Linking Gut to Skin

The Microbiome and Chronic Inflammatory Diseases

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Colophon

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De link tussen darm en huid
Het microbioom en chronische inflammatoire aandoeningen

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VOORWOORD

Een persoonlijke noot staat soms in schril contrast met de harde wetenschap. Alhoewel wij, als zijnde wetenschappers, de resultaten op een zo objectief mogelijke manier trachten weer te geven, zal de subjectiviteit in dit proefschrift door de regels heen te lezen zijn. Dit is echter geen zeldzaamheid. Hierbij spreek ik in het bijzonder over de onderzoeksvelden waar (nog) geen eenduidige gedeelde zienswijze over bestaat. Het thema van dit proefschrift is hier bij uitstek een voorbeeld van. De complexiteit van de gezondheid van de mens zal immer tot discussie en verdeeldheid leiden. Men zou subjectiviteit een valkuil van de wetenschap kunnen noemen, echter zal de interesse snel verloren gaan bij het ontbreken hiervan. De ervaring schept de mens, alsmede de wetenschap. Bewust zijnde van de valkuilen van de geest en van de wetenschap, is dit proefschrift na het voeren van vele discussies met de co-auteurs met uiterste zorg tot stand gekomen. Er is getracht de diverse interpretatie mogelijkheden van de resultaten te belichten vanuit een open zienswijze. Want de mens die gelooft dat hij alleen de waarheid in pacht heeft en alleen binnen zijn eigen voorspelde paden blijft lopen, zal de grootste wonderen des levens aan zich voorbij zien gaan. Laat een ieder die dit proefschrift leest met een open visie de discussie aanvangen, want alleen dat zal leiden tot de beste vooruitgang van de wetenschap, en daarmee uiteindelijk tot de beste patiëntenzorg.

"The greatest tragedy of human existence is the illusion of separateness"

(Albert Einstein)

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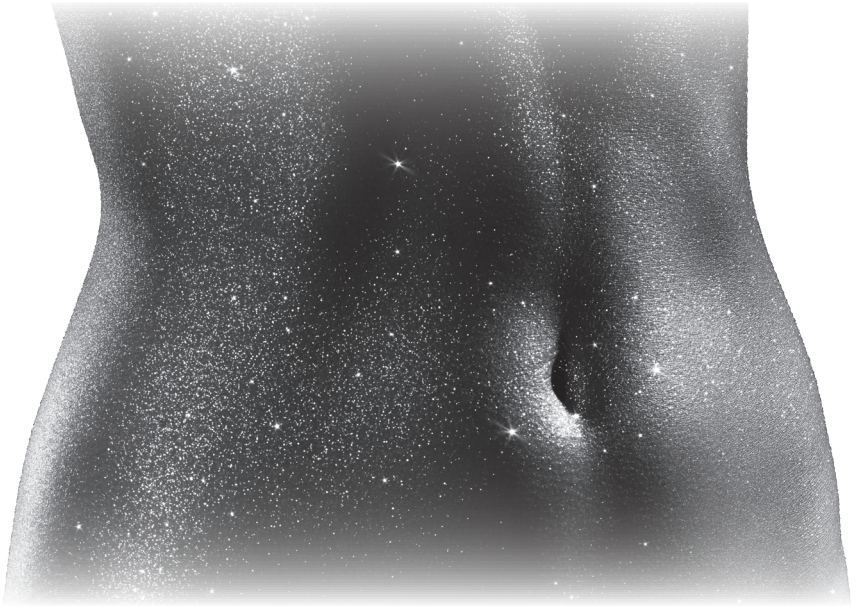

CHAPTER 1

Introduction and outline of the thesis

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M.P. Peppelenbosch
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The microbiome and psoriatic arthritis. Current Rheumatology Reports 2014
The gut microbiome dysbiosis and its potential role in psoriatic arthritis. International Journal of Clinical Rheumatology 2014

INTRODUCTION

Background

The past decades, chronic inflammatory diseases have become one of the major health concerns of modern life. Their incidence continues to increase while their complex etiology is still not fully explored. Chronic inflammatory diseases as a group consist of a large variety of diseases, which can manifest in different organs such as the gut, skin and joints. Although different organs can be affected, different chronic inflammatory diseases share common pathogenic pathways. Most current studies and treatments focus on one specific disease, which leaves the link between the diverse chronic inflammatory diseases unexposed. From previous studies it is known that the occurrence of one inflammatory immune-mediated disease in a patient increases the chances of developing a second disease in the same patient. In this thesis we will emphasize the presence of a link between gut and skin, focusing on the chronic inflammatory diseases inflammatory bowel disease (IBD), psoriasis, and hidradenitis suppurativa (HS). The role of the microbiome in these diseases, as explained below, will play a major part in this thesis.

The link between IBD, psoriasis and HS

The complex etiology of chronic inflammatory diseases is highly intriguing. Genetic, immunological, microbial and environmental factors all seem to contribute to their pathogenesis, and appear to overlap between these diseases. Chronic inflammatory diseases are most seen as systemic diseases, each with their specific primary target organ. For example IBD can be seen as a systemic disease with the primary disease site at the intestines, presenting with extra-intestinal manifestations on the skin.

IBD, comprising Crohn's disease (CD) and ulcerative colitis (UC) is a chronic inflammatory disease entity of the intestines. In CD, transmural inflammation can be found in the entire gastrointestinal tract from mouth to anus, while in UC inflammation is limited to the mucosal layer in the colon. The prevalence of IBD in the general population in the Netherland is estimated to be 0.4%[1]. Extra-intestinal manifestations including skin manifestations are common in IBD and frequently described IBD-associated skin diseases are psoriasis and HS[2, 3].

