Analysis of the skin microbiome in health and disease

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The major aim is to apply deep sequencing technology to obtain a detailed unbiased overview of the bacterial microbiome of human skin. This will be performed in healthy individuals, psoriasis patients and atopic dermatitis patients. Furthermore, we...

Ethical review	Approved WMO
Status	Recruiting
Health condition type	Skin and subcutaneous tissue disorders NEC
Study type	Observational non invasive

Summary

ID

NL-OMON34787

Source
ToetsingOnline

Brief title
The skin microbiome

Condition

- Skin and subcutaneous tissue disorders NEC

Synonym
psoriasis and atopic dermatitis

Research involving
Human

Sponsors and support

Primary sponsor: Universitair Medisch Centrum Sint Radboud
Source(s) of monetary or material Support: Ministerie van Economische Zaken

Intervention

Keyword: deep sequencing, inflammation, microbiology, skin

Outcome measures

Primary outcome

The outcome of the study is a nearly complete microbiome of each of the samples. The composition of the microbiomes (at various taxonomical levels: from phylum to genus) will be compared between groups: controls vs psoriasis, controls vs atopic dermatitis, and within groups (psoriasis patients during therapy, healthy controls during healing of superficial skin wounds).

Secondary outcome

n.a.

Study description

Background summary

Humans have evolved facing a continuous exposure to infectious agents, and it is likely that our genetic make-up has been shaped by selective pressures from external microorganisms and our commensal flora of skin and gut. Recent data on two major inflammatory skin diseases, psoriasis and atopic dermatitis ('eczema') have indicated that the chemical and physical barrier of the skin, which protects us against infection, plays a crucial role in the development of these diseases (refs 1-3). Atopic dermatitis is a disease characterized by inflammation and itch, affecting up to 15% of all children. Skin of these patients has a poor antimicrobial defense as demonstrated by several labs including our own. Skin of atopic dermatitis patients is often infected and nearly always colonized by Staphylococcus aureus. Recently it was found that mutations in filaggrin, a gene involved in skin barrier function, explains a large part of the heritability of this disease. Psoriasis is also a common inflammatory skin disease affecting 2% of the adult population. A major genetic association was found with HLA-Cw6, and streptococci have been implicated in disease development. Recent findings by our lab have elucidated a major part of the genetic basis (>60%) when we found that polymorphisms in antimicrobial proteins (beta-defensins) and skin barrier proteins (LCE3C/B) were associated

with the disease. Collectively, these data have prompted us to investigate the role of microorganisms in psoriasis and atopic dermatitis.
Recent investigations have started to make an inventory of skin and gut microbiome by high-throughput approaches such as microarrays or by conventional sequencing approaches (refs 4-5). Both have their drawbacks, as microarrays are restricted towards known sequences, and clone-by-clone sequencing yields incomplete coverage. The deep-sequencing technology as will be used in this project overcomes these problems, and allows unbiased identification of virtually all skin-inhabiting organisms. Our aim is to establish causal relationships between microbiome and human skin disease. This would not only shed a completely different light on the pathogenesis, but also provide means to investigate a different approach to prevention and therapy which would entail the normalization of the bacterial skin, throat and gut flora, e.g. by use of natural products like food or probiotics.

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Study objective

The major aim is to apply deep sequencing technology to obtain a detailed unbiased overview of the bacterial microbiome of human skin. This will be performed in healthy individuals, psoriasis patients and atopic dermatitis

patients. Furthermore, we will study the dynamics of skin microbiome composition in regressing lesions (patients) and following superficial injury (tapestripping of healthy individuals). A second aim is to collect throat swaps and stool samples from these individuals for sequencing of the corresponding microbiomes. Bioinformatics will be used to defect patterns of microbiome composition that correlate with disease or disease activity.

Study design

STUDY DESIGN

Methodological considerations
This is a pilot study on the microbiome of relatively small groups of individuals. These analyses will generate large amounts of experimental data (> 10.000 datapoints per sample, comparable to microarray studies), creating the problem of family wise errors. For this reason, the analysis will not primarily focus on the quantitative comparison of individual taxa but rather on the shifts of the microbial communities between samples (at higher taxonomical aggregation levels, from genus to phylum).

