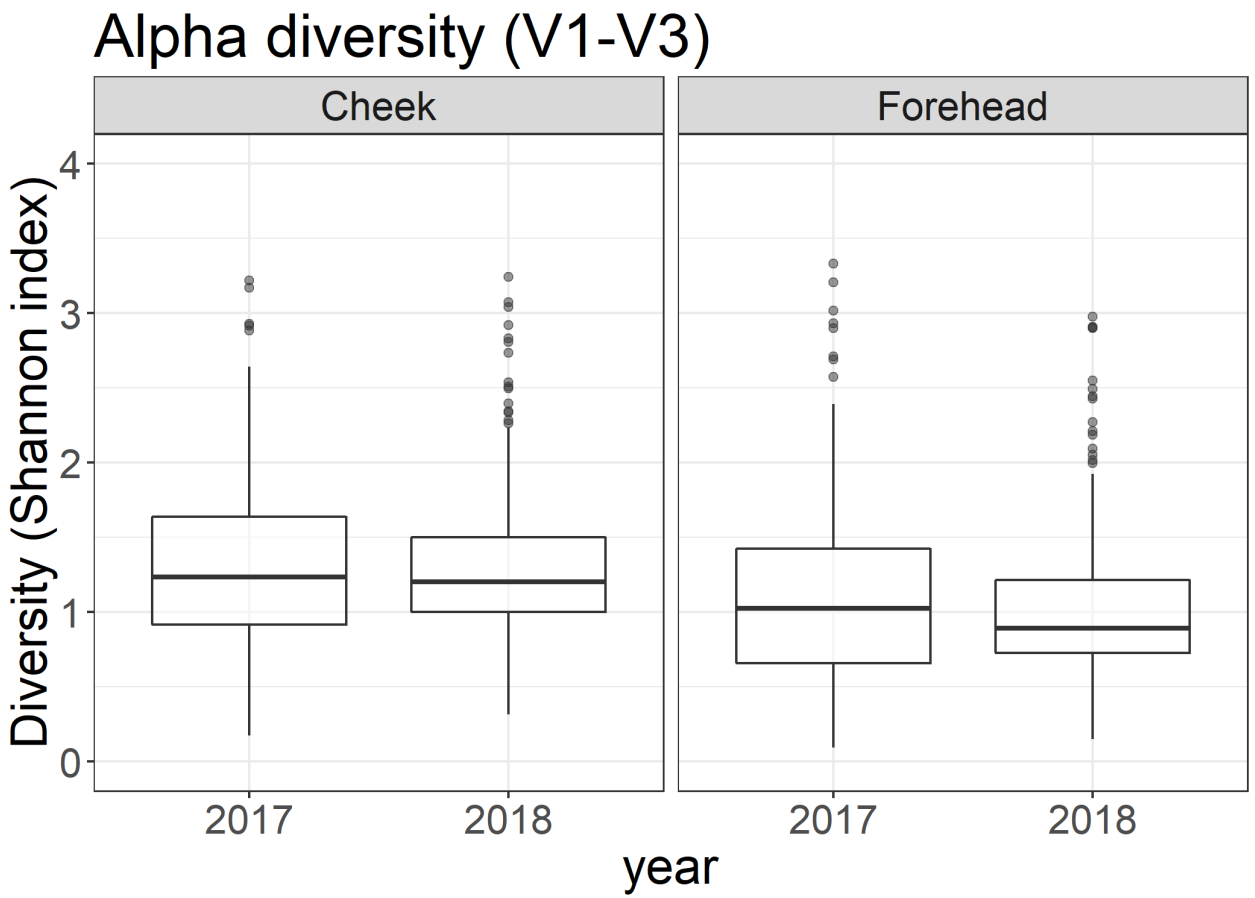
Alpha diversity – Diversity within a single microbial ecosystem –

Shannon Index –

- No significant change in alpha diversity over the one-year (slightly lower in 2018)



Similar results were noted with V4 Primer.

STABILITY OF TEMPORAL DIVERSITY

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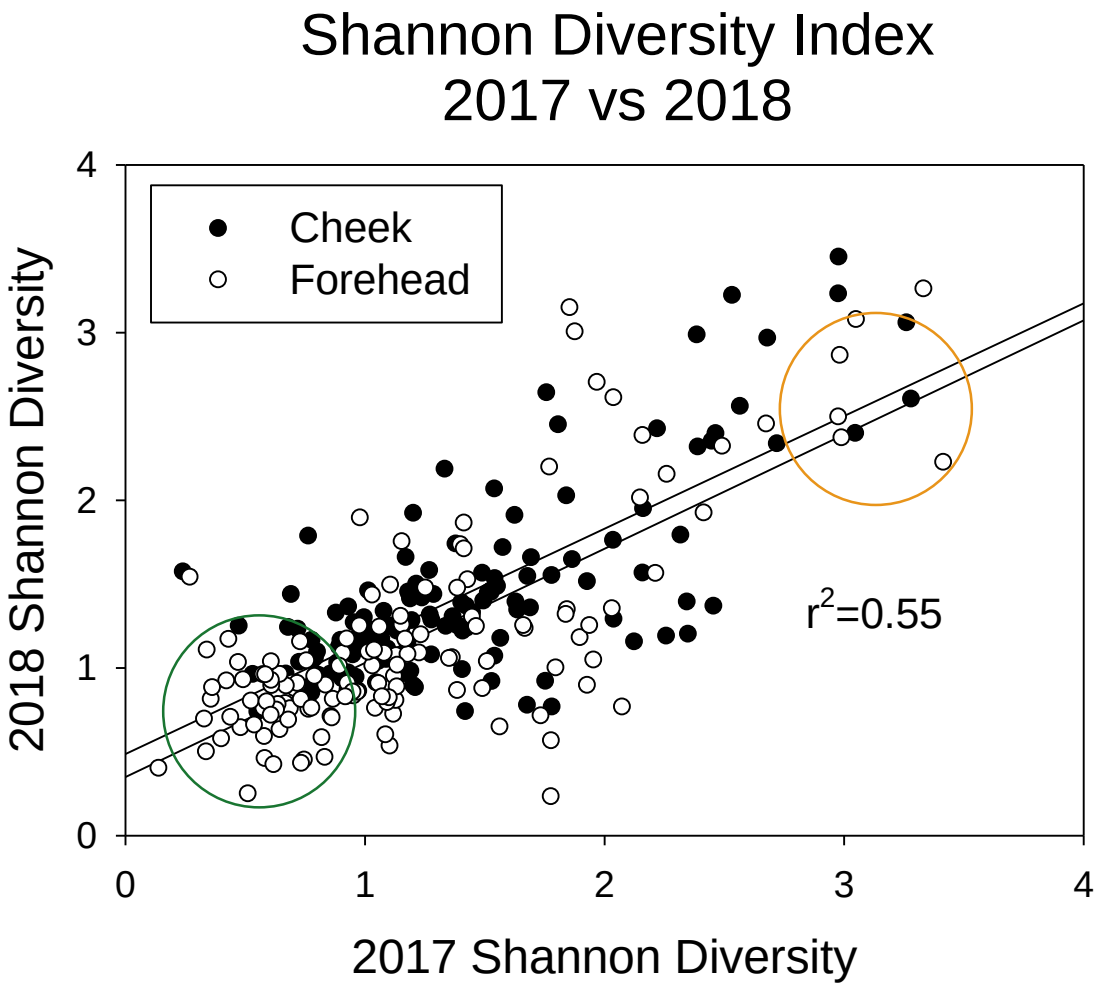
Within Individual – Microbiome diversity remains stable over time from 2017 and 2018

- In general, subjects with high (or low) diversity in 2017 maintained their high (or low) diversity over the one-year.