Psoriasis, a chronic inflammatory skin disease characterized by erythematosquamous plaques, affects approximately 2-3% of the general population. In CD the prevalence of psoriasis is estimated to be 10%, while in UC this is 5.7%[4, 5]. Psoriasis is strongly associated with the chronic inflammatory joint disease, psoriatic arthritis (PsA). PsA belongs to the family of spondylarthritides (SpA) and presents with pain and stiffness in the joints[6]. Members of the SpA family include ankylosing spondylitis, reactive arthritis, undifferentiated SpA, arthritis in IBD, and the pauci-articular and axial forms of PsA. All subtypes

share clinical, radiological, and genetic characteristics evidently different from those of other chronic inflammatory joint diseases, for example rheumatoid arthritis (RA). In a large cross-sectional observational study, one-third of the psoriasis patients were diagnosed with PsA after 30 years of being diagnosed with psoriasis[7]. Psoriasis lesions precede the presence of PsA in almost 70% of patients; in 20% arthritis starts before the onset of psoriasis; and in 10% both develop simultaneously[8]. However, the severity of PsA does not reflect the severity of the psoriasis, although both diseases are mediated by the immune system. Both psoriasis and PsA are associated with several environmental influences, including streptococcal pharyngitis, stressful life events, low humidity, drugs, HIV infection, trauma, smoking, and obesity[9]. Traumatic injury and tissue damage can cause inflammation and, consequently, T cell activation. A characteristic of psoriasis is the "Koebner phenomenon", referring to the development of psoriatic skin lesions at the sites of physical trauma.

In addition, a less familiar skin disease, HS, has more recently also been reported to be associated with IBD, and is able to even mimic CD. HS is thought to affect up to 4% of the general population[10, 11]. In IBD its prevalence is estimated to be between 6.8-23%[12, 13]. HS is a chronic inflammatory skin disease manifesting primarily in inverse regions such as the axillary, inguinal and perianal region. Similarities with CD include abscess and fistula formation in the perianal area. HS is a disabling skin disease due to excessive scaring and the symptoms occurring in private areas.

IBD, psoriasis and HS are chronic inflammatory immune-mediated diseases of the intestines and skin, respectively. Environmental factors seem to induce these inflammatory diseases in individuals with underlying genetic susceptibility. In this thesis, we hypothesize that common inflammatory pathways of the "gut-skin-axis" could be induced or mediated by the microbiome.

The human bacterial microbiome

Human intestinal microorganisms colonizing different anatomical locations within the human body (the so-called microbiome) are one of the multiple factors involved in the genesis and regulation of pathogenic inflammation. The notion that the human microbiome has a profound effect on chronic inflammatory immune-mediated diseases has become increasingly accepted in recent years. Although the skin, oral cavity, upper respiratory tract, and female genital tract are all colonized with microorganisms, the human gastrointestinal (GI) tract contains by far the most densely populated ecosystem, consisting of bacteria, archaea, and eukaryotic microorganisms. The intestine of a healthy adult harbors ~10^{14} non-eukaryotic cells, of which bacteria are by far the most abundant and well-studied microorganisms. All bacterial life can be categorized in a number of phyla, which taxonomic rank is further subdivided into classes, orders, families, genera and species of bacteria. It is believed that the bacterial intestinal community structure consists of

a relatively limited number of dominating phyla, which include *Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria, Verrucomicrobia, and Fusobacteria*. At the genus level, *Bacteroides* species outcompete other microbes, being the most abundant colonizers of the human gut, followed by species of the genera *Faecalibacterium*, *Bifidobacterium*, *Lachnospiraceae*, *Roseburia*, and *Alistipes*[14]. The composition of the microbiome is specific to each individual. In the postnatal period, and childhood the microbiome composition is formed, and remains relatively stable during most of adult life. However, in the elderly, changes of composition are often observed again[15]. Of note, aging in the elderly is often concurrent with other factors, including drug use, comorbidities, and malnutrition, which taken together might possibly explain this observation. Other environmental factors, for example diet, infections, stress, disease, and drug use (e.g. antibiotics) can distort the composition of the microbiome throughout life[16-19]. Note that these environmental factors are the same risk factors which are thought to trigger the development of chronic inflammatory diseases. The magnitude of the effect of the microbiome on the human entity is not yet fully elucidated. The intestinal microbiome is able to modulate both the mucosal and the systemic immune systems, thereby exerting extra-intestinal effects at distant human body sites[20]. Thus the gut microbiome may be involved in the development of chronic inflammatory diseases beyond the intestines. Many recent association and case-control studies have linked alterations in gut microbiome composition with diseases and traits including inflammatory bowel disease (IBD), allergic diseases, RA, type I diabetes, and encephalomyelitis[21-24]. It has been established that the intestinal microbiota in mice is able to convert L-carnitine from red meat into substrates that are thought to promote atherosclerosis[25]. An important recent finding regarding the intestinal bacterial community is that individuals with a low GI-tract bacterial richness have an inflammatory phenotype when compared with high-bacterial-richness individuals[26]. Furthermore, only a few bacterial species are needed to distinguish between individuals with high and low bacterial richness and to indicate the inflammatory phenotype[27]. Among these species is *Faecalibacterium* (formerly *Fusobacterium*) *prausnitzii*, which belongs to the *Clostridium leptum* subgroup (cluster IV) of the phylum *Firmicutes* and is one of the most abundant bacteria in the human gut eco-system. A decrease in the abundance of *F. prausnitzii* and a decreased microbial diversity are common to the intestinal microbiome of different IBD populations (Europe, Japan and the USA)[28]. Therefore, analyses of specific dysbiosis-associated bacteria like *F. prausnitzii* introduces new opportunities to identify targets and predictive biomarkers as well as to define new strategies for pharmacological, immunomodulatory vaccines and nutritional applications relevant to patients with chronic inflammatory diseases.

The human fungal microbiome

The human fungal and yeast microbial community (mycobiome) is only recently recognized as being complex and important for health. Although some fungi are associated with the human skin, most interactions of the human host with yeast and fungi take place in the intestines. Most fungi in skin, genital, and gastrointestinal mucosa are present without causing disease. Although the commensal *Candida* can become pathogenic, this typically happens in immunosuppressed individuals. Immune system defects, genetic predispositions, a breached mucosal barrier, and microbial dysbiosis can all contribute to fungal infection and invasion. Upon a defect in the fungal recognition, specific pathogenic species can gain access to the host tissues and increase the severity of the inflammation[29]. Skin, epithelial, and mucosal surfaces prevent fungi from invading the deeper tissue through the expression of antimicrobial peptides, the action of the complement system, and phagocytosis assisted by the production of secretory antibodies that target fungi. Inflammatory reactions to fungi drive the adaptive immune responses. Of particular importance for the anti-fungal adaptive responses is the generation of Th17 cells[30]. IL-17 and IL-17RA signaling have been revealed to be essential for controlling *Candida* overgrowth and invasion[31]. Inborn IL-17/R defects are strongly correlated with a severe form of IBD, and genetic mutations in genes coding for IL-17RA and IL-17 F lead to chronic mucocutaneous candidiasis, characterized by recurrent or persistent infection of the skin, nails, and oral and genital mucosa caused by *C. albicans*[31]. This suggests that host immune responses to *Candida* and other members of the fungal microbiome are important for preventing invasive disease and for control of the commensal growth at mucosal surfaces.

