Skin Microbiota Landscape: Cause or consequence ?

Richard MARTIN
MERCURIALIS Biotech

You, Me, Us…

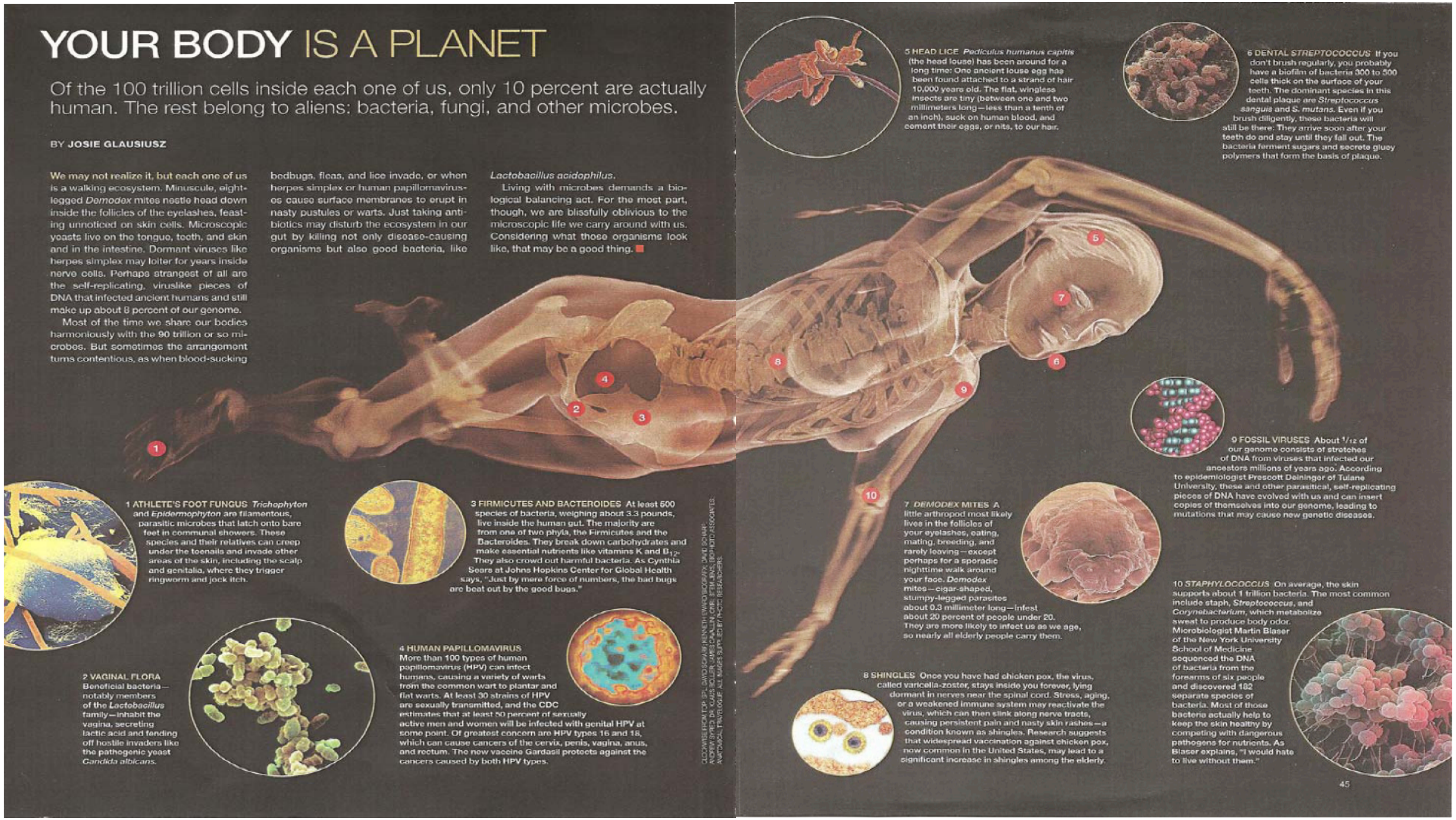
« People are not just people.
They are an awful lot of microbes, too »

Gut, skin but also in each human cell:
mitochondria

The Economist , August 18th 2012

A NEW ORGAN: MICROBIOTA HARBOURING ON OUR SKIN.

THE BACTERIAL POINT OF VIEW:



YOUR BODY IS A PLANET

Of the 100 trillion cells inside each one of us, only 10 percent are actually human. The rest belong to aliens: bacteria, fungi, and other microbes.

BY **JOSIE GLAUSIUSZ**

We may not realize it, but each one of us is a walking ecosystem. Minuscule, eight-legged *Demodex* mites nestle head down inside the follicles of the eyelashes, feasting unnoticed on skin cells. Microscopic yeasts live on the tongue, teeth, and skin and in the intestine. Dormant viruses like herpes simplex may loiter for years inside nerve cells. Perhaps strangest of all are the self-replicating, viruslike pieces of DNA that infected ancient humans and still make up about 8 percent of our genome.

Most of the time we share our bodies harmoniously with the 90 trillion or so microbes. But sometimes the arrangement turns contentious, as when blood-sucking bedbugs, fleas, and lice invade, or when herpes simplex or human papillomaviruses cause surface membranes to erupt in nasty pustules or warts. Just taking antibiotics may disturb the ecosystem in our gut by killing not only disease-causing organisms but also good bacteria, like *Lactobacillus acidophilus*.

Living with microbes demands a biological balancing act. For the most part, though, we are blissfully oblivious to the microscopic life we carry around with us. Considering what those organisms look like, that may be a good thing. ■

1 ATHLETE'S FOOT FUNGUS *Trichophyton* and *Epidermophyton* are filamentous, parasitic microbes that latch onto bare feet in communal showers. These species and their relatives can creep under the toenails and invade other areas of the skin, including the scalp and genitalia, where they trigger ringworm and jock itch.

2 VAGINAL FLORA Beneficial bacteria—notably members of the *Lactobacillus* family—inhabit the vagina, secreting lactic acid and fending off hostile invaders like the pathogenic yeast *Candida albicans*.

3 FIRMICUTES AND BACTEROIDES At least 500 species of bacteria, weighing about 3.3 pounds, live inside the human gut. The majority are from one of two phyla, the Firmicutes and the Bacteroides. They break down carbohydrates and make essential nutrients like vitamins K and B_{12}. They also crowd out harmful bacteria. As Cynthia Sears at Johns Hopkins Center for Global Health says, "Just by mere force of numbers, the bad bugs are beat out by the good bugs."

4 HUMAN PAPILLOMAVIRUS More than 100 types of human papillomavirus (HPV) can infect humans, causing a variety of warts from the common wart to plantar and flat warts. At least 30 strains of HPV are sexually transmitted, and the CDC estimates that at least 50 percent of sexually active men and women will be infected with genital HPV at some point. Of greatest concern are HPV types 16 and 18, which can cause cancers of the cervix, penis, vagina, anus, and rectum. The new vaccine Gardasil protects against the cancers caused by both HPV types.

5 HEAD LICE *Pediculus humanus capitis* (the head louse) has been around for a long time: One ancient louse egg has been found attached to a strand of hair 10,000 years old. The flat, wingless insects are tiny (between one and two millimeters long—less than a tenth of an inch), suck on human blood, and cement their eggs, or nits, to our hair.

6 DENTAL *STREPTOCOCCUS* If you don't brush regularly, you probably have a biofilm of bacteria 300 to 500 cells thick on the surface of your teeth. The dominant species in this dental plaque are *Streptococcus sanguis* and *S. mutans*. Even if you brush diligently, these bacteria will still be there: They arrive soon after your teeth do and stay until they fall out. The bacteria ferment sugars and secrete gluey polymers that form the basis of plaque.