Within Individual - Temporal Diversity is Stable

CHANGE IN MICROBIOME DIVERSITY

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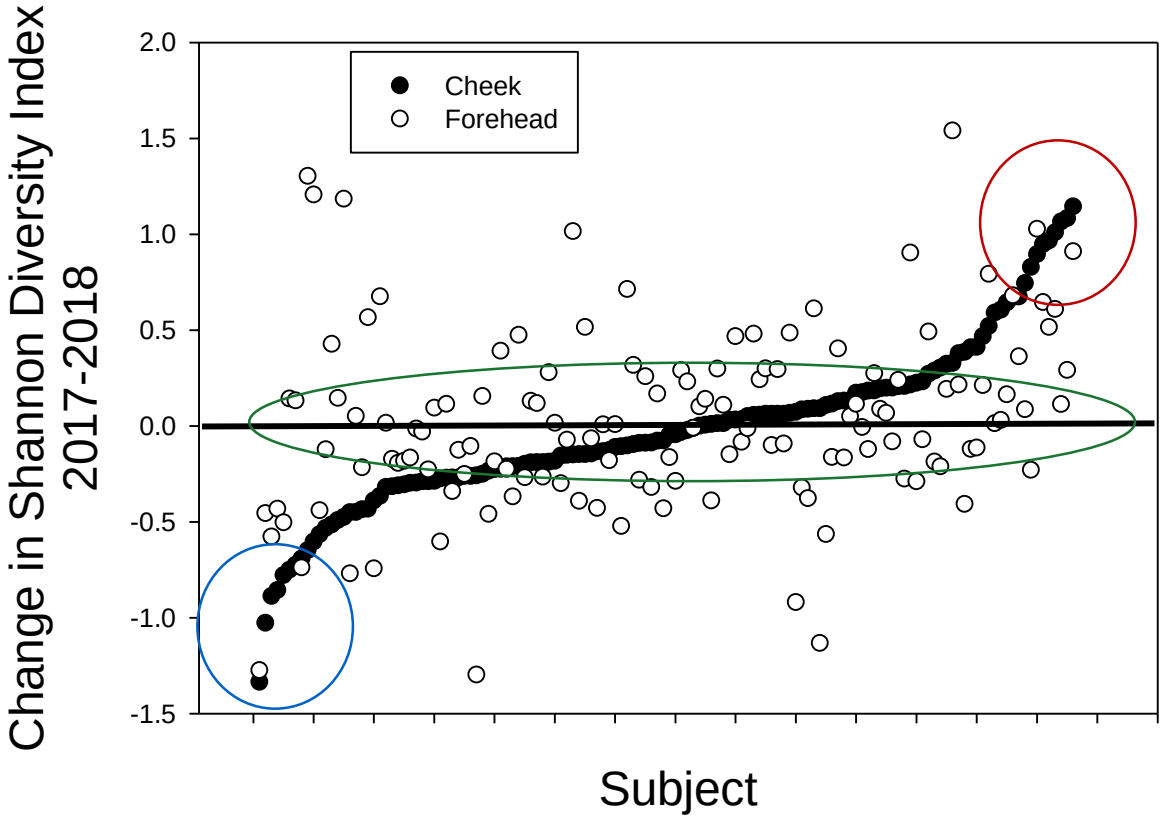
Change in Cheek Diversity (●) = Diversity in 2017 – Diversity in 2018

- Most subject's showed no change in diversity from 2017 to 2018.
- A few subjects showed significant increase or decrease in diversity from 2017 to 2018.

Subjects rank-ordered based on change in cheek diversity (●)



COMMUNITY DISSIMILARITY

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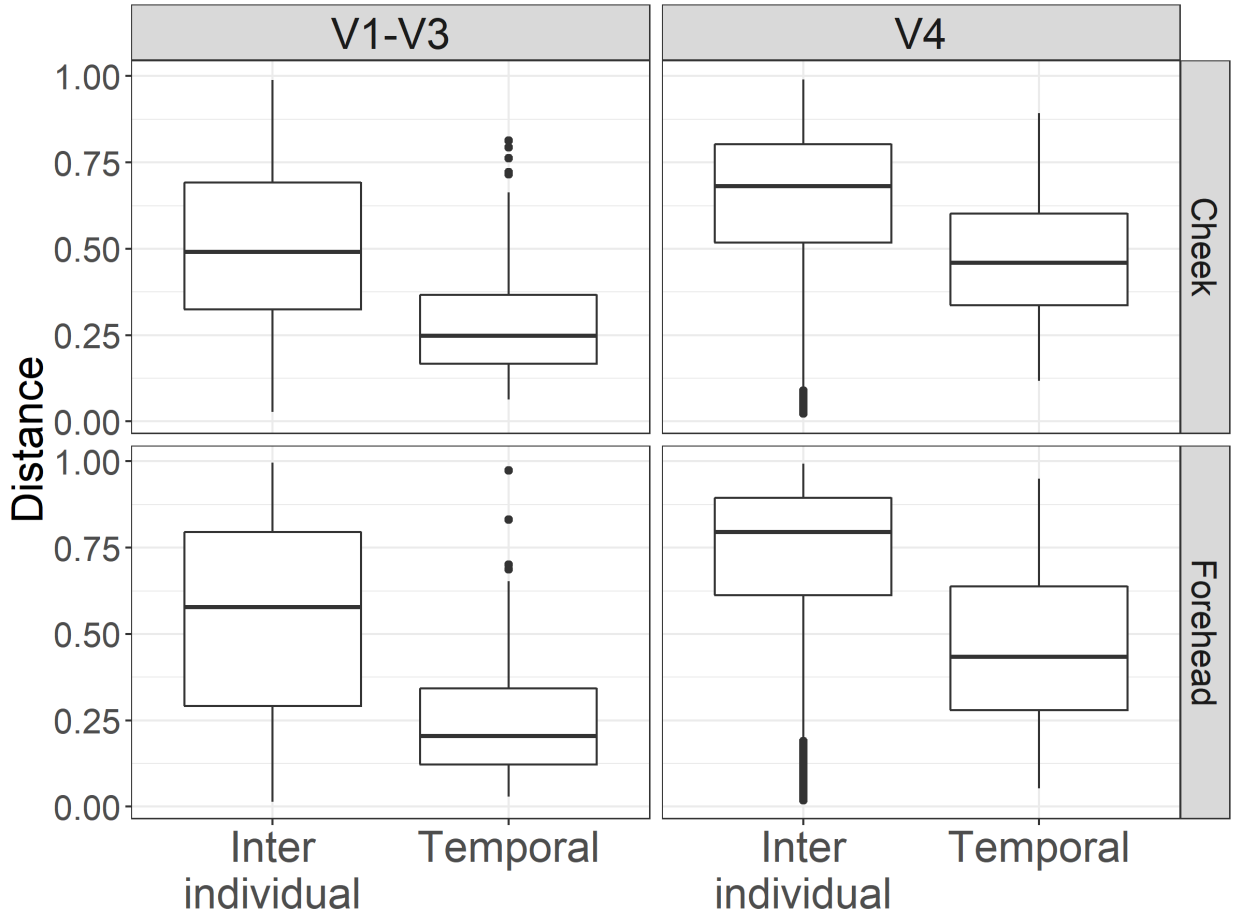
Beta Diversity - Variability

Microbiome dissimilarity between subjects is greater than the dissimilarity within a subject from 2017 to 2018.

- High inter-individual dissimilarity
- High temporal stability



Distance: 0 = Identical Communities 1 = Dissimilar Communities

Inter-individual Community Dissimilarity > Intra- Individual

Facial Microbiome and Skin Parameters

MICROBIOME DIVERSITY vs. TEWL

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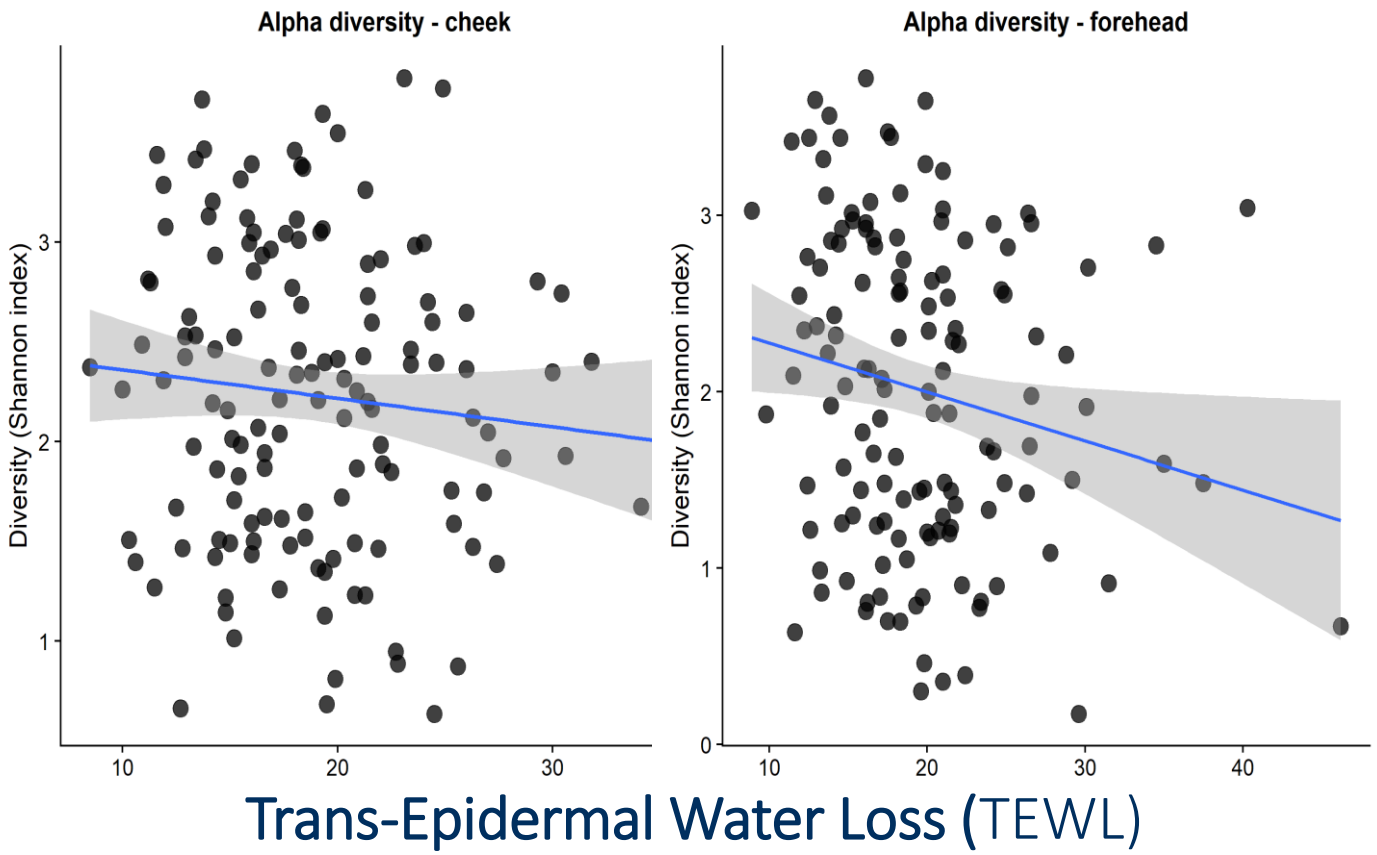
Subjects with better stratum corneum barrier function (lower TEWL) tend to show higher diversity , however, the relationship is very weak.