Studies in mice have revealed increased *Candida* colonization in the stomachs of antibiotic-treated mice[32, 33], and germ-free mice are highly susceptible to *Candida* infection[34]. Similarly, prolonged antibiotic treatment can predispose humans to fungal infection[30]. These results indicate that the bacterial community is of utmost importance acting as a barrier against the outgrowth of fungal pathogens. Vice versa, commensal fungi can also protect the host from bacterial pathogens. For instance, strains of *S. cerevisiae boulardii* have been successfully used for treating *Clostridium difficile*-induced diarrhea[35, 36] and *Salmonella*-induced gastroenteritis[37]. The mechanism of protection seems to be a combination of direct competition with intestinal pathogens, interaction with the host immune system, and interaction with the intestinal epithelium[37, 38]. These studies underline the fact that fungi and bacteria have often been seen as pathogens and that their function as commensals and protection for the host has been underappreciated.

Intestinal diseases are likely to compromise the host's ability to control the fungal microbiome, leading to a shift in its composition. This could be of importance for patients suffering from IBD or from related chronic inflammatory diseases, such as psoriasis, PsA and HS.

The Microbiome in Immunity

The intestinal microbiome has an important function in the development of a healthy immune system[39]. In a healthy individual the host and microbes interact in a harmonious way. Auto-immunity is a complex process, in which misdirected immune responses can cause chronic inflammatory diseases. It is not clear what initiates this process. It is known that the presence of one auto-immune disease in a patient increases the risk of developing another auto-immune disease in the same patient, and in their family. An overlap between different auto-immune diseases clearly exists, an example of which is the prevalent co-existence of psoriasis, PsA and IBD, which share several genetic and environmental factors[40]. The gut microbiome could be a crucial factor in this observation, because a disbalanced composition of the microbiome, also known as "dysbiosis", can affect the immune homeostasis and thereby cause inflammation and auto-immune disease[41]. A reduced abundance of commensal bacteria, for example *lactobacilli*, *bifidobacteria*, and *Faecalibacterium prausnitzii*, and/or increased abundance of such pathogens as *Escherichia coli*, *Salmonella*, and *Helicobacter* might contribute to this dysbiosis (Figure 1). In addition, a primary defect of the immune system might result in recognition of commensal bacteria as pathogens, and therefore cause inflammation. Consequently, chronic inflammatory immune-mediated diseases might develop.

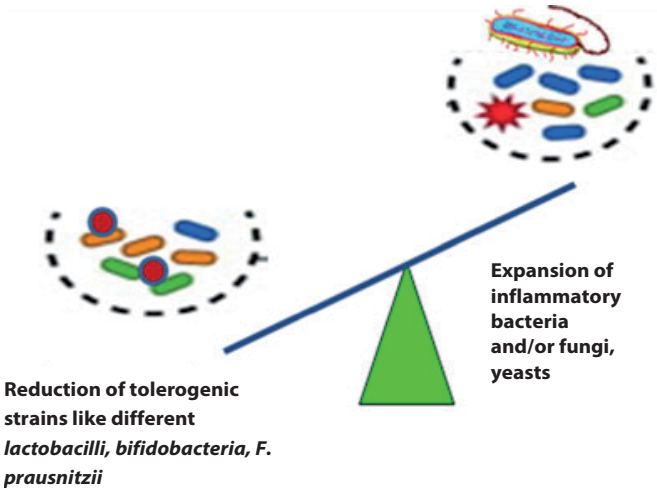

Figure 1. Gut dysbiosis may contribute to chronic inflammatory diseases by overgrowth of inflammatory strains of bacteria and fungi/yeasts, by reduction of tolerogenic strains including *F. prausnitzii*, or by acombination of both.

Interaction between the Microbiome and the T cell populations

The immune system is able to affect the composition of the microbiome, and the microbiome can modulate the immune system, and therefore the interaction is bidirectional[42]. Aberrant activation of T-cells plays an important role in IBD, PsA, psoriasis and HS, however, the trigger for this over-activation is as $CD8^+$ T cell expansions have been observed[44-46], indicating that T cells are important in the pathogenesis. Gut dysbiosis can determine the direction of differentiation of naive $CD4^+$ T cells into either effector T cells or regulatory T cells (Tregs). The direction of differentiation of $CD4^+$ naïve T cells is affected by a variety of transcription factors and cytokines, and the expression of these can be modulated by the gut microbiome. The balance between Tregs and the T cell effector subsets Th1, Th2, and Th17 is of crucial importance for immune homeostasis, and determine tolerance for non-pathogenic antigens, including antigens from food. An imbalance in T-cell subsets can lead to chronic inflammation in the gut, skin or joint.

A lot of research into the effect of the microbiome on immunity has been done with germ-free animals, which are animals raised in a sterile environment and thus unexposed to microorganisms. Hormannsperger et al. found that these germ-free animals had Th2 dominated immune responses. Interestingly, when the gut of the germ-free mice was colonized by microbial communities the T cell balance was easily restored. This indicates that microorganisms are essential for maintaining balance in the differentiation of naïve T cells. However, the complexity of the interaction of the microbiome and the immune system extends beyond this observation. Using specific colonization of germ-free mice, Th17 induction was observed to be dependent on the presence of a particular bacterial species, the segmented filamentous bacteria. Microbial ATP was revealed to be responsible for the differentiation of Th17 cells, whereas lipopolysaccharide A from *Bacteroides fragilis* was found to induce Treg cells, thereby reducing the response of Th17[47]. Another study found that the segmented filamentous bacteria induced auto-immune arthritis through the ability to specifically promote the Th17 subset[48]. Segmented filamentous bacteria have been found to be essential for maturation of $CD4^+$ and $CD8^+$ T cells in mice[49]. Both $CD4^+$ and $CD8^+$ T cells are capable of secreting IL-17[50]. The segmented filamentous bacteria, however, have so far not been detected in the human small intestine. This indicates that in humans, other species of the bacterial or fungal gut microbiome might have a large effect on the immune system and cause extra-intestinal inflammatory disease. Therefore we have to interpret microbiome data retrieved from mice studies with caution before applying it to humans.