7 *DEMODEX* MITES A little arthropod most likely lives in the follicles of your eyelashes, eating, mating, breeding, and rarely leaving—except perhaps for a sporadic nighttime walk around your face. *Demodex* mites—cigar-shaped, stumpy-legged parasites about 0.3 millimeter long—infest about 20 percent of people under 20. They are more likely to infect us as we age, so nearly all elderly people carry them.

8 SHINGLES Once you have had chicken pox, the virus, called varicella-zoster, stays inside you forever, lying dormant in nerves near the spinal cord. Stress, aging, or a weakened immune system may reactivate the virus, which can then slink along nerve tracts, causing persistent pain and nasty skin rashes—a condition known as shingles. Research suggests that widespread vaccination against chicken pox, now common in the United States, may lead to a significant increase in shingles among the elderly.

9 FOSSIL VIRUSES About 1/12 of our genome consists of stretches of DNA from viruses that infected our ancestors millions of years ago. According to epidemiologist Prescott Deininger of Tulane University, these and other parasitical, self-replicating pieces of DNA have evolved with us and can insert copies of themselves into our genome, leading to mutations that may cause new genetic diseases.

10 *STAPHYLOCOCCUS* On average, the skin supports about 1 trillion bacteria. The most common include staph, *Streptococcus*, and *Corynebacterium*, which metabolize sweat to produce body odor. Microbiologist Martin Blaser of the New York University School of Medicine sequenced the DNA of bacteria from the forearms of six people and discovered 182 separate species of bacteria. Most of those bacteria actually help to keep the skin healthy by competing with dangerous pathogens for nutrients. As Blaser explains, "I would hate to live without them."

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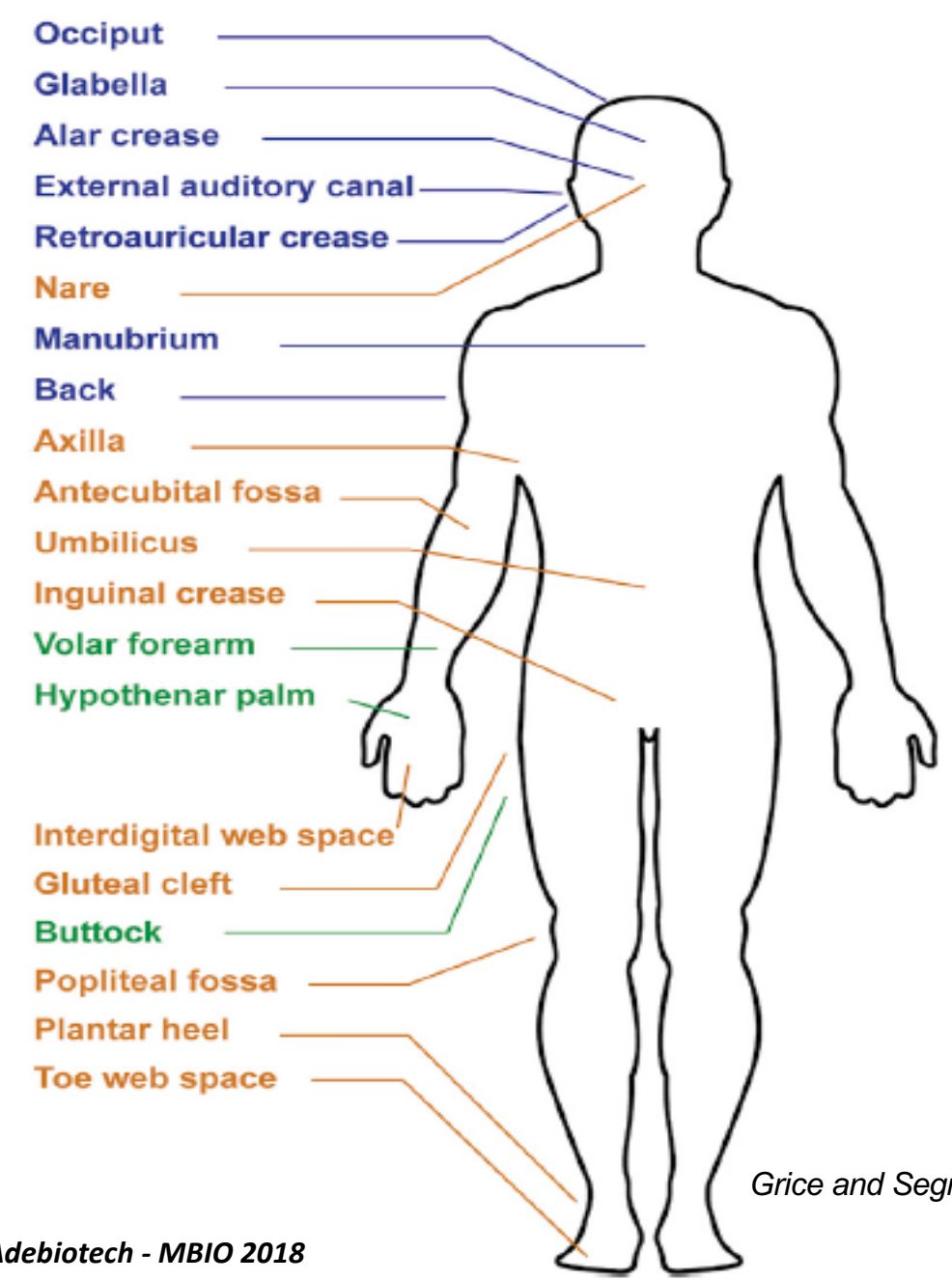
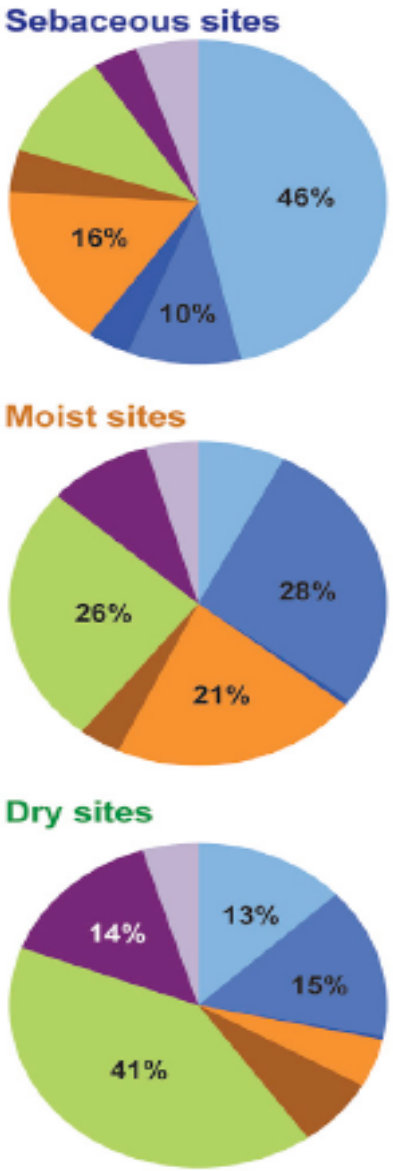
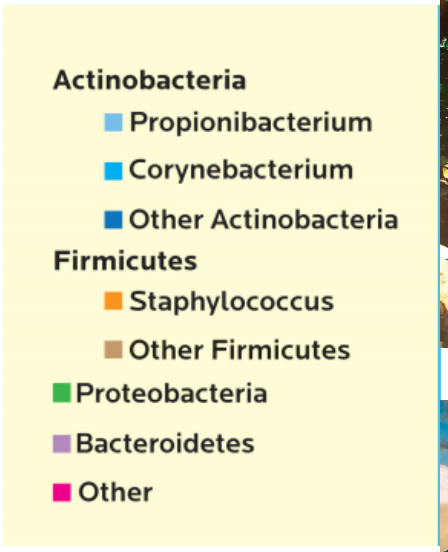
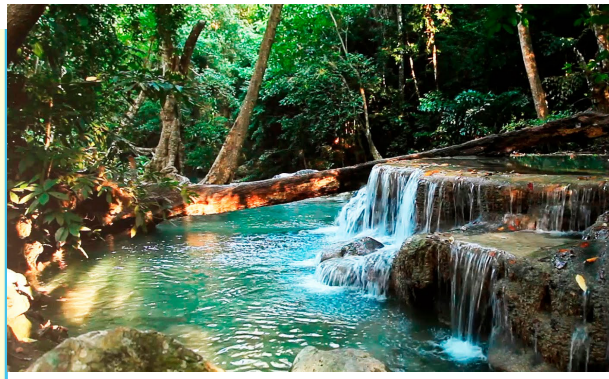
SKIN MICROBIOTA: MANY BIOTOPES