- Shaded areas: with 95% confidence limits.



Microbiome Diversity is weakly correlated with trans-epidermal water loss

STABILITY OF SKIN MICROBES - FOREHEAD

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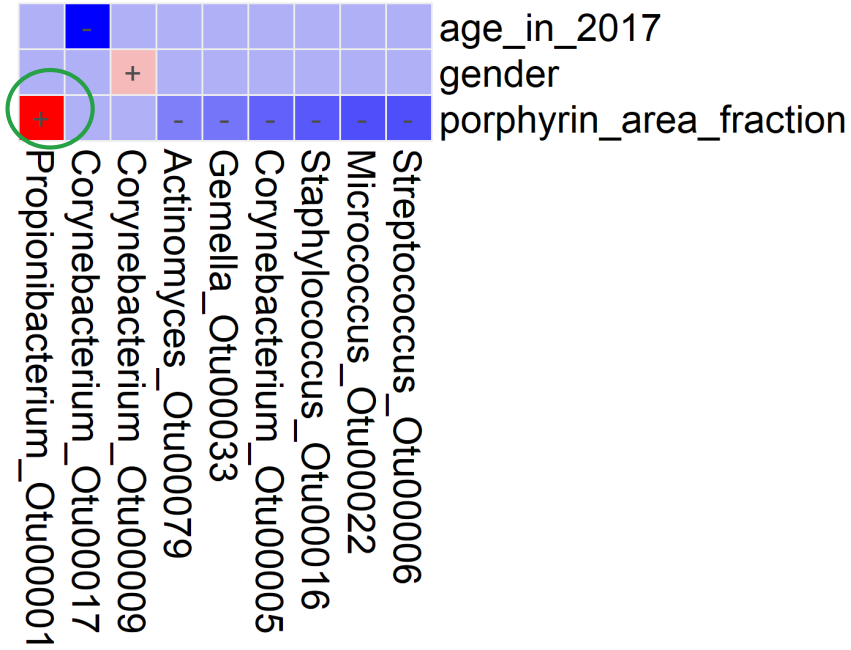
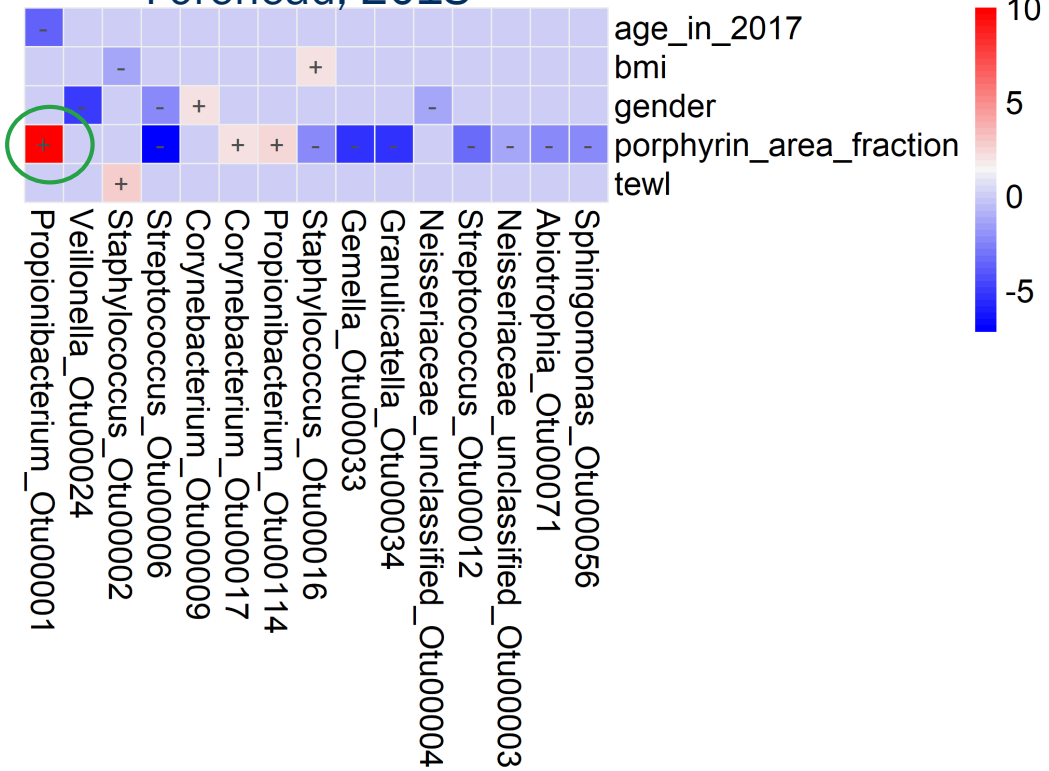
Forehead - Certain demographic and skin parameters were correlated with specific OTUs.