Recent literature has focused on the effect of Th17, an important T cell subset with a significant role in the pathogenesis of several inflammatory diseases, including CD, RA, psoriasis, and PsA[21, 51-53]. Th17 is a producer of the cytokines IL-17A, IL-17F, TNF, IL-21, and IL-22, and is able to stimulate osteoclast formation and bone resorption[44]. In a recent psoriasis study, specific blocking of Th17 signaling in patients induced a larger response than blocking either TNF-α or IL-22. Th17 would therefore seem to be the main inducer

of inflammation in psoriasis[51]. Both psoriasis and PsA are associated with an increased risk of cardiovascular events[54, 55], and IL-17 may be responsible for this association[56]. Thus it seems that the effect of Th17 extends beyond the skin and gut, and might be able to affect multiple organs. And, because there is an important relationship between Th17 and the bacterial and/or fungal microbiome, this may be one of the mechanisms by which the microbiome affects the development of chronic inflammatory diseases. Whether bacteria or fungi/yeasts might be the primary or secondary factor in the etiology of chronic immune-mediated inflammatory disease is as yet unknown.

The Skin Microbiome

Similar to the gut, the skin represents another important interface between the host and the environment. Microbial profiling has revealed the presence of highly diverse commensal communities along distinct topographical skin sites[57, 58]. The skin microbiome has been implicated in the pathogenesis of psoriasis[59, 60] and given that up to 30% of psoriasis patients develop PsA, the skin microbiome may also be involved in the etiopathogenesis of PsA. Recently, Castelino et al. postulated a causal effect for the skin microbiome in PsA, suggesting that individuals with psoriasis who develop PsA might have a different composition of the skin microbiome compared with psoriasis patients without PsA[60]. However, Naik et al. revealed that the skin seems to contain an autonomous microbiome, affecting the local T cell subsets and local inflammation, but does not seem to affect distant sites[58]. This seems in contrast to the gut microbiome, which does have the ability to affect the systemic immune system and distant organs[20]. Taurog et al. found that a germ-free environment was able to prevent development of gut and joint inflammatory diseases in HLA-B27 transgenic rats. In contrast, the skin and genital lesions were unaffected by the germ-free environment in this study[61]. Possibly, a Th17-dominated immune response in the gut and joint will not necessarily manifest in the skin as well, because distinct microbes in the skin may prevent the development of psoriasis plaques in some individuals. However, no data about this subject is available to date.

Linking Gut, Skin and Joint

The microbiome has now been appreciated as separate "organ" to emphasize its importance to health and disease, similar to the immune system. Microbiome dysbiosis may be the conductor, consequence or mediator, of the common inflammatory pathways seen in these chronic inflammatory diseases. In the etiology of IBD, the role of the gut microbiome has been widely recognized. However, the role of the gut microbiome in skin and joint diseases had not been investigated until date.

Research indicating the relationship of PsA and the gut has been primarily performed within the SpA group[62]. The presence of gut inflammation in psoriasis and/or PsA has been reported in several studies[63-66]. As well as the connection between skin and joint

and between gut and joint, a link also exists between gut and skin. Psoriasis and IBD share several have similarities in genetics and pathogenesis[67, 68]. Furthermore, because stress-induced skin inflammation is associated with an alteration of the composition of the intestinal microbiome, even a gut – brain – skin axis might be present[69].

Since the microbiome plays a role in IBD, a role for these intestinal microbes in psoriasis and PsA might also be envisioned. Previous studies already have shown microbiome involvement in diseases such as RA and atopic dermatitis[70, 71].

Unlike the skin and intestines, where pathogens and commensals live together in symbiosis, the joint does not contain its "own" microbiome. This begs the question as to how microbes, in particular intestinal microbes, may affect local synovial inflammation. One such mechanism could be the above mentioned triggering of the immune system in the gut, resulting in systemic effects. In addition, while the joint was previously regarded as a sterile environment, it has been demonstrated that intestinal microbes or their components are able to translocate into the blood stream[72, 73], and may thus end up elsewhere in the body. This bacterial (component) translocation is partly due to a leaky gut caused by, for example, the production of inflammatory cytokines in the intestinal mucosa. In addition, bacteria are taken up by phagocytes at the mucosal barrier, and ineffective apoptosis and clearance of phagocytes, as has been suggested to occur in IBD, may contribute to translocation of bacterial components to distant sites. It is conceivable that bacteria or fungi translocate from the gut into the circulation toward the joints, a location easily accessible for antigens. This may result in a rapid T cell invasion and inflammation since these T cells might be previously activated in the gut. In genetically susceptible individuals, inflammatory arthritis might transit into a chronic form of arthritis such as PsA, whereas in subjects without an underlying susceptibility the body could potentially resolve the inflammation itself. In such a scenario, a microbial dysbiosis in the gut, potentially in interaction with the skin, can result in joint symptoms via migration from the circulation (Figure 2).

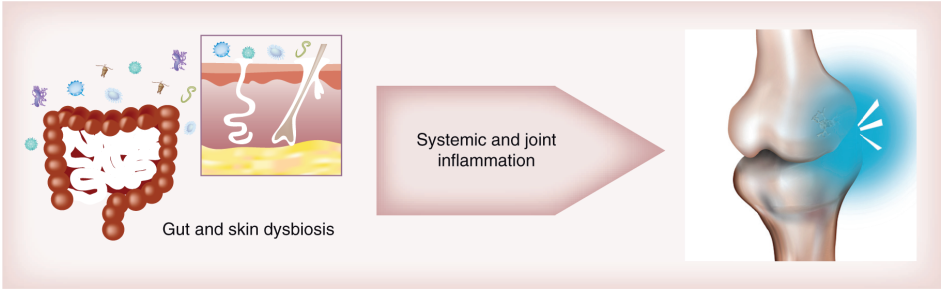

Figure 2. Gut dysbiosis, possibly in interaction with skin dysbiosis, can underlie auto-immune reactions associated with joint inflammation.

Thus, microbial factors might form a connection between the skin, joint and gut diseases and the immune disturbances found in these diseases. The interaction of microbes with genetic, immunologic and other environmental factors such as lifestyle and diet makes this a highly complex study matter.