Experimental groups:

Group 1. normal skin of healthy controls (n=10)
Group 2. tape stripped skin of healthy volunteers (see below for tapestripping) (n=10)
Group 3. lesional and non-lesional skin of un treated atopic dermatitis patients (n=10)
Group 4. lesional and non-lesional skin of untreated plaque psoriasis patients (n=10)
Group 5. lesional and non-lesional skin of untreated psoriasis inversa patients (n=10)
Group 6. lesional skin and non-lesional skin, throat swabs and stool of plaque psoriasis patients during therapy with systemic medication (n=10); as controls we will study samples of normal skin, throat swabs and stools of healthy controls (n=10)

Before the start of the actual study, optimization of skin sampling and bacterial DNA extraction is performed, which requires a small number of volunteers:

Group 7. optimization studies: controls (n=5) and psoriasis patients (n=5)

Analysis of skin microbiome

groups 1,3,4,5 and 7
Volunteers will be recruited from our list or via our outpatient department. An

anamnesis will be taken by a clinician who will be part of the project team. In this way we will recruit individuals for experimental groups 1 to 5 and 7.
We will start by optimizing our sampling and DNA extraction procedure on skin of healthy volunteers and psoriasis patients (group 7). We will compare skin sampling by scraping with a scalpel and standard swabs. The best method (DNA yield, microbial diversity) will be selected for further study.
DNA from skin samples of groups 1,3,4 and 5 will be extracted and amplified with universal 16S RNA primers and subjected to deep sequencing using the Roche 454 technology. Bioinformatic analysis will be performed in collaboration with NIZO (The Dutch Dairy Institute, Ede). In this way we will obtain a comprehensive analysis of the relative quantities of various bacterial taxa in the samples, under steady state conditions (normal skin, lesional psoriasis and atopic dermatitis skin).
Volunteers will receive for sample collection in one visit a ¤20 fee and travel expenses.

group 2: tape-stripped skin
In this group we will subject normal skin to superficial injury by repeated application of adhesive tape on skin, which removes one layer of stratum corneum with its constituent microorganisms. This can be done 20-30 times before hitting the first viable cell layer which is where the sterile compartment of the epidermis is likely to start. This procedure creates a superficial injury whilst the normal skin flora is largely removed. The epidermis will recover within a week by increasing cell proliferation and altered differentiation. We will sample control skin at day 0 and day 14; tape-stripped skin is samples at day 0, day 1, day 2, day 4, day 7 and day 14. Bacterial DNA extraction and analysis is performed as described above. Volunteers will receive a ¤180 fee and travel expenses.

group 6: patients during therapy
Individuals from group 6 are patients treated by biologicals (anti-TNF). These are patients that receive regular therapy and will be asked to participate in the following study: During a 3 month period following the start of the treatment they will be asked to provide stool samples, throat swabs and skin swabs/scrapings during regular control visits in our clinic (4 times). They will receive a fee of ¤160 for participation. The samples will be stored for further analysis (deep sequencing of the microbiome). In parallel, control skin, throat swabs and stools from healthy volunteers will be analysed at two time points (3 month interval). They will receive a fee of ¤80 and reimbursement of travel expenses.
The analyses of these samples will teach us if the skin microbiome of lesional skin normalizes under therapy, and if changes precede or follow clinical improvement. It also allows us to correlate the skin changes with changes of the microbiome in other locations (throat and gut).

Study burden and risks

No significant risks or burden. No benefit.

Contacts

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Trial sites

Listed location countries

Netherlands

Eligibility criteria

Age
Adults (18-64 years)
Elderly (65 years and older)

Inclusion criteria

Patients with:
1. chronic plaque psoriasis
2. psoriasis inversa
3. atopic dermatitis

Exclusion criteria

individuals < 18 y of age are excluded

Study design

Design

Study type:	Observational non invasive
Intervention model:	Other
Allocation:	Non-randomized controlled trial
Masking:	Open (masking not used)
Control:	Active
Primary purpose:	Basic science

Recruitment

NL

Recruitment status:	Recruiting
Start date (anticipated):	01-09-2010
Enrollment:	80
Type:	Actual

Ethics review

Approved WMO

Date:	02-06-2010
Application type:	First submission
Review commission:	CMO regio Arnhem-Nijmegen (Nijmegen)

Study registrations

Followed up by the following (possibly more current) registration

No registrations found.

Other (possibly less up-to-date) registrations in this register

No registrations found.

In other registers

Register	ID
CCMO	NL30955.091.10