Forehead, 2017



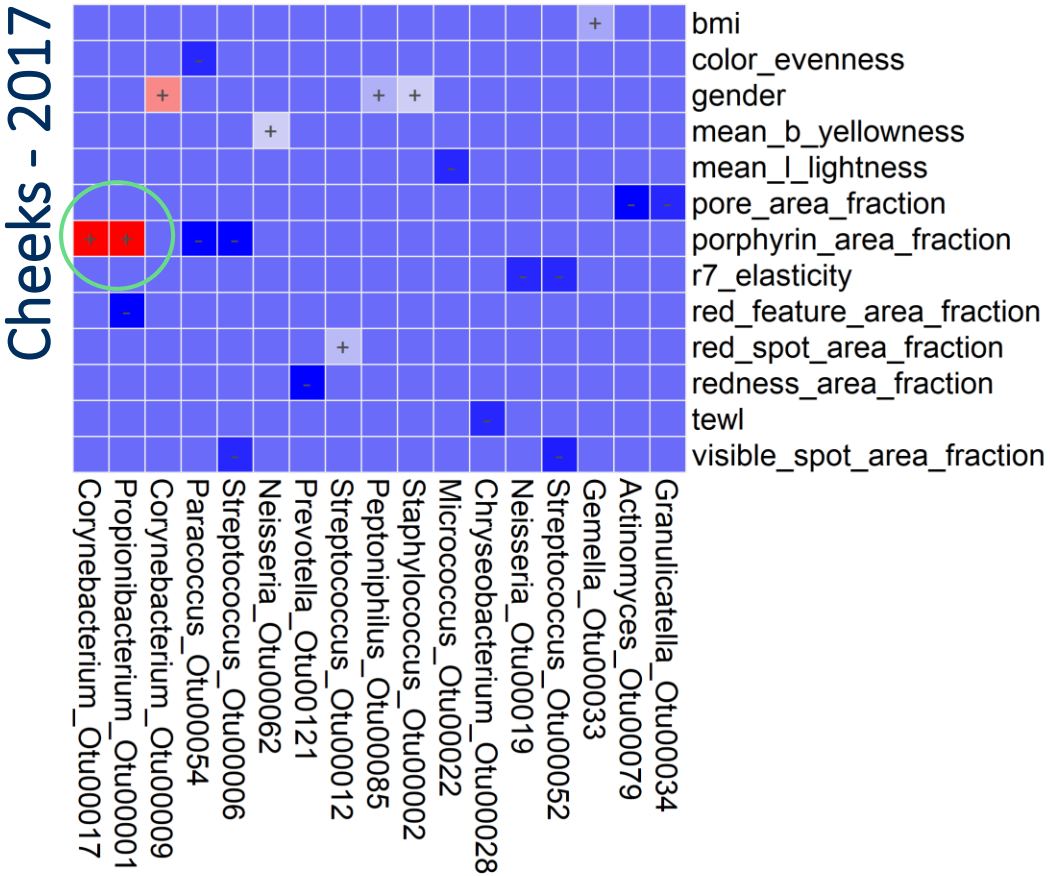
Forehead, 2018

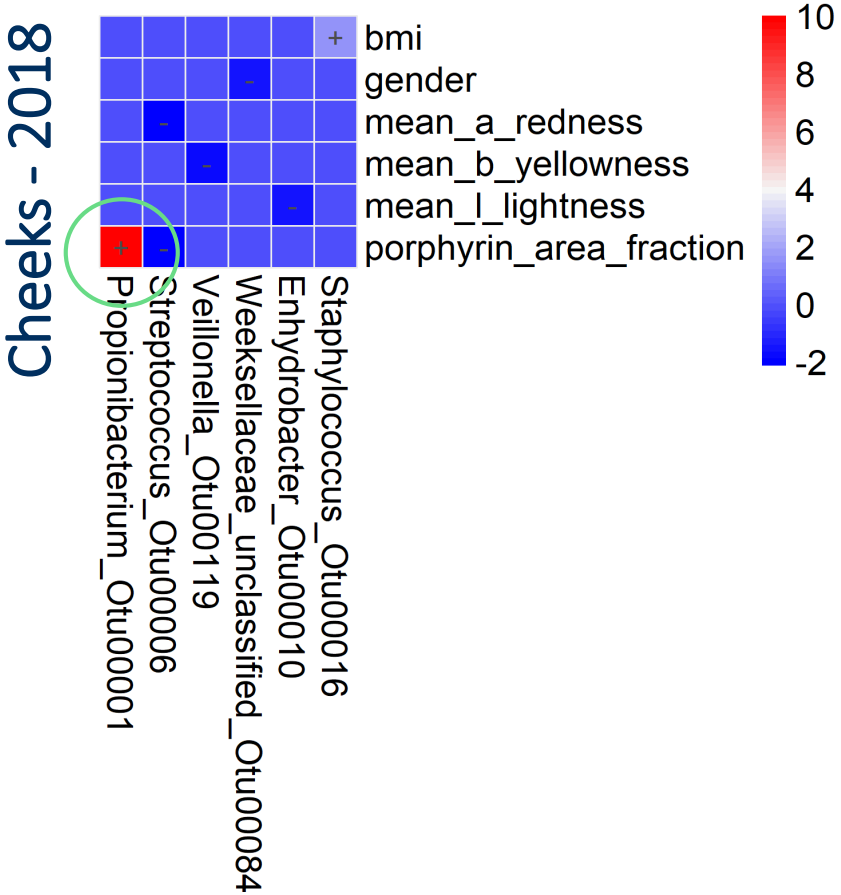


Strongest correlation was between forehead porphyrins and *Propionibacterium*

STABILITY OF SKIN MICROBES - CHEEKS

Cheeks- Stability of associations between skin parameters and microbes





CUTIBACTERIUM ACNES AND PORPHYRINS

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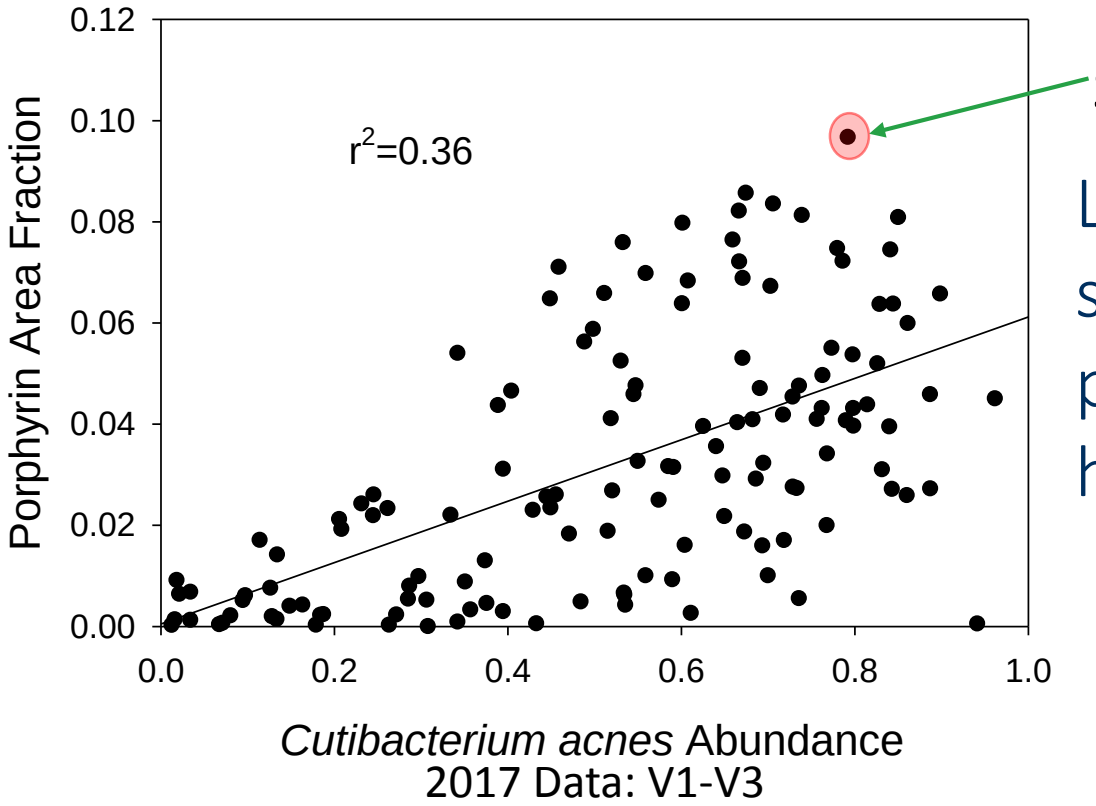
Good co-relation between *C. acnes* abundance and cheek porphyrins

Abundance of *C. acnes* seems to be necessary for having porphyrins.

Cheek *Cutibacterium acnes* vs Cheek Porphyrins



Subject #: 205

Let's look at this subject with high porphyrins and high *C. acnes*.

C. acnes Abundance - Strongly Correlated with Porphyrins

CUTIBACTERIUM ACNES AND PORPHYRINS

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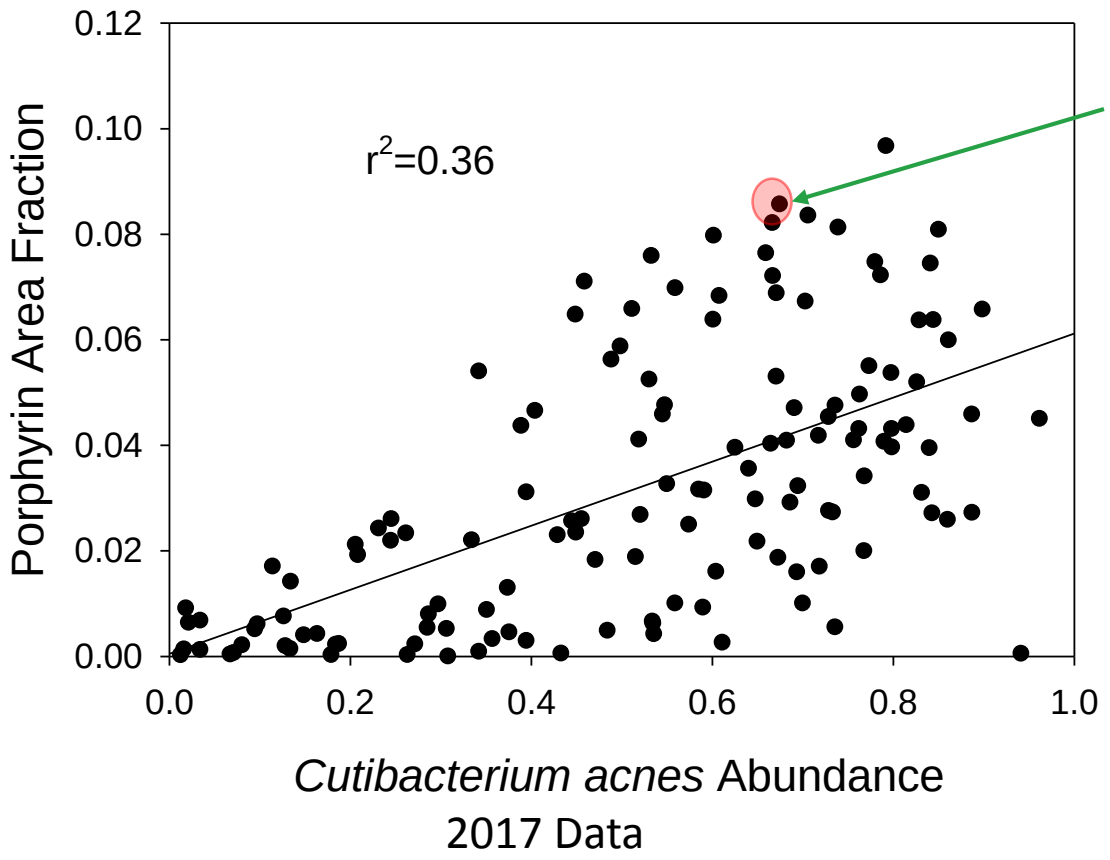
Subject #205 –
Despite very high porphyrins, visually skin showed clear complexion, healthy looking, evenness of color tone and no acne.