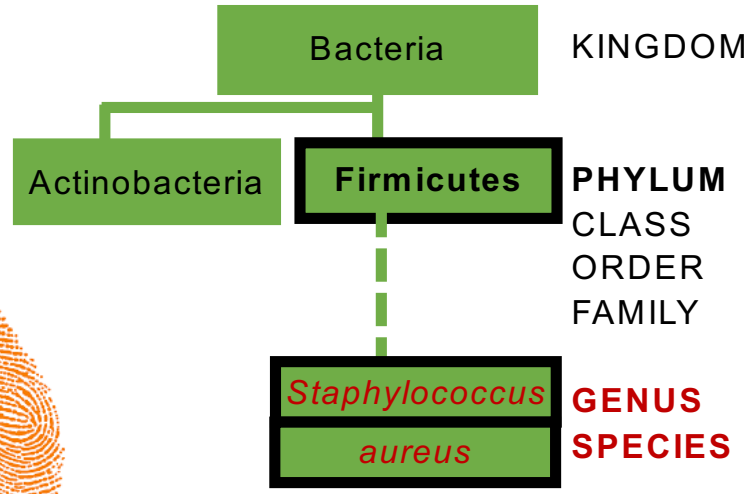


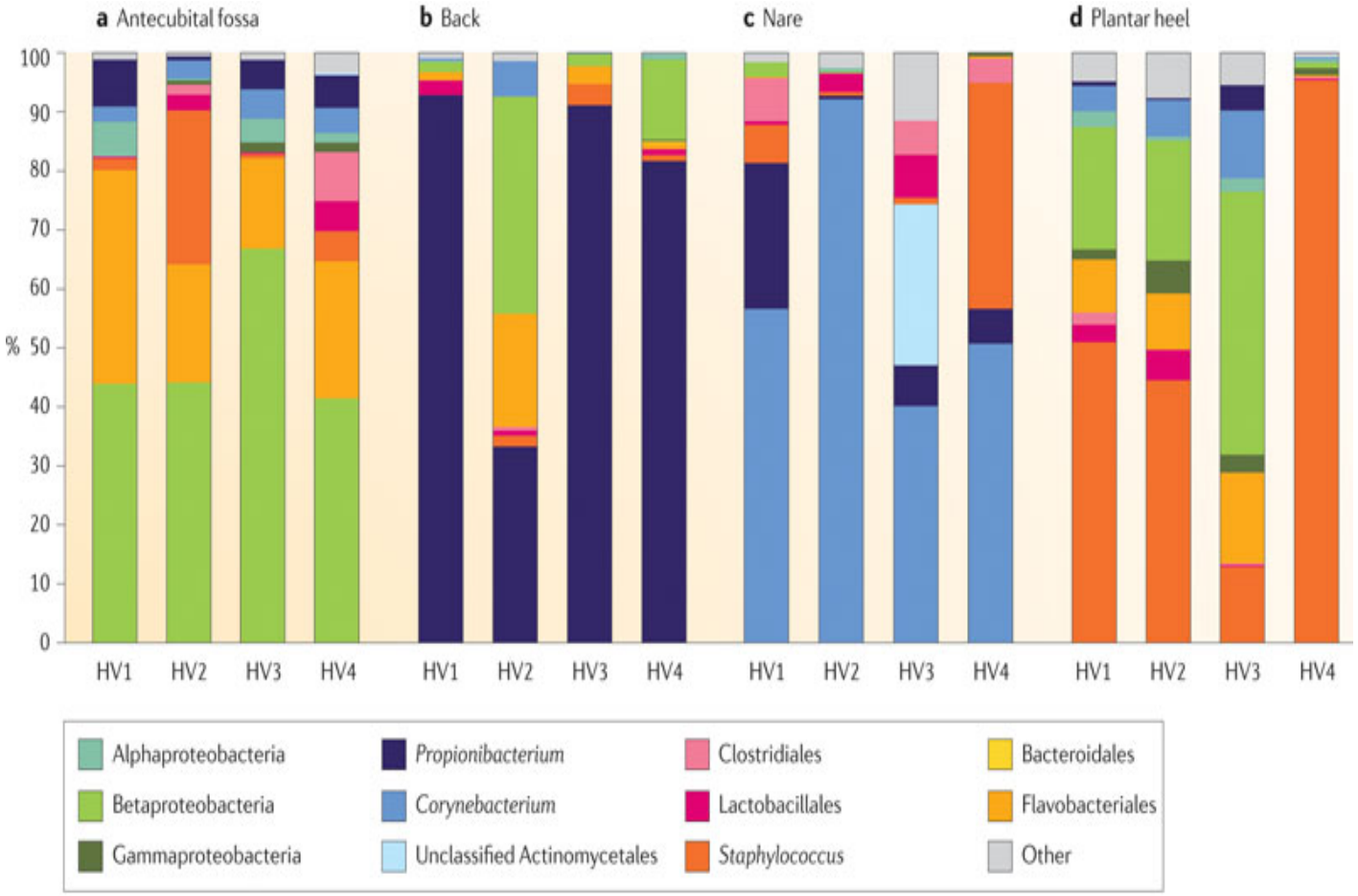




Grice and Segre, Ann Rev Genomics Hum Gen, 2012

ZOOM: EACH INDIVIDUAL HAS THEIR OWN MICROBIOTA FINGERPRINT





Nature Reviews | Microbiology

Interpersonal variation of the skin microbiota

The microbial distribution of four sites on four healthy volunteers
(HV1, HV2, HV3 and HV4)

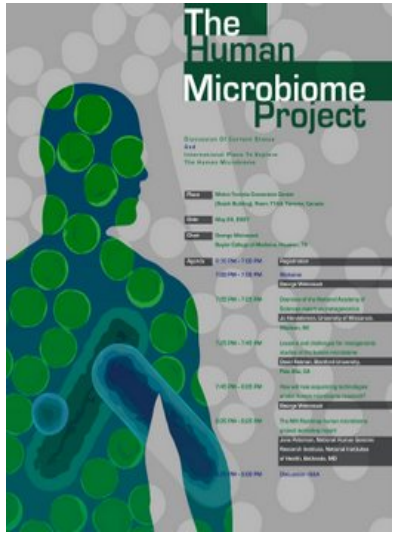
SKIN MICROBIOME: OUR SECOND GENOME

- The human body contains 2 to 5 times more bacteria than human cells (10^{13} cells and 2 to 5.10^{13} bacteria)

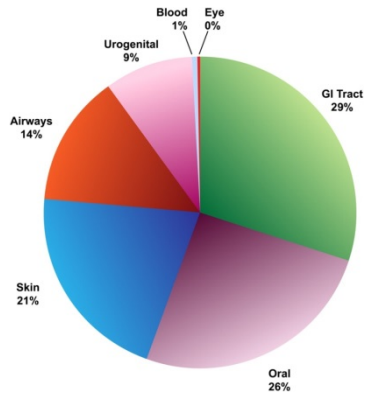
- **10^{3}~10^{6} bacteria** inhabit each cm^2 of skin.