OUTLINE OF THE THESIS

As discussed above, IBD, psoriasis and HS are all chronic inflammatory incurable diseases of respectively intestines and skin sharing several similarities in etiology and therapy. There is a high co-occurrence of these diseases, although the underlying mechanism and characteristics of this category of patients is unclear. In this thesis, we investigated whether patients with both IBD and a skin disease such as psoriasis or HS exhibit specific characteristics and have a distinct disease course. Furthermore, to date, we know that intestinal microbes are involved in the etiology of IBD, but for the skin diseases this is unknown. Therefore, in this thesis, we focused on the microbial signatures of these diseases.

In the first part of the thesis, we examined the clinical characteristics of patients with concurrent IBD and a skin disease. In **Chapter 2** we investigated the prevalence of IBD in a psoriasis cohort. In addition, we investigated whether patients with concurrent IBD and psoriasis carry a distinct phenotype compared to a cohort of patients with only psoriasis and only IBD. In **Chapter 3** we described the clinical characteristics of patients with concurrent IBD and HS. In **Chapter 4**, we investigated whether dietary factors worsened bowel and/or skin symptoms in IBD patients with and without concomitant skin diseases.

In the second part of the thesis we focused on the microbial involvement in IBD and/or the associated skin diseases psoriasis or HS. In **Chapter 5** we investigated whether the faecal abundance of *F. prausnitzii* and/or *E. coli* was similarly altered in the skin diseases psoriasis and HS in comparison to IBD patients and healthy controls. Following the results of Chapter 5, we investigated the composition of the gut- and skin microbiota of HS patients compared to healthy controls on a deeper level in **Chapter 6**, in search for the missing pathogenic link between HS and IBD. We subsequently investigated the influence of UVB light on the gut- and skin microbiome in patients with skin diseases in **Chapter 7**. In **Chapter 8** we transited to the fungal microbiome and investigated the abundance of the "baker's yeast" *S. cerevisiae* in psoriasis patients, and whether dimethylfumarate therapy affects the abundance of this yeast (*in vivo* and *in vitro*). Finally, in **Chapter 9** we reviewed the state-of-the-art of gut microbiota developments, with emphasis on IBD.

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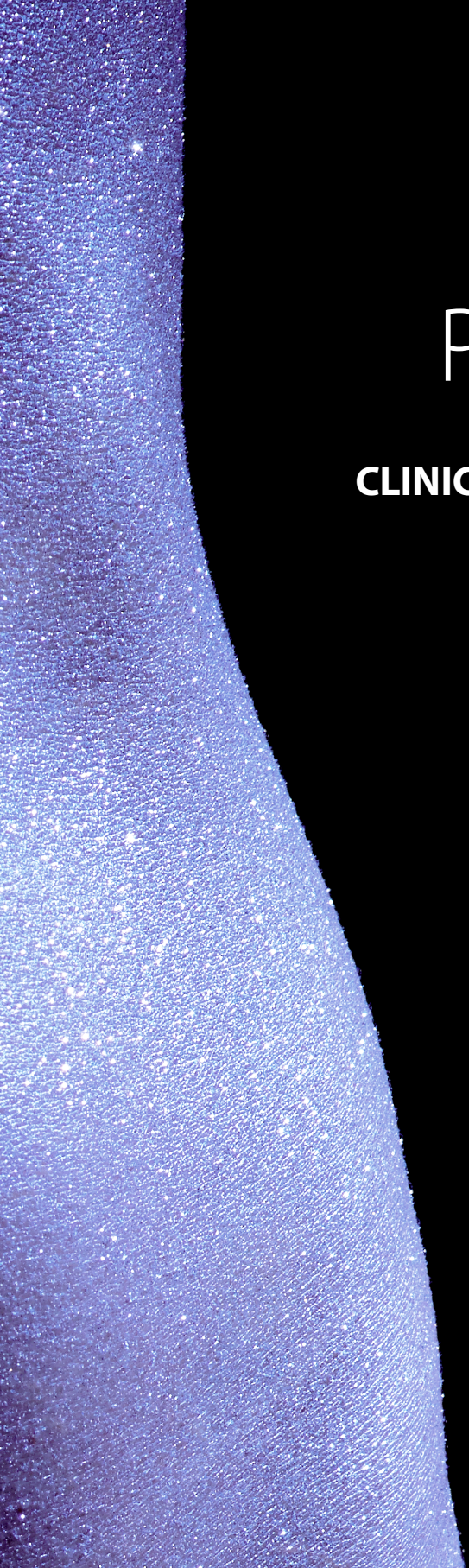
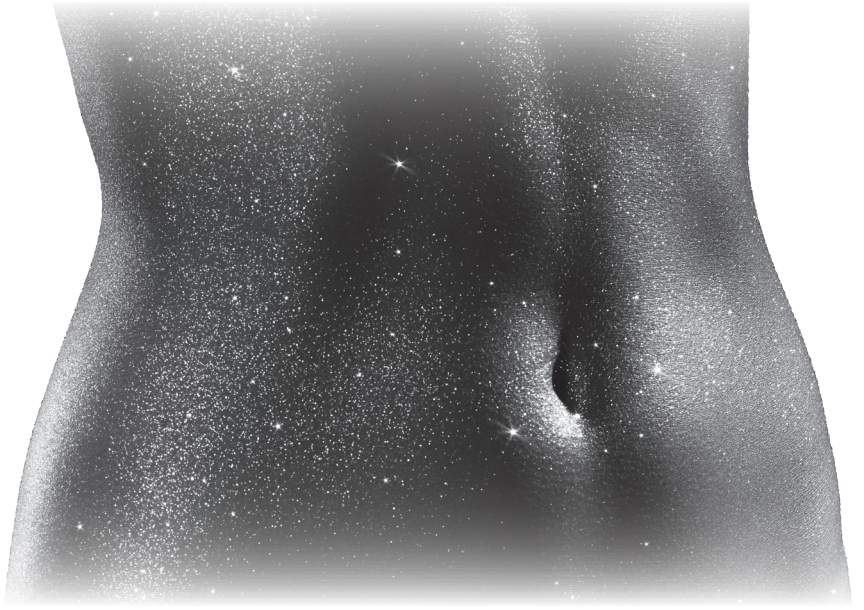


PART I

CLINICAL SIGNATURES



CHAPTER 2

Prevalence and phenotype of patients with concurrent psoriasis and inflammatory bowel disease

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ABSTRACT

Background

Psoriasis, psoriatic arthritis (PsA) and inflammatory bowel disease (IBD) are related inflammatory immune-mediated diseases, with considerable overlap. However, it is as yet unclear whether co-occurrence of these diseases affects disease course and characteristics of the individual complaints.