Imaging Analysis showed very high porphyrin fluorescence redness

CUTIBACTERIUM ACNES AND PORPHYRINS

Cheek *Cutibacterium acnes* vs Cheek Porphyrins



Another person (subject #155) with High *C. acnes*. and High porphyrins

Image Analysis Results

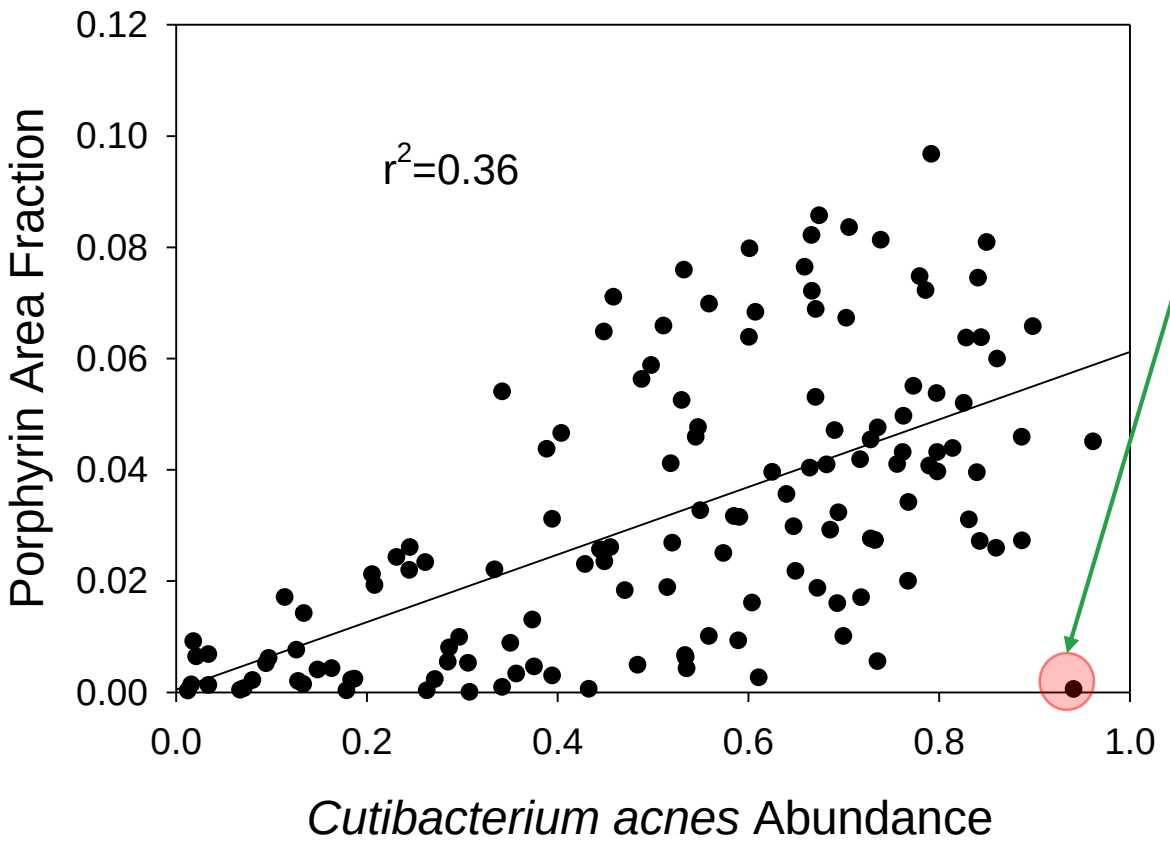
- Visually some redness
- Very high porphyrins fluorescence
- Moderate redness and red blotchiness.

CUTIBACTERIUM ACNES AND PORPHYRINS

Although high *C. acnes* seems to be necessary for having porphyrins -

Some subjects showed high *C. acnes* and low porphyrins level.

Cheek *Cutibacterium acnes* vs Cheek Porphyrins



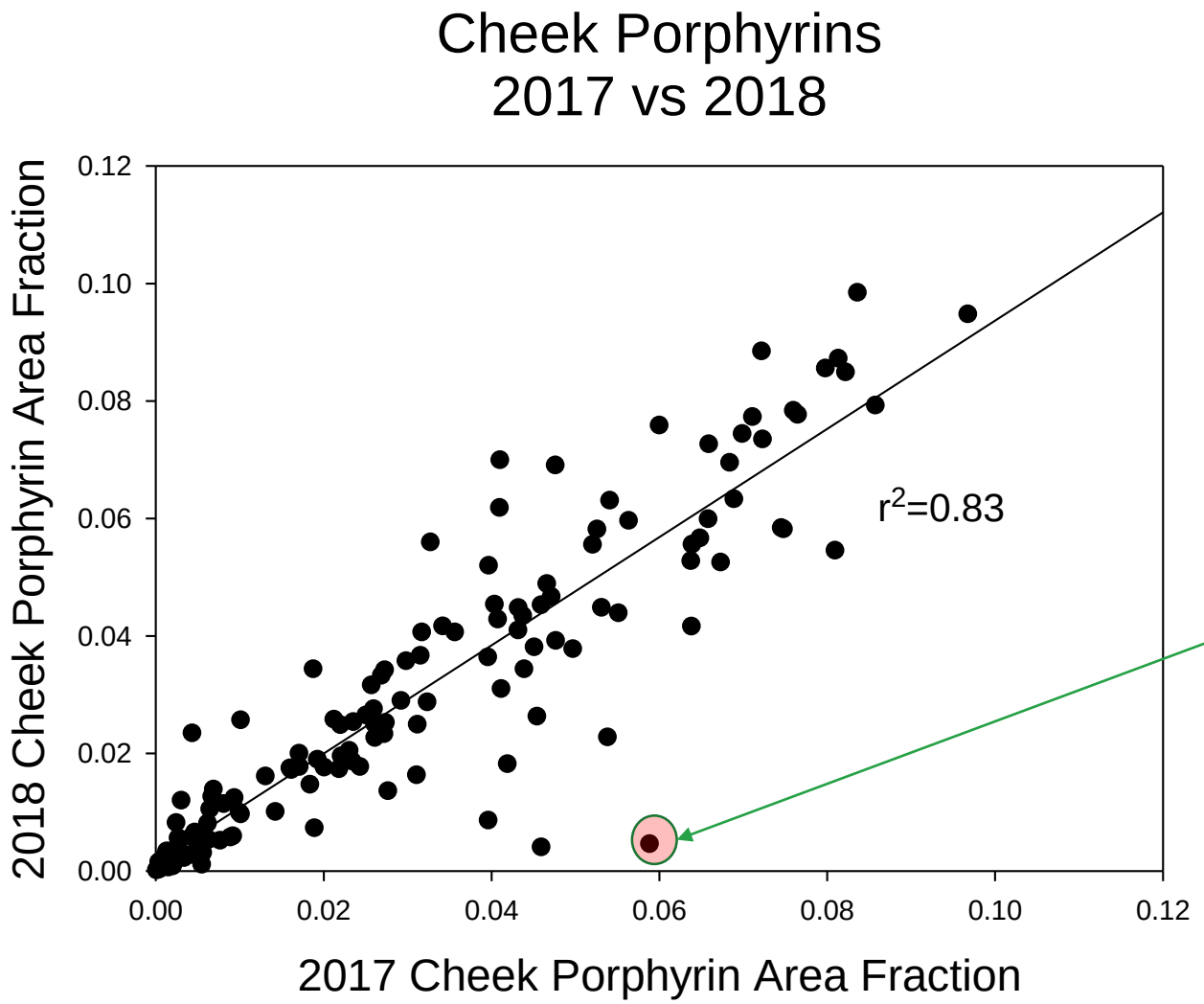
2017 Data: V1-V3

Subject #004 - with high *C. acnes* and very low porphyrins

Image Analysis

- Clear complexion
- A few Porphyrins
- No Inflammation

PORPHYRINS - TEMPORAL STABILITY



Subjects with low porphyrins in 2017 showed low porphyrins in 2018 and visa versa.

Let's look at this subject #003 with moderately high porphyrins in 2017 but very low porphyrins in 2018.