33:1

All in contact with tissue



- The most abundant microbe represents less than **1%** of the total microflora.



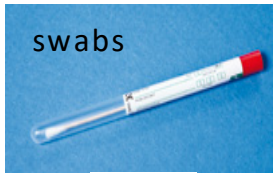
- To date, **more than 500 bacterial species** have been detected on healthy skins, potentially expressing more than **3,5 millions of genes**

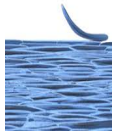
- A holistic view versus a targeted view: no bad, no good bacteria

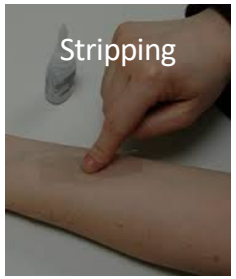
The NIH HMP Working Group et al. Genome Res. 2009;19:2317-2323

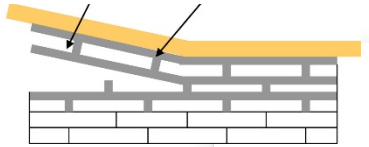
SAMPLING AND ANALYSIS PROTOCOL

Axenic conditions ensure only skin flora is collected

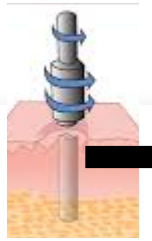


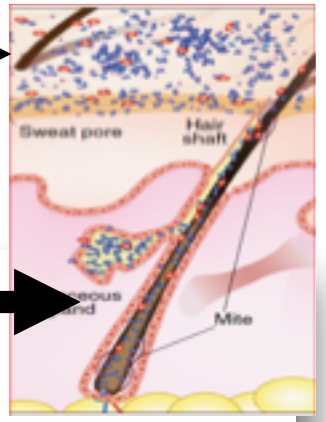






Biopsy

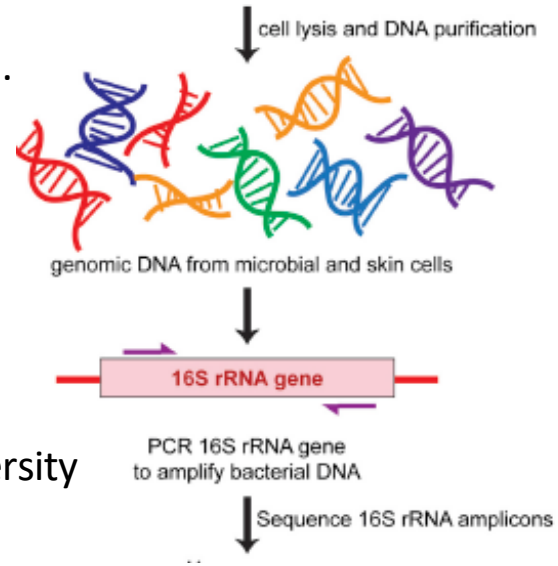




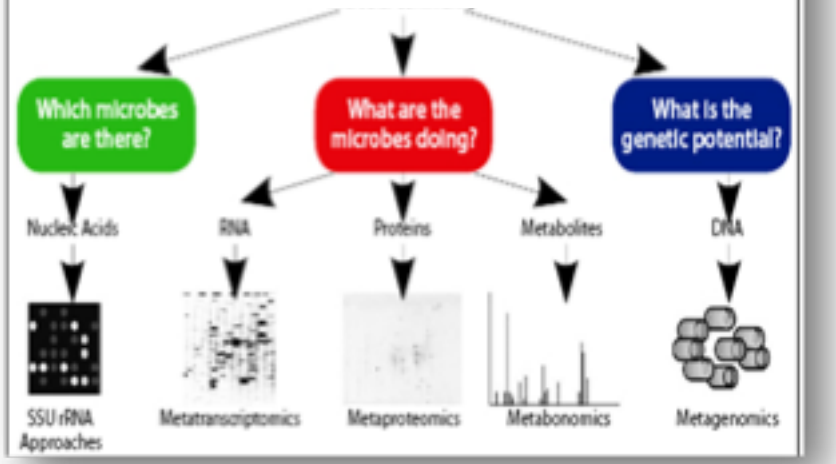
Select specific rRNA for Bacteria. (16S rRNA)

Identify Bacterial landscape of the sample area

Quantify the bacterial diversity



Microbial Community Description



Shannon diversity index

WHAT IS THE SHANNON DIVERSITY INDEX?

$$\text{Shannon Index (H)} = -\sum_{i=1}^{s} p_i \ln p_i$$

Based on the species richness (the number of species present)
and species abundance (the number of individuals per species)