Objectives

To identify the prevalence of IBD and PsA in a psoriasis cohort and to examine whether patients with concurrent psoriasis and IBD carry a distinct phenotype.

Methods

Data of all psoriasis patients visiting a general hospital in the Netherlands between 2009-2014, were retrospectively retrieved from electronic patient files. In addition, clinical characteristics of patients with concurrent psoriasis and IBD (n=40) were compared with psoriasis-only (n=1643) and IBD-only (n=385) cohorts.

Results

Among 1669 hospital-based psoriasis patients, the prevalence of PsA was 12.2% (n=203, 95%CI 10.5-13.7) and of IBD 1.6% (n=26, 95%CI 1.0-2.2), including 12 Crohn's disease (CD) and 14 ulcerative colitis (UC) patients. Psoriasis-PsA patients were more likely to have IBD than psoriasis-only (3.0 vs 1.4%).

Psoriasis-CD patients were younger at CD diagnosis (20.0 vs 32.0 yrs, p=0.001), and psoriasis diagnosis (28.0 vs 43.5 yrs, p=0.004) than psoriasis-only patients. Psoriasis-IBD patients had a mild psoriasis phenotype similar to psoriasis-only patients, but the CD phenotype was significantly more severe than in CD-only patients.

Conclusions

The prevalence of IBD in psoriasis was approximately four times higher than in the general population, with the highest risk for psoriasis-PsA patients. Psoriasis-IBD patients have a mild (early-onset) psoriasis but an earlier-onset and severe CD phenotype.

INTRODUCTION

Psoriasis, psoriatic arthritis (PsA) and inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), are chronic inflammatory diseases sharing similar pathogenic pathways while targeting different organs, respectively skin, joints and intestines[1]. The prevalence of psoriasis in the general population is around 2%[2], but increases to approximately 10% in CD and 5.7% in UC[3, 4]. The prevalence of IBD in the general population is approximately 0.4%[5], while in a previous study, the prevalence of IBD in psoriasis was around 1.0%[6]. Furthermore in a case-control prospective study it was described that of all IBD patients being dermatological referred (n=251), 25% obtained the diagnosis of psoriasis (n=62)[7].

While limited data is available regarding the phenotype of patients with concomitant psoriasis and IBD, they might exhibit different characteristics compared to patients with one disease[7]. Knowledge about co-occurrence of PsA is hereby of importance, as psoriasis and PsA are closely related.

The coexistence of psoriasis, PsA and IBD, can potentially be explained by their common genetic background[8, 9] and similar pathogenic pathways[1, 10]. The current assumption is that psoriasis and/or IBD develop as a result of a combination of environmental and immunological factors in genetically susceptible individuals. An impaired interaction between the immune system[11, 12] and the microbiota contributes to IBD's etiology[13], but also possibly to that of psoriasis[14-16]. Therapies for both diseases are overlapping, as immunosuppressants are the cornerstone of their treatment.

To be able to recognize the significance of the associations of these disorders, and to further explore their etiology, it is fundamental to expand the knowledge about the coexistent prevalence and shared features of these diseases. The aim of this study was to determine the prevalence of IBD and PsA in a psoriasis cohort. In addition, we investigated whether patients with both psoriasis and IBD carry a distinct phenotype compared to psoriasis-only and IBD-only patients.

PATIENTS AND METHODS

Prevalence of IBD and PsA in a psoriasis cohort

To assess the prevalence of IBD in a psoriasis cohort, a retrospective chart review was performed in a general hospital, Medical Center Leeuwarden (MCL), in the north of the Netherlands. The institutional research board approved the protocol. All patients diagnosed with psoriasis by a dermatologist between 01-01-2009 and 01-06-2014 were included (n=1669). The patient records were screened for the comorbidities PsA and IBD. The diagnosis of psoriasis, IBD and/or PsA was confirmed by the Dutch DBC system

(diagnose-treatment combination), a detailed Dutch health care code which all physicians are required to generate for health care insurance reimbursement purposes.[17] Furthermore PsA diagnosis was only scored when made by a rheumatologist, whereas IBD diagnosis was confirmed by a gastroenterologist, based on endoscopy, as registered in the electronic patients' charts. Clinical characteristics were collected from the patients' medical charts, these data included visit reports, letters and registrations from Departments of Dermatology, Rheumatology and Gastroenterology and Hepatology. Characteristics that were retrieved were age at diagnosis of psoriasis, age at diagnosis of PsA, age at diagnosis of IBD, gender, smoking history, psoriasis type, psoriasis severity, medications, comorbidities, character and location of CD, location of UC.

Phenotype of patients with IBD and psoriasis

In order to better investigate the phenotype of the psoriasis-IBD subgroup, we supplemented the psoriasis-IBD patients from the MCL (n=26), with 14 patients with both psoriasis and IBD from a biobank of the Erasmus Medical Center, Rotterdam, The Netherlands (Erasmus MC). These patients all signed written informed consent after the Medical Ethical Committee of the Erasmus MC approved the study. Again, all patients had a confirmed IBD diagnosis by a gastroenterologist based on endoscopy, a confirmed PsA diagnosis by a rheumatologist and a confirmed psoriasis diagnosis by a dermatologist. The clinical features of patients with both IBD and psoriasis were assessed and the analyses were performed separately for CD and UC, and as well for IBD as a group.

Phenotype of patients with psoriasis and IBD compared to patients with only psoriasis

To compare the phenotype of psoriasis-IBD patients to patients with only psoriasis, we compared the features of psoriasis-IBD patients with psoriasis-only patients from the MCL cohort (n=1643). The following characteristics were compared between the groups: gender, age of onset of psoriasis, BMI, medications, psoriasis type, psoriasis severity, and the presence of comorbidities.