CHEEK PORPHYRIN ANALYSIS

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Visually -

- Improved Skin Health in 2018 as compared to 2017

Porphyrin Analysis Results – Decrease in Porphyrins Abundance

- 2017 - 0.026 Porphyrin Area Fraction
- 2018 - 0.004 Porphyrin Area Fraction

Skin Redness Analysis

- A marked reduction in red blotchy skin on the cheeks

PORPHYRINS AND *C. ACNES* ANALYSIS

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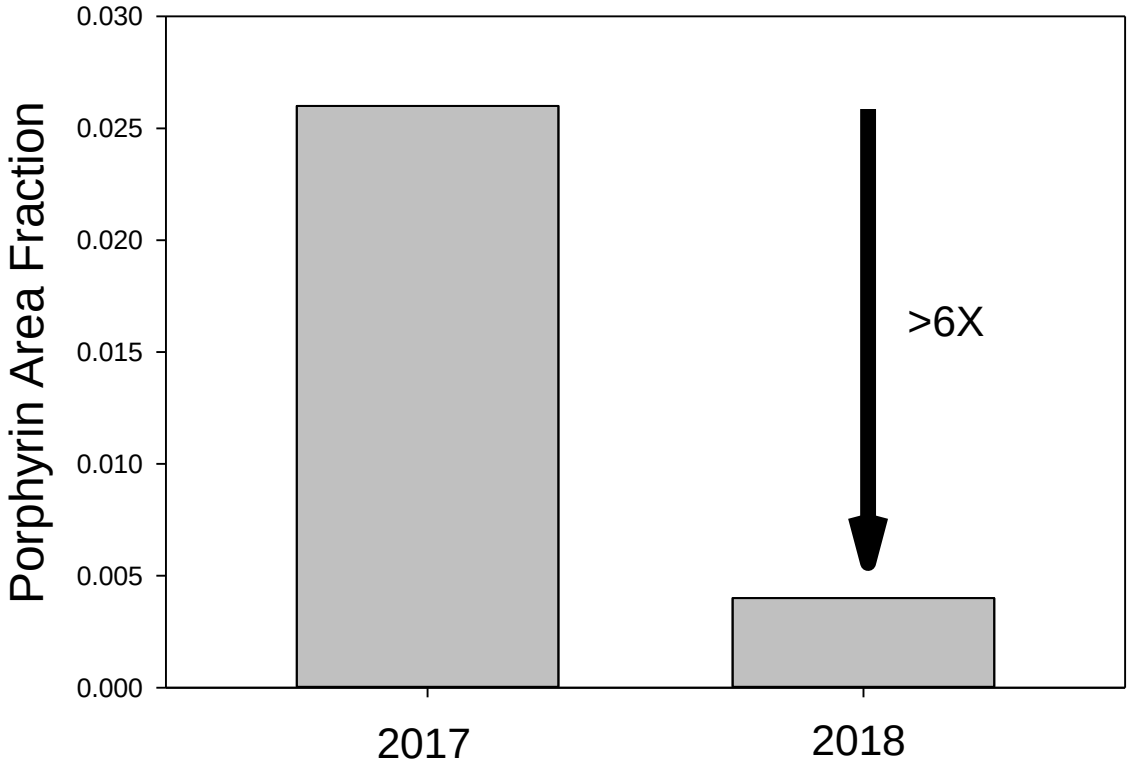
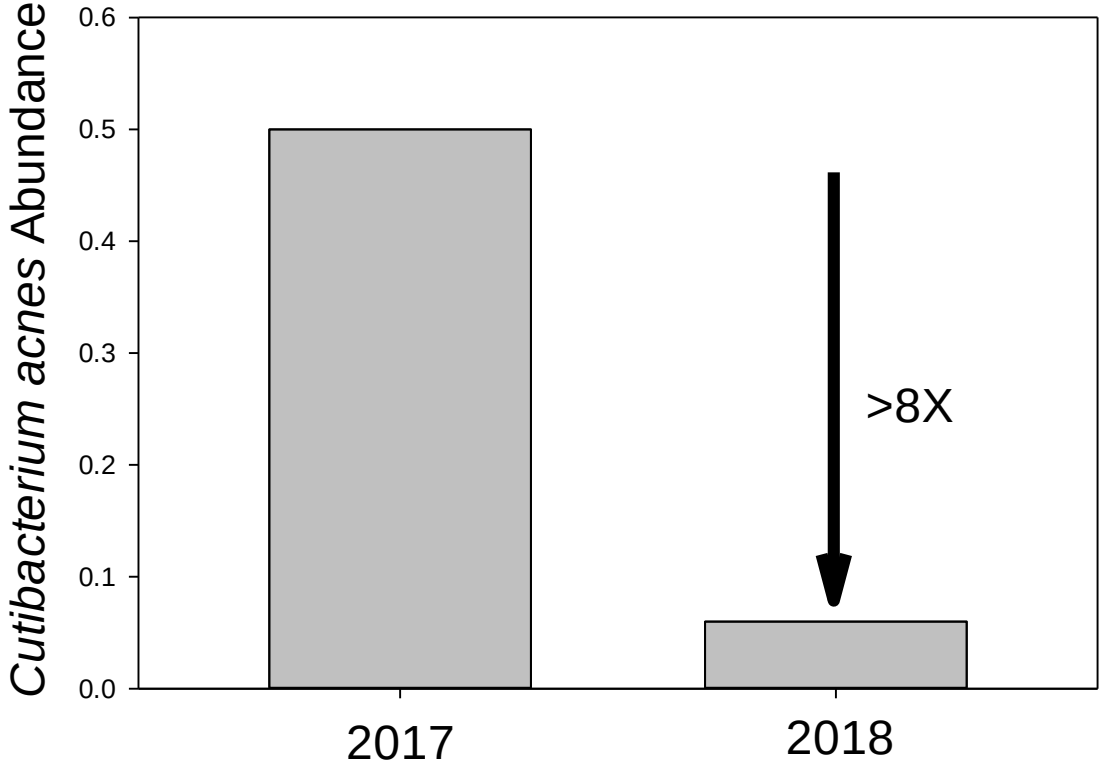
Porphyrins declined >6-fold



Decrease in *C. acnes* abundance >8-fold



A 1:1 post-study interview with this subject revealed no obvious explanation for the dramatic decrease in porphyrins or *C. acnes*.

RESULTS – DATA ANALYSIS

Corynebacterium kroppenstedtii (CK)

Age Dependent

BACKGROUND: ARTPRIZE COHORT

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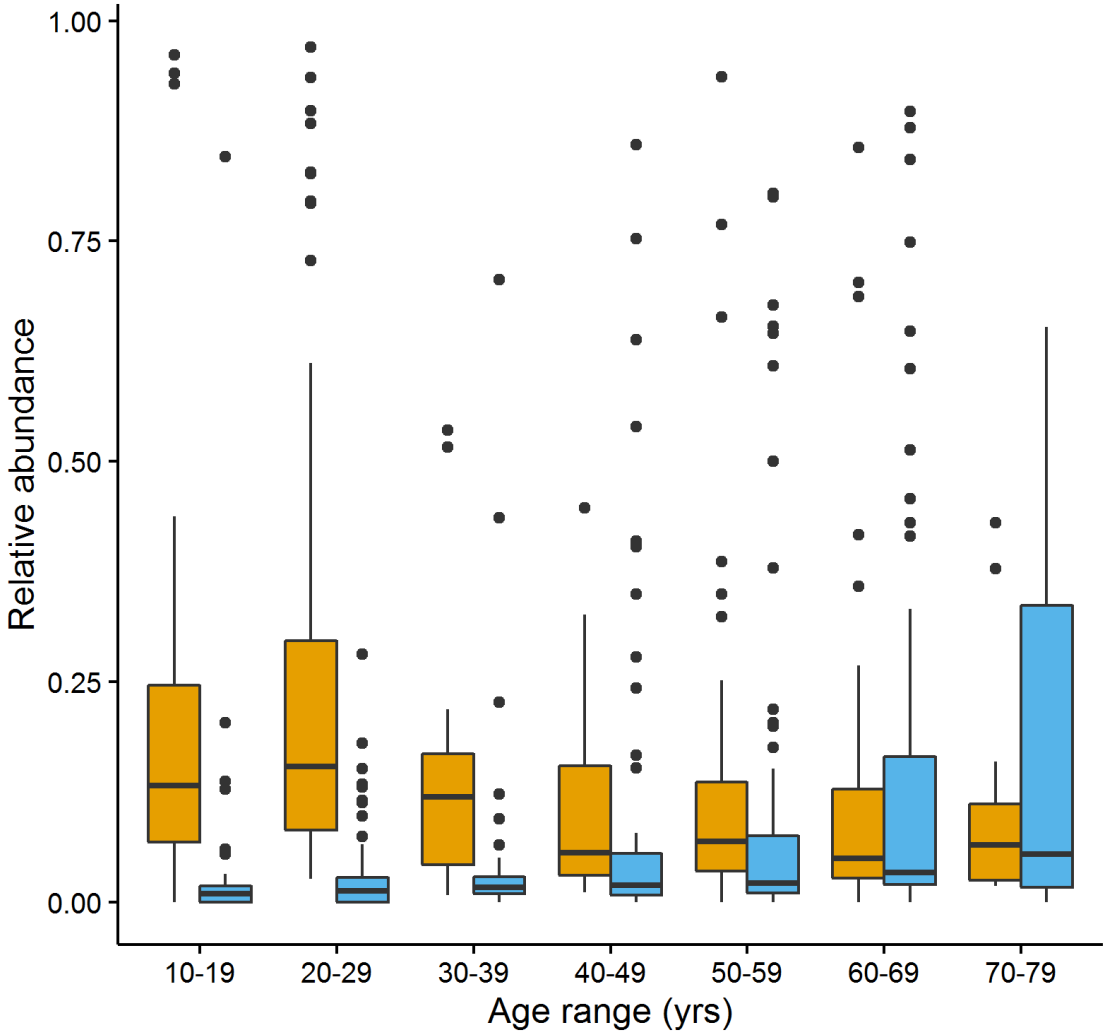
In 2015, we characterized the skin microbiomes of 500 subjects in a cross-sectional study ('ArtPrize Study'), where we found two mutually exclusive and age-dependent *Corynebacteria OTUs*.