SKIN DISBIOSYS AND BACTERIA LANDSCAPE COMPARISONS: SOME PUBLISHED EXAMPLES

Same sampler **Same sampling method** **Same Pipe Line**

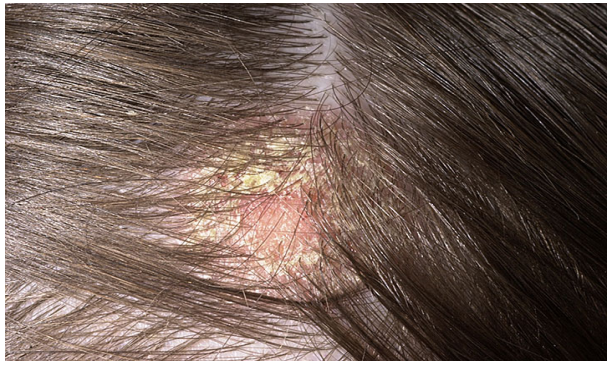
N ≥ 30

Acne

Greasy Skin

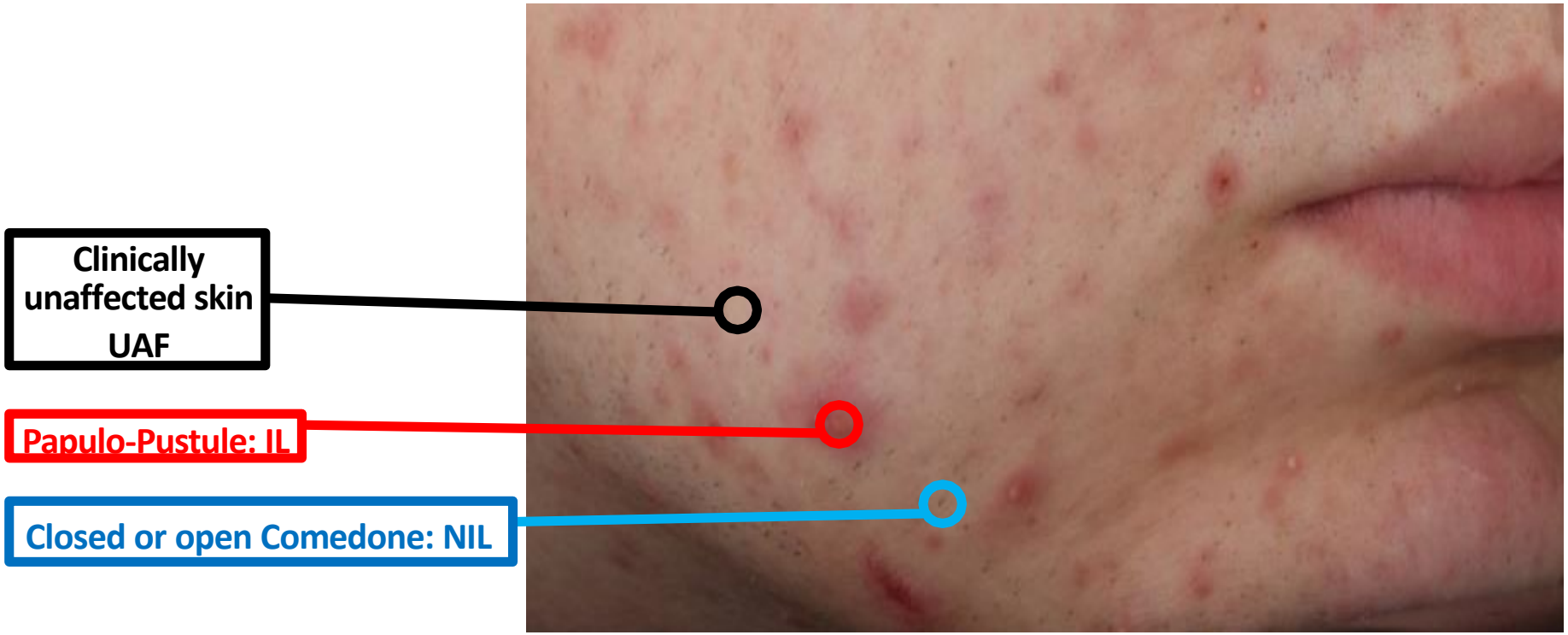
Atopic Dermatitis

Dandruff



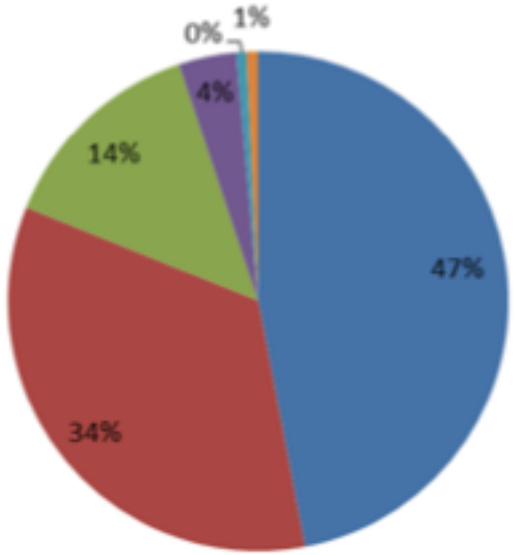
MICROBIOTA CARTOGRAPHY ON ACNE AFFECTED SKIN SURFACE (LA ROCHE-POSAY) PR B. DRÉNO / S. SEITE

- Does the microbiota of papulo-pustules and of comedones differ and differ from adjacent unaffected skin?
- Does the microbiota of superficial acneic skin differ from a normal healthy skin?
- How an antibiotic changes the bacterial landscape compared to a dermobiotic ?

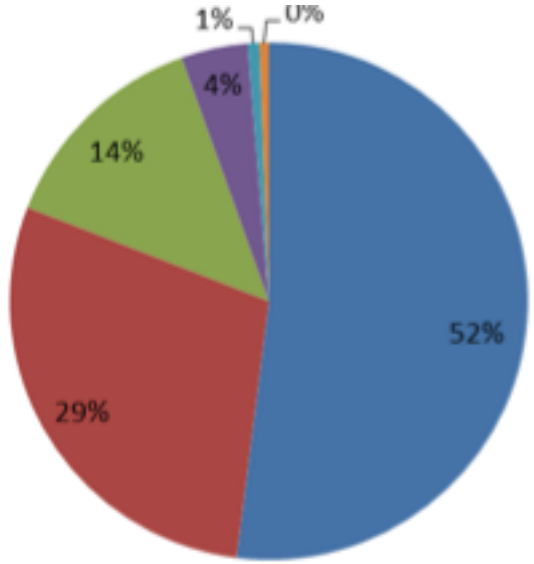


BACTERIAL PHYLA ON THE SKIN SURFACE IN ACNE-AFFECTED PATIENTS

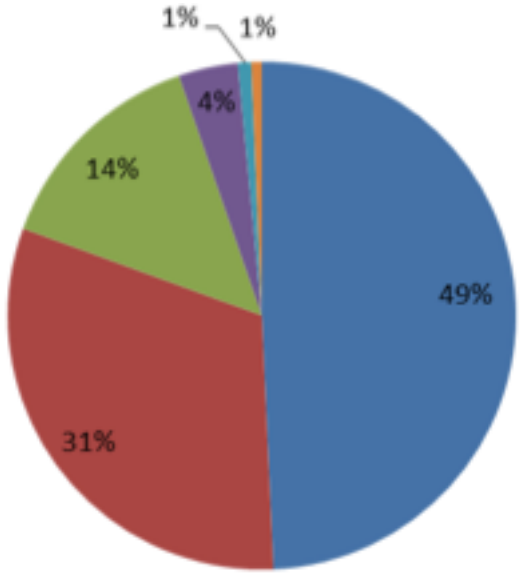
Unaffected skin (UAF)

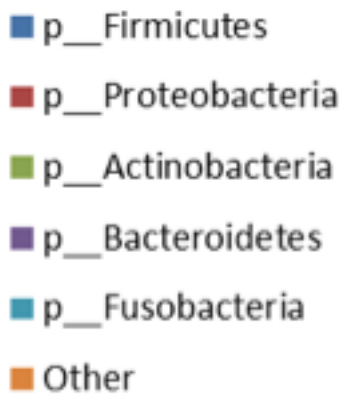


closed or open comedones (NIL)



Papulo-Pustules (IL)





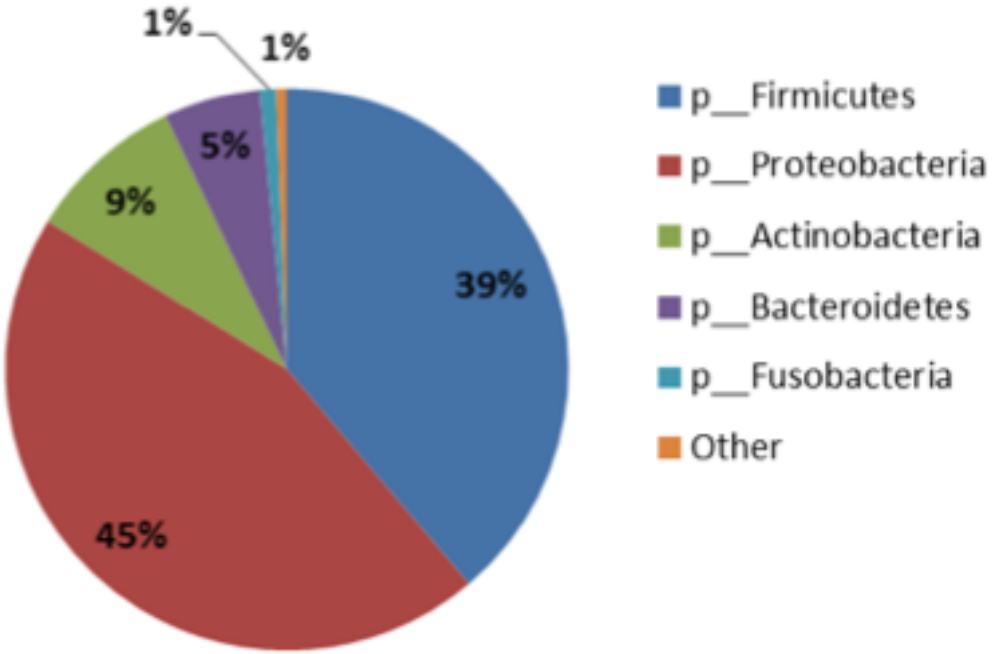
Similar profile on the 3 skin sampled areas