Phenotype of patients with psoriasis and IBD compared to patients with only IBD

To compare the phenotype of psoriasis-IBD patients to IBD-only patients, we compared the features of psoriasis-IBD patients with IBD-only patients of the Delta Cohort, a population-based inception cohort in the Netherlands[18]. In this cohort psoriasis comorbidity was registered, which were excluded for this study (n=4). Patients with undifferentiated IBD (n=27) were also excluded, resulting in a total inclusion of 385 IBD-only patients. The following characteristics were compared between the groups: gender, age of onset of IBD, smoking history, BMI, medications, CD location, behavior and UC location.

Statistical analysis

The prevalence of IBD and PsA in a cohort of psoriasis patients was calculated as the percentage of the total, with a confidence interval (CI). Data were expressed as median, interquartile range (IQR). The prevalence was compared to the prevalence of IBD in the Netherlands demonstrated in an earlier study[5]. Descriptive statistics, independent Mann-Whitney U- tests and chi-square tests were used. Missing data were excluded in the analyses. A two-sided p-value of <0.05 was considered statistically significant. Statistical analyses were performed with SPSS21 (IBM Corp, Armonk, NY).

RESULTS

The prevalence of PsA in psoriasis

PsA was diagnosed in 203 patients in a total of 1669 psoriasis patients (12.2%, 95%CI 10.5-13.7, see Supplementary Table 1 for characteristics).

In patients with both psoriasis and PsA, the majority (n=141, 69.5%) was first diagnosed with psoriasis at median 37.7 years (IQR 26.1-50.3), followed by PsA at 45.2 years (36.4-57.2) with median 5.1 years between both diagnoses (0.9-11.7). In 24.1% (n=49), PsA was the first diagnosis at median 43.4 years (32.1-53.1) with psoriasis as second diagnosis at median 51.8 years (38.5-57.4) and with median 3.0 years between both diagnoses (1.1-8.0). In 6.4% (n=13) of patients, psoriasis and PsA were diagnosed simultaneously at a median of 41.1 years (34.6-66.6).

The prevalence of IBD in psoriasis

Of all 1669 psoriasis patients, 26 patients were diagnosed with IBD (1.6%, 95%CI 1.0-2.2), of which 12 (46.2%) had CD and 14 (53.8%) UC (Supplementary Table 1). With a prevalence of 1.6%, IBD was four times more common in psoriasis than in the general population in the Netherlands (0.4%)[5]. Separate CD and UC analyses revealed that the CD prevalence in 1669 psoriasis patients was 0.7% (95% CI 0.4-1.3), while for UC the prevalence was 0.8% (95%CI 0.5-1.4).

In addition, the IBD prevalence was higher in patients suffering from both psoriasis and PsA (3.0%) compared to psoriasis-only patients (1.4%). Of the six patients suffering from all three diseases, four had CD and two UC.

Phenotype of patients with both psoriasis and IBD compared to patients with only psoriasis

A total of 40 psoriasis-IBD patients were compared with 1643 psoriasis-only patients (Table 1). For none of the psoriasis-IBD patients a conclusive trigger (such as anti-TNF-α treatment) was described for the development of psoriasis.

Sixty percent of psoriasis-IBD patients were female, versus 51.2% of psoriasis-only patients. The BMI of psoriasis-only patients was comparable to that of psoriasis-IBD patients (26.8 vs 25.3). Psoriasis-IBD patients more often used anti-TNF-α therapy than psoriasis-only patients (p=0.025, 42.3% vs 21.9%). The same was observed for steroids (p<0.001, 61.5% vs 2.3%), methotrexate (p=0.001, 46.2% vs 16.7%) and NSAIDs (p=0.006, 23.1% vs 6.4%). As expected, IBD-specific medications (azathioprine, mesalazine, sulfasalazine, mercaptopurine) were significantly more frequently prescribed in psoriasis-IBD patients than in psoriasis-only patients (p<0.001).

Psoriasis was diagnosed at a significant younger age in psoriasis-CD patients compared to psoriasis-only patients (p=0.001, 28.0 vs 43.5 years). Most psoriasis-IBD patients had a mild psoriasis phenotype, similar to psoriasis-only patients (57.7% vs 58.0%). Plaque type was the most common subtype in both psoriasis-IBD patients and psoriasis-only patients (65.0% vs 70.9%). Notably, psoriasis-UC patients had more psoriasis capitis than psoriasis-only patients (p=0.045). PsA frequency was significantly higher in psoriasis-IBD (22.5%), than in psoriasis-only patients (12.0%, p=0.045), and more often associated with psoriasis-CD (28.6%) than with psoriasis-UC (15.8%). Furthermore, psoriasis-IBD patients had significantly more concomitant auto-immune diseases, other than PsA, than psoriasis-only patients (p=0.033, 10.0% vs 2.9%).

Table 1. Characteristics of psoriasis-IBD vs psoriasis-only patients

	Psor - CD	**Psor - UC**	**Psor - IBD**	**Psor - only**
Patients n (%)	21 (52.5)	19 (47.5)	40 (100)	1643 (100)
Gender n female (%)	12 (57.1)	12 (63.2)	24 (60.0)	841 (51.2)
BMI[1] median(IQR)	26.8 (24.4-30.0)	24.9 (20.7-27.8)	25.3 (21.7-31.5)	26.8 (24.4-30.0)
Age at Psor diagnosis[2] median (IQR)	28.0 (25.0-37.0)	46.0 (38.7-54.8)	37.0 (25.6-49.6)	43.5 (28.4-59.2)
Type Psor[3] n (%)				
Plaque	15 (71.4)	11 (57.9)	26 (65.0)	1165 (70.9)
Guttate	4 (23.8)	1 (5.3)	5 (12.5)	237 (14.4)
PPP	2 (9.5)	3 (15.8)	5 (12.5)	104 (6.3)
Inverse	3 (14.3)	4 (21.1)	7 (17.5)	205 (12.5)
Capitis	6 (28.6)	9 (47.4)	15 (37.5)	374 (22.8)
Unguium	0 (0)	0 (0)	0 (0)	100 (6.1)
PsA[4]	6 (28.6)	3 (15.8)	9 (22.5)	197 (12.0)

Table 1. Characteristics of psoriasis-IBD vs psoriasis-only patients (continued)