C. Unclassified

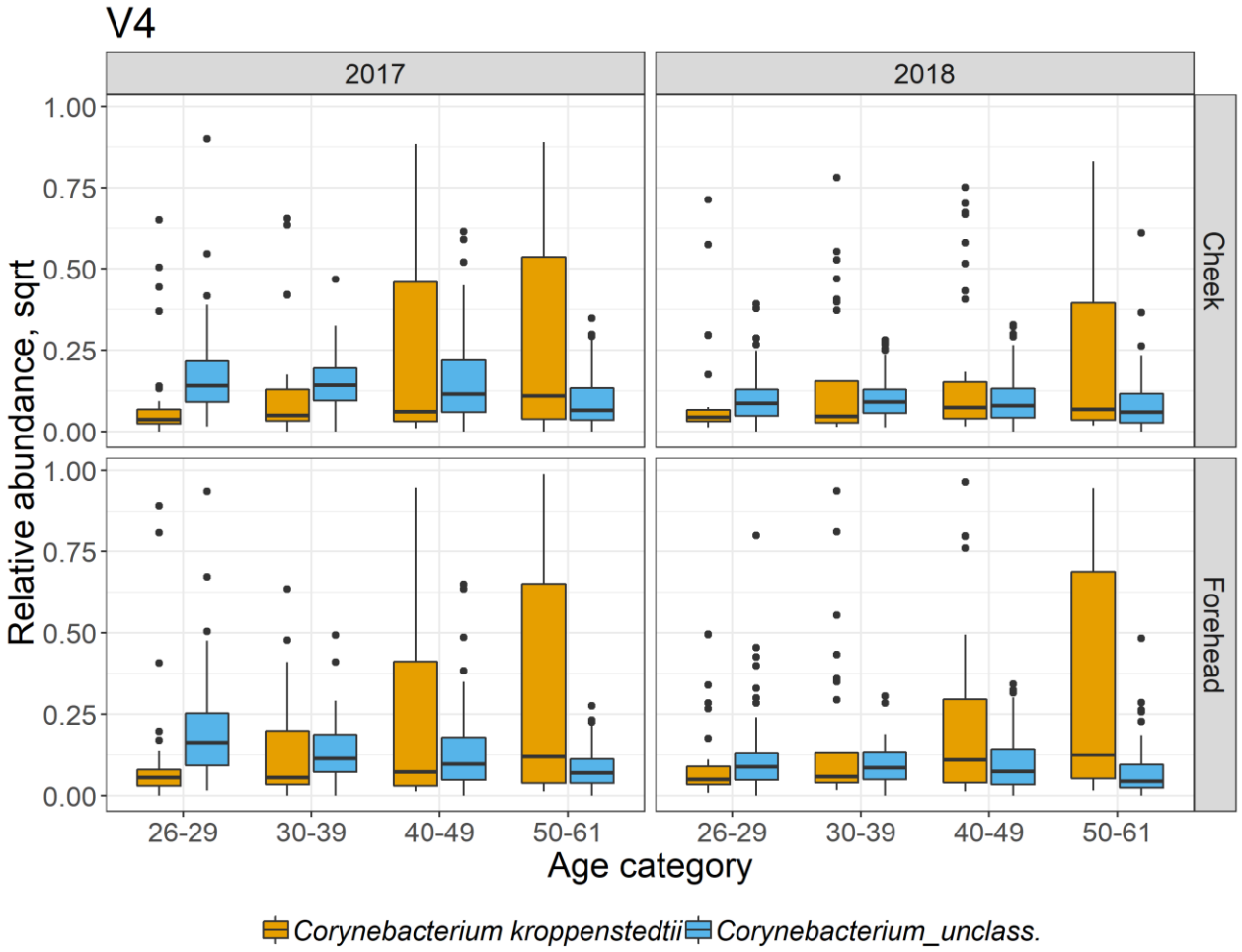
C. kroppenstedtii

AGE DEPENDENT *CORYNEBACTERIUM OTUs*

Cheek and the Forehead –
(2017 and in 2018 using V4 Primer)

With age, there is a shift in abundance of Corynebacteria spp.

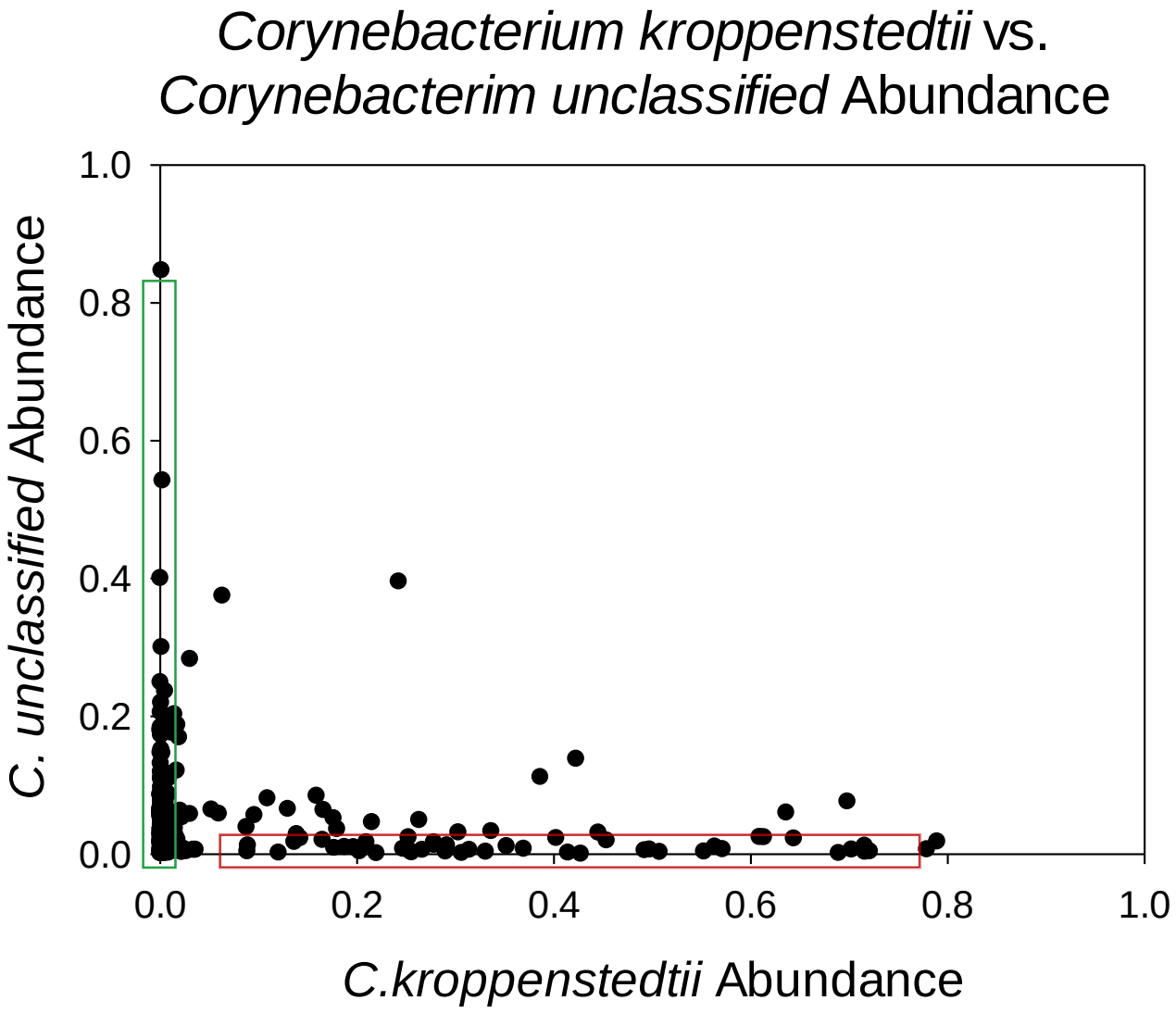
- Increase in ***Corynebacterium kroppenstedtii***
- Decrease *in* ***Corynebacterium sp.*** (unclassified)



Similar results were noted with V1-3 Primer.

CORYNEBACTERIA SPP. ARE CO-EXCLUSIVE

Corynebacterium kroppenstedtii and *Corynebacterium unclassified* are usually "Co-exclusive" in their abundance