There is NO difference with severity of the pathology

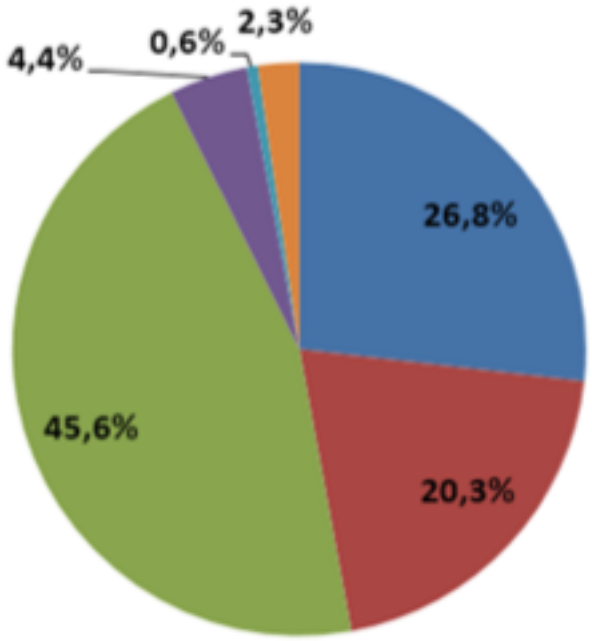
(GEA-2 or 3)

MAIN BACTERIAL PHYLA DIFFER FROM NORMAL HEALTHY SKIN

Acne - cheeks



Healthy - cheeks

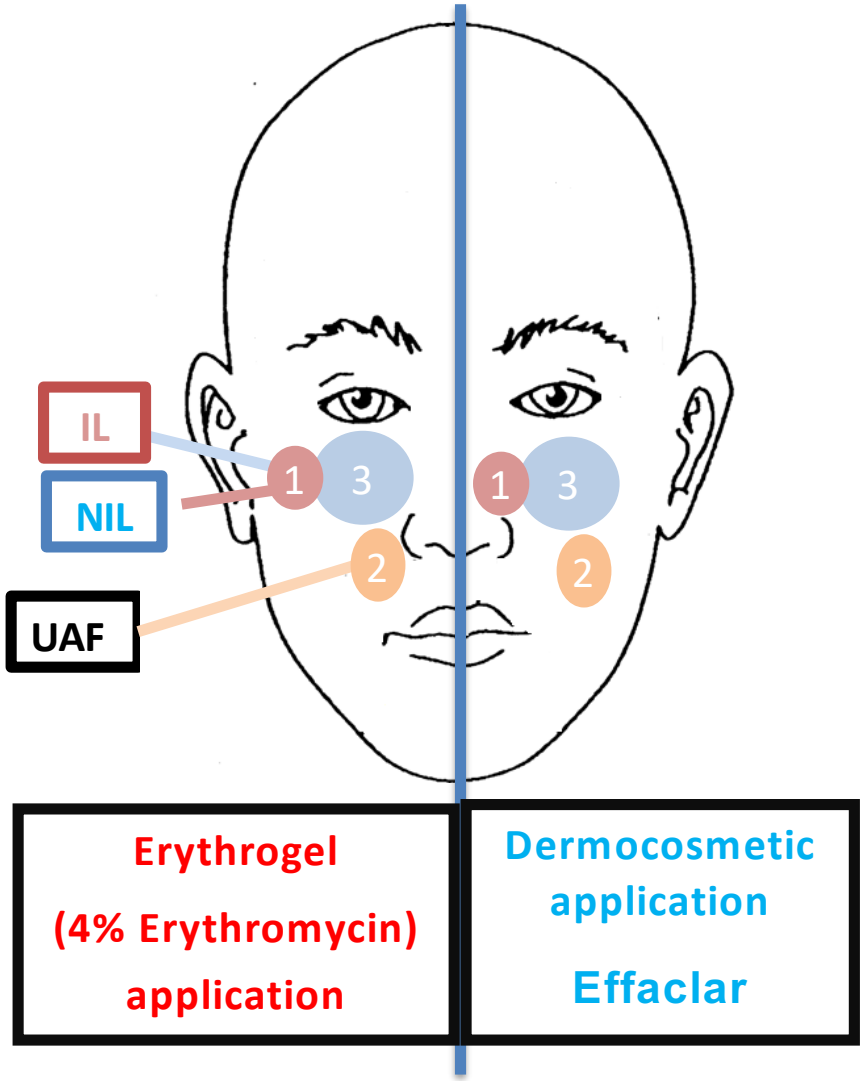


Versus healthy subjects, skin surface of the cheeks of acne patients showed an **over-abundance of Proteobacteria** (p=0.0003) and **Firmicutes** (p=0.023) and an **underrepresentation of Actinobacteria** (p<0.0001).

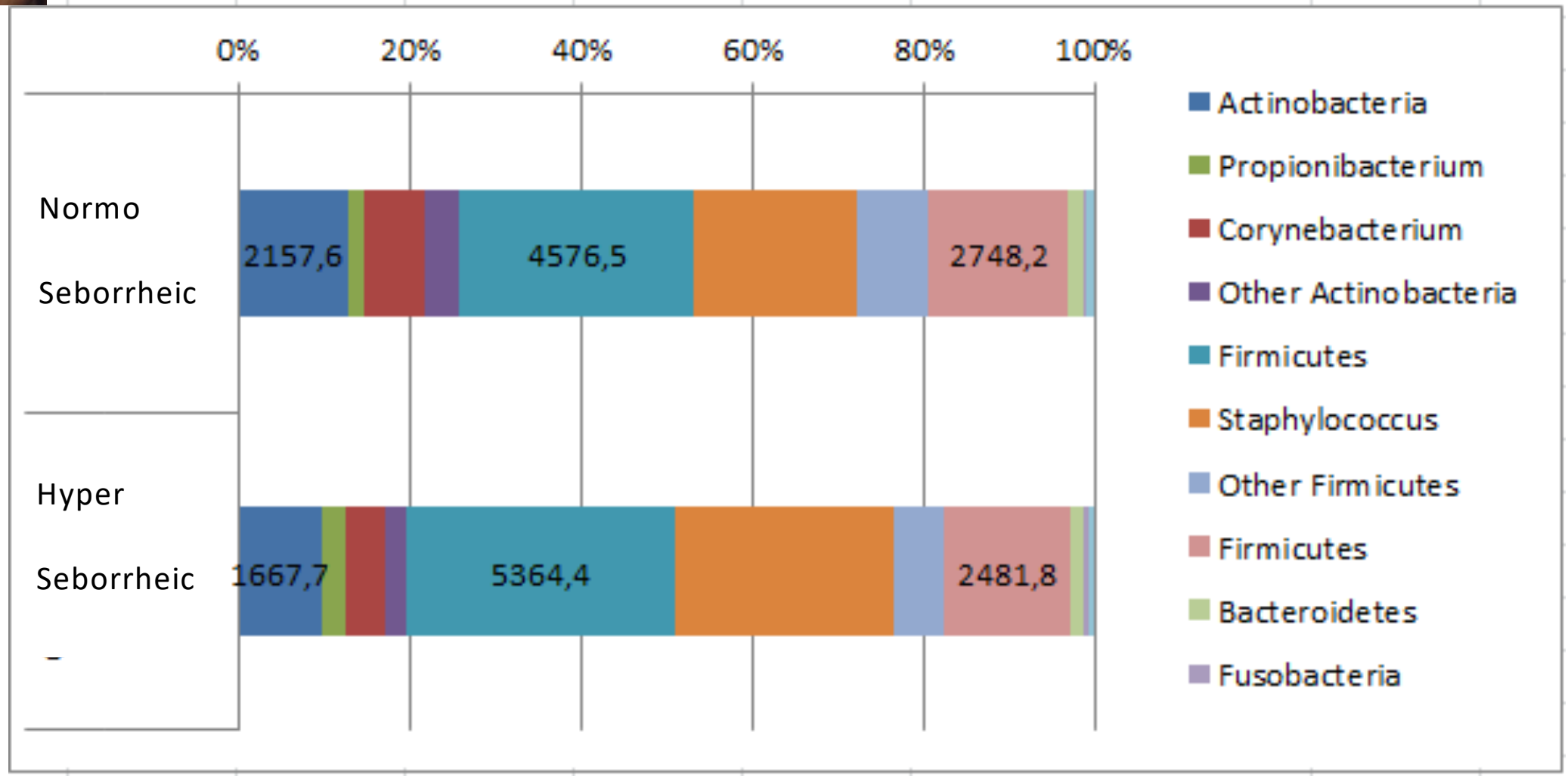
ERYTHROMYCIN VERSUS EFFACLAR DUO

- Can the microbiota of acne-affected skin be modified after applying a topical antibiotic or a specific dermocosmetic?