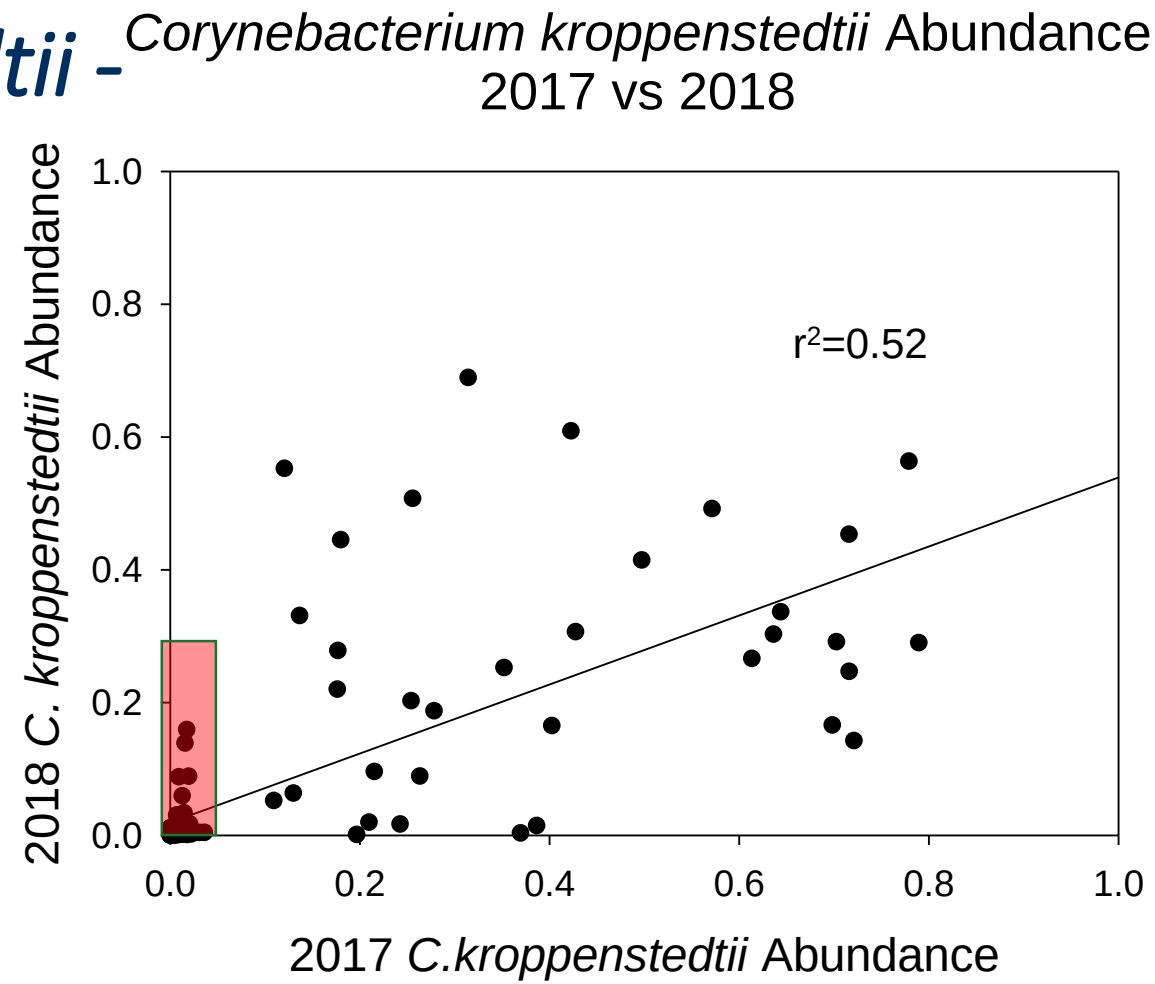


C. KROPPENSTEDTII - TEMPORAL STABILITY

Corynebacterium kroppenstedtii -

High intra-individual and temporal stability …..

If relative abundance of *C. kroppenstedtii* was low in 2017, it remained low in 2018 and visa versa.

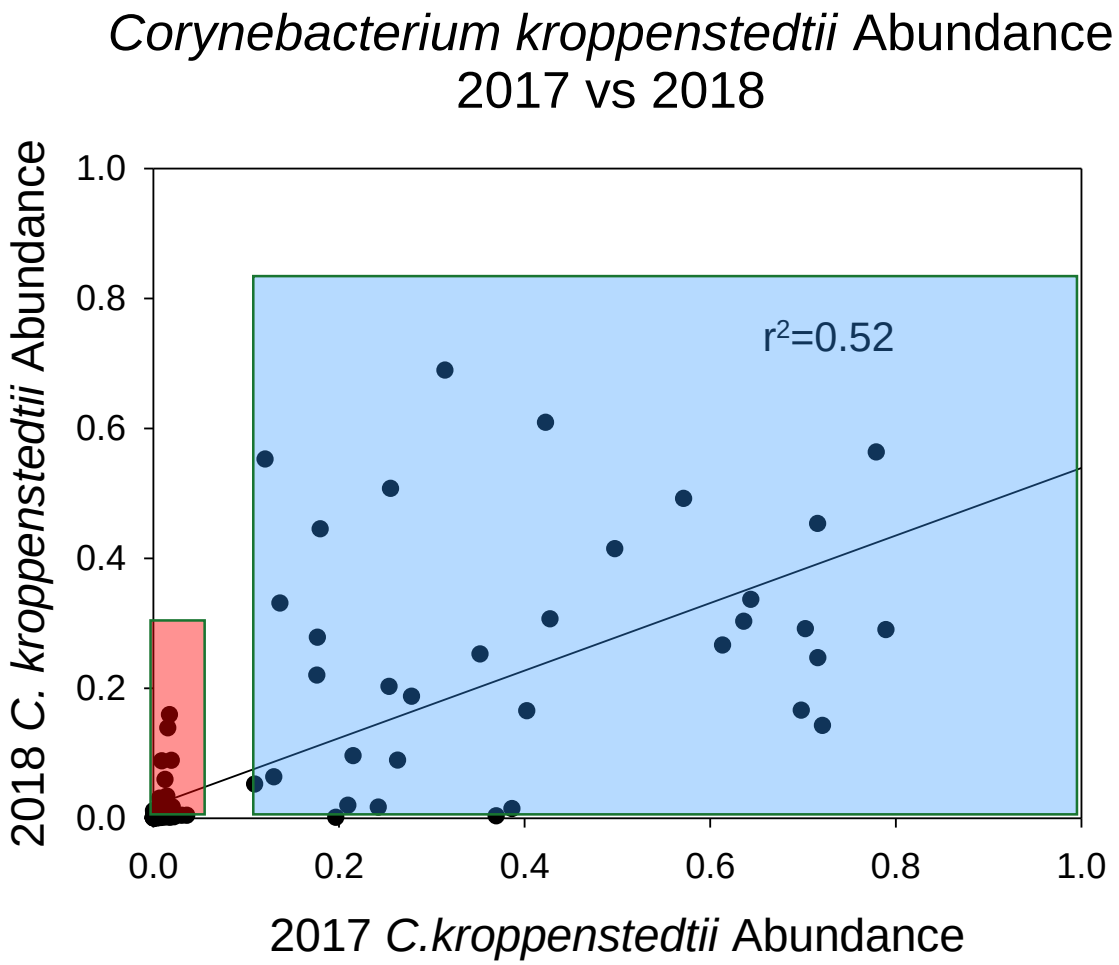


C. KROPPENSTEDTII - TEMPORAL STABILITY

Based on *C. kroppenstedii* abundance, the subjects were grouped into:

- < 0.1 relative abundance – **Low *C. Kropp*.**
- ≥ 0.1 relative abundance – **High *C. Kropp*.**

The skin parameters (age, BMI, Redness etc.) of these two groups were compared using a t-test.



C. KROPPENSTEDII - ABUNDANCE

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The group with higher *Corynebacterium kroppenstidii* (>0.1) had significantly higher Skin Redness (2.5a*)

Skin Measurements

Parameter	Low *C. kropp.* (<0.1)	High *C. kropp.* (>0.1)	P-value
Age	42	50	0.001
BMI	26.2	28.9	0.03
Red Features	0.08	0.11	2E-05
Visible Spots	0.036	0.045	0.02
Red Spots	0.005	0.008	0.004
Redness	0.39	0.51	0.005
Evenness	0.51	0.47	0.001
a*	23.1	25.6	0.0002
L*	64.7	63.1	0.001

Increase in *Corynebacterium kroppenstedtii*- Increase in Skin Redness

C. KROPPENSTEDII - LIETERATURE REVIEW

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***Finegoldia magna* and *Corynebacterium kroppenstedtii* are significantly enriched in rosacea independent of rosacea subtype: Results of a case-control study**

BM Rainer[2,1], E Mongodin[3], J Bui[3], A Fischer[1], H Pasieka[1], LA Garza[1], S Kang[1] and A Chien[1]
1 Dermatology, Johns Hopkins School of Medicine, Graz, Austria, 2 Dermatology, Medical University of Graz, Graz, Austria and 3 Institute for Genome Sciences, Baltimore, MD

In "Rosacea Patients" as compared to controls, the cheek and nose data showed that the relative abundance was significantly;

- **Higher** for *Corynebacterium kroppenstedtii* (4.35; $p < 0.005$) – Nose
- **Higher** for *Finegoldia magna* (+4.44 log; $p<0.005$) – Cheek
- **Lower** for *Propionibacterium acnes* (-3.48 log; $p<0.005$) - Cheek

Rosacea Patients:

C. kroppenstedtii and F. magna may have important roles in rosacea pathophysiology

SKIN MICROBIOTA DYSBIOSES -

Amway
RESEARCH & DEVELOPMENT

Shift in skin microbiota of Western European women across aging

R. Jugé et al, 23 May 2018. Journal of Applied Microbiology - R&D department, SILAB, Brive-la-Gaillarde, France

Concluded: At the genus level, older skin exhibited: **A significant increase in *Corynebacterium* and a decrease in *Propionibacterium* relative abundance.**

Cinco De Mayo Study -

- With increase in age- Increase in *C. kroppenstedii.*
- With increase in *C. kroppenstedii* abundance of - Increase in Skin Redness

Rosacea Patients (Literature) –

- Higher abundance of *C. kropppenstedii* and *Finegoldia magna*
- *Lower P. acnes*

Food for thoughts: Is Rosacea a result of dysbiosis i.e. – high abundance of C. kroppenstedii and F. magna* and low *P. acnes?

SUMMARY

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Results observed in two separate cohorts, multiple skin sites, two different primers (V4 and V1-V3) and over 1 year period.

1. Microbiome diversity and composition is relatively STABLE (over 1 year in this study cohort).
2. Microbiome diversity and composition are significantly associated with important parameters of skin health (e.g., redness, porphyrins).
3. *Corynebacterium kroppenstedtii* is:
    - Associated with age and skin redness parameters.
    - Mutually exclusive with other *Corynebacterium spp. (unclassified)*.

THANK YOU!

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