- **The answer is clear:**
  - Same efficacy: acne resolution in both cases
  - Different bacterial landscape after treatment
  - Long lasting effect with the dermocosmetic
  - Shannon index higher with dermocosmetic



BACTERIAL GENUS ON THE SKIN SURFACE: GREASY (N=119)

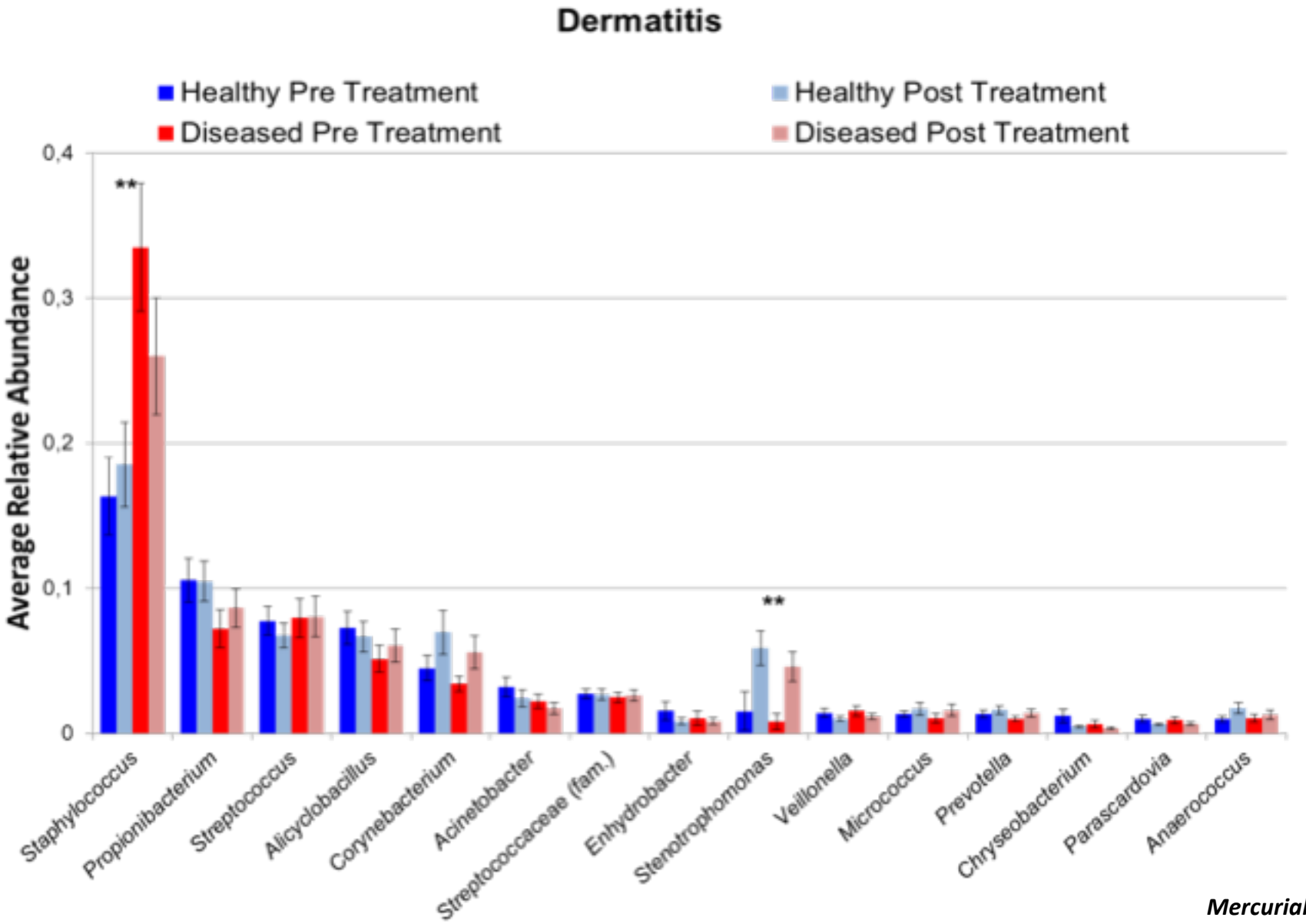


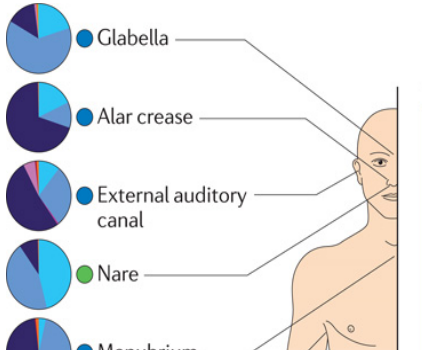
GREASY SKIN: LESS ACTINO MORE STAPHYLO

ATOPIC DERMATITIS (N=55) LA ROCHE-POSAY

We have dramatically increased the efficacy:

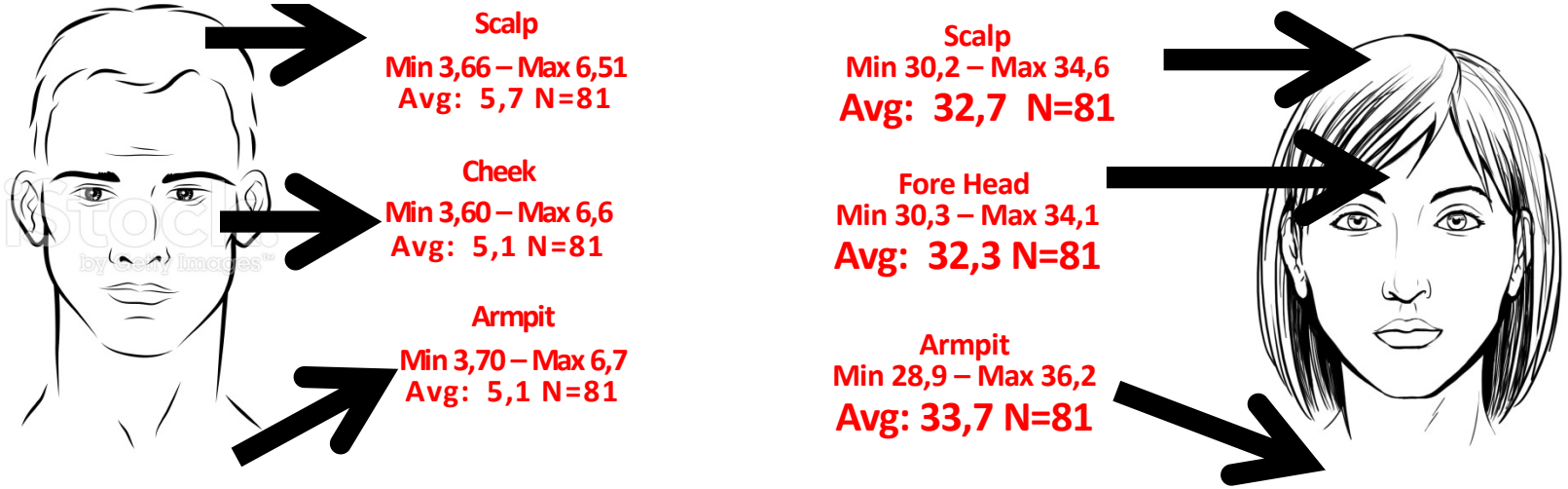
- PAMs production by keratinocytes induced by *Vitreoscilla filiformis* to decrease Staph.
- We added Mannose to increase the keratolytic Xanthomonadaceae familly *(Stenotrophomonas)*
- We increased water proportion to increase aw





SYNTHESIS: WHAT CAN INFLUENCE SKIN BACTERIAL LANDSCAPE

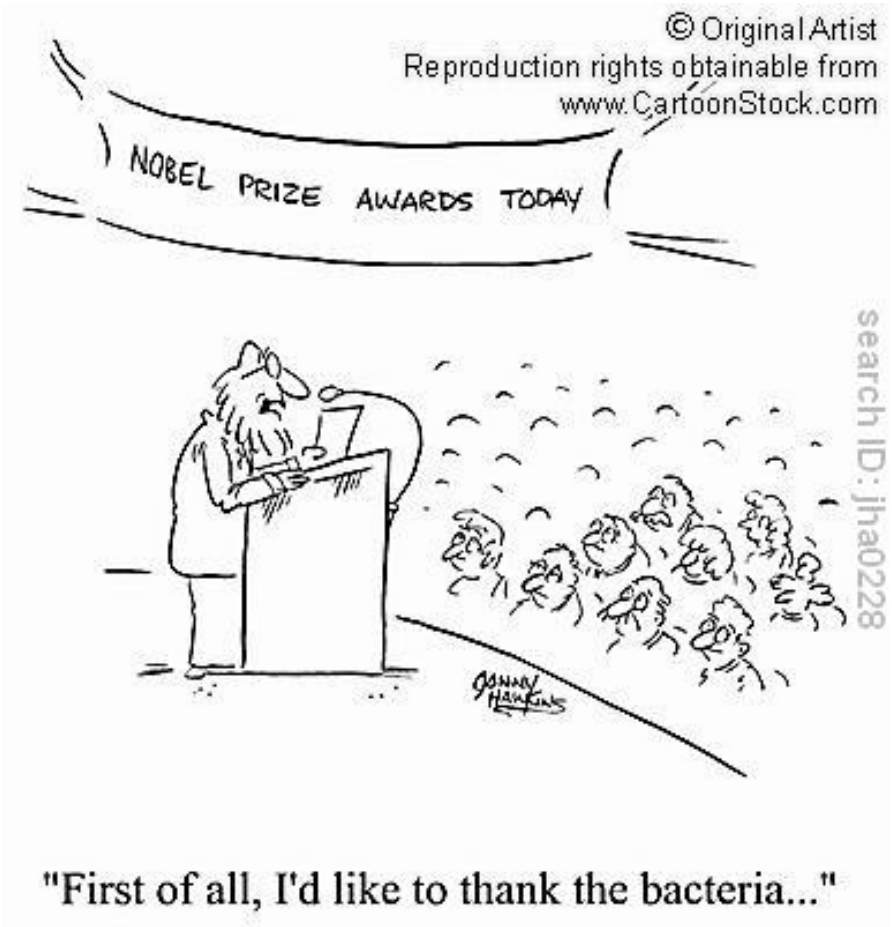
We have seen that : antibiotics, water, sugar, dead and surely living bacteria (MIT/Cholera), chemicals can influence dramatically the bacteria landscape of the skin



Distribution of pH and temperature of a healthy human skin

Data not published

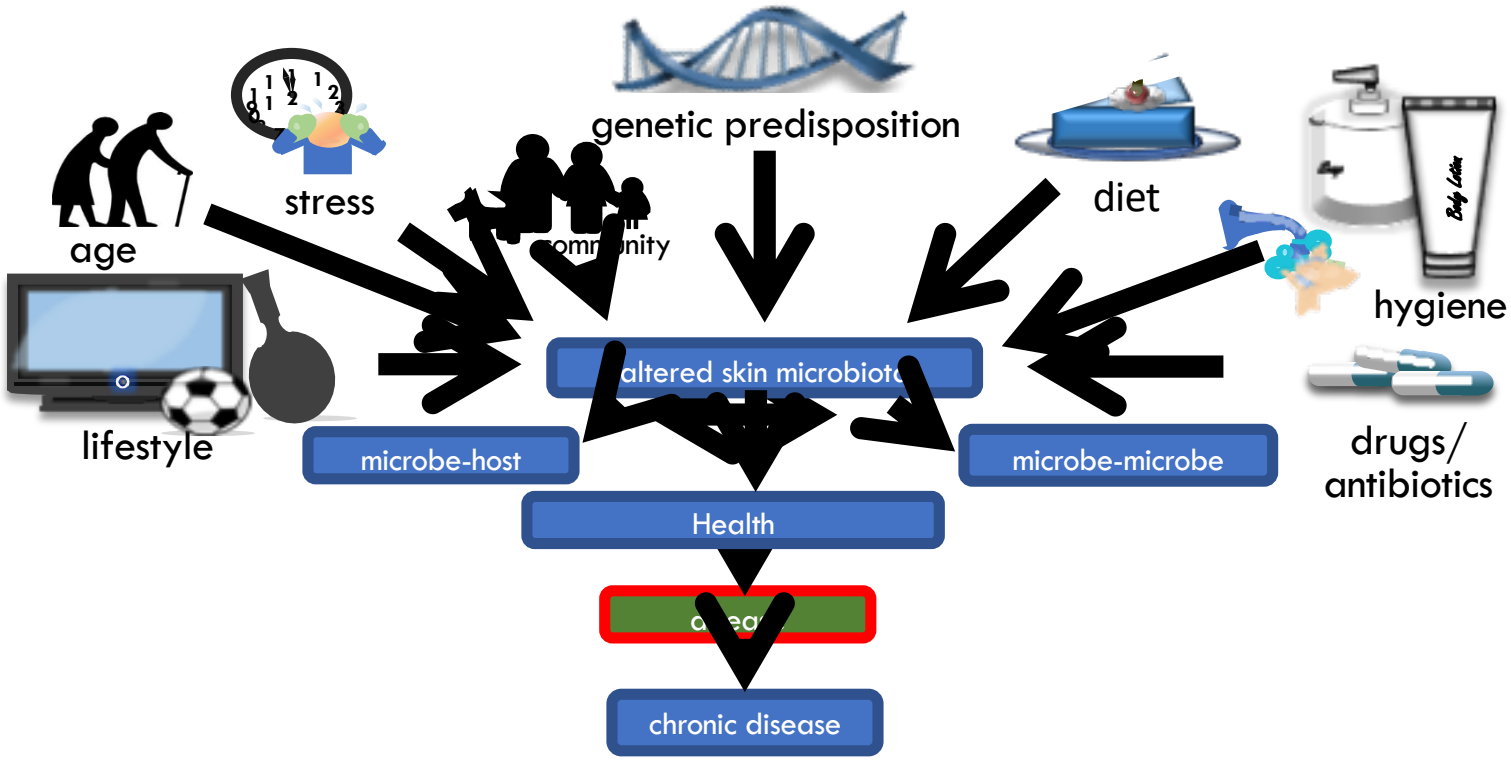
TO SUMMARIZE:



"Everything is everywhere, the environment selects"
Said the Dutch microbiologist Martinus Beijerink (1851-1831)

Change the skin environment and you change the bacteria landscape, It is what makes the cosmetic for centuries without knowing it

WE ARE AT THE BEGINNING OF A NEW UNDERSTANDING OF SKIN BIOLOGY



Schommer NN., and Gallo RL. *Trends Microbiol* 2013

Thank you for your attention