	Psor - CD	Psor - UC	Psor - IBD	Psor - only
Severity Psor[5] n (%)				
Mild	6 (50.0)	9 (64.3)	15 (57.7)	953 (58.0)
Mild-moderate	4 (33.3)	1 (7.1)	6 (23.1)	290 (17.7)
Moderate	1 (8.3)	3 (21.4)	4 (15.4)	294 (17.9)
Moderate-Severe	0 (0)	0 (0)	0 (0)	34 (2.1)
Severe	1 (8.3)	0 (0)	1 (3.8)	72 (4.4)
Systemic Medication[6] n (%)				
Anti-TNF-α therapy	6 (50.0)	5 (35.7)	11 (42.3)	360 (21.9)
Dimethylfumarate	2 (16.7)	2 (14.3)	4 (15.4)	220 (13.4)
Methotrexate	4 (33.3)	8 (57.1)	12 (46.2)	274 (16.7)
Ustekinumab	3 (25.0)	0 (0)	3 (11.5)	50 (3.0)
Ciclosporin	0 (0)	5 (35.7)	5 (19.2)	331 (20.1)
Acitretin	0 (0)	1 (7.1)	1 (3.8)	103 (6.3)
NSAIDs	4 (33.3)	2 (14.3)	6 (23.1)	105 (6.4)
Azathioprine	6 (50.0)	6 (42.9)	12 (46.2)	1 (0.1)
Mesalazine	0 (0)	9 (64.3)	9 (34.6)	0 (0)
Sulfasalazine	0 (0)	4 (28.6)	4 (15.4)	37 (2.3)
Steroids	7 (58.3)	9 (64.3)	16 (61.5)	37 (2.3)
Mercaptopurine	1 (8.3)	3 (21.4)	4 (15.4)	0 (0)
Other	1 (8.3)	0 (0)	1 (3.8)	20 (1.2)
Comorbidities[7] n (%)				
Dermatologic	4 (19.0)	4 (21.1)	8 (20.0)	247 (15.0)
Diabetes Mellitus	1 (4.8)	4 (21.1)	5 (12.5)	154 (9.4)
Cardiovascular	0 (0)	4 (21.1)	4 (10.0)	207 (12.6)
Hypertension	3 (14.3)	4 (21.1)	7 (17.5)	310 (18.9)
Auto-immune[8]	2 (9.5)	2 (10.5)	4 (10.0)	48 (2.9)
Psychic	3 (14.3)	1 (5.3)	3 (10.0)	146 (8.9)

Abbreviations: Psor, psoriasis; CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease; BMI, body mass index; PPP, palmoplantar pustulosis; PsA, psoriatic arthritis

[1] BMI missing Psor-UC 14, Psor-CD 6, Psor-only 1512

[2] p=0.004 age at diagnosis Psor-CD vs Psor-only; p=0.928 Psor-UC vs CD-only; p=0.014 Psor-CD vs Psor-UC; missing Psor-only 316

[3] p=0.046 Capitis Psor-UC vs Psor-only

[4] p=0.045 PsA Psor-IBD vs Psor-only

[5] Severity missing Psor-CD 9, Psor-UC 5

[6] History of systemic medication Psor-IBD vs Psor-only: p=0.025 anti-TNF-α- therapy; p=0.001 methotrexate; p=0.006 NSAIDs; P<0.001 steroids, azathioprine, mesalazine, sulfasalazine, mercaptopurine; missing Psor-CD 9, Psor-UC 5; Other Psor-CD 1 olsalazine; Psor-only 11 hydroxychloroquine, 6 leflunomide, 1 colchicine, 1 cyclofosfamide, 1 isotretinoin

[7] Patients could have more than one comorbidity, IBD, Psor and PsA excluded

[8] p=0.033 other auto-immune diseases Psor-IBD vs Psor-only

Phenotype of patients with both psoriasis and IBD compared to patients with only IBD

The characteristics of patients with both psoriasis and IBD (n=40) were compared with IBD-only patients (n=385, Table 2). In the psoriasis-IBD patients, 60% were female, versus 53.0% of IBD-only patients. Psoriasis-IBD patients had a comparable BMI (slightly higher) with IBD-only patients (25.3 vs 23.9). Of the psoriasis-IBD patients 12.5% reported smoking, compared to 27.4% of IBD-only patients. Anti-TNF-α therapy was significantly more frequent prescribed in psoriasis-IBD patients than in IBD-only patients (p=0.023, 42.3% vs 21.0%. In addition, immunosuppressants were more often prescribed in psoriasis-IBD than in IBD-only patients (p<0.001, 88.5% vs 42.1%). Mesalazine was less often prescribed in psoriasis-IBD compared to IBD-only patients (p<0.001, 34.6% vs 84.4%). CD was diagnosed in psoriasis at approximately the same frequency (52.5%) as UC (47.5%), a distribution that was comparable to IBD-only patients (51.7% CD and 48.3% UC). CD was diagnosed at a significant younger age in patients suffering from psoriasis than in CD-only patients (p=0.001, 20.0 vs 32.0 years), which was not the case for UC (p=0.928). Indeed, at IBD diagnosis, psoriasis-CD patients were significantly younger than psoriasis-UC patients (p=0.001, 20.0 vs 41.0 years, Table 2).

The CD location in psoriasis-CD patients was in most patients extensive (ileocolonic, 60.0%), compared to 40.1% of CD-only patients. Psoriasis-UC patients had extensive disease (pancolitis) in 33.3% compared to 18.9% of UC-only patients. Psoriasis-CD patients suffered significantly more often from perianal disease than CD-only patients (p=0.001). In addition, psoriasis-CD patients had significantly more often penetrating disease than CD-only patients (p<0.001).

Phenotype of psoriasis-CD compared to psoriasis-UC patients

At psoriasis diagnosis, psoriasis-CD patients were significantly younger than psoriasis-UC patients (p=0.014, 28.0 vs 46.0 years, Table 1). Also, at IBD diagnosis, psoriasis-CD patients were significantly younger than psoriasis-UC patients (p=0.001, 20.0 vs 41.0 years, Table 2).

Table 2. Characteristics of psoriasis-IBD vs IBD-only patients

	Psor - CD	CD - only	Psor - UC	UC - only	Psor - IBD	IBD - only
Patients n (%)	21 (52.5)	199 (51.7)	19 (47.5)	186 (48.3)	40 (100)	385 (100)
Gender n female (%)	12 (57.1)	113 (56.8)	12 (63.2)	91 (48.9)	24 (60.0)	204 (53.0)
BMI[1]	26.8	23.6	24.9	24.8	25.3	23.9
median (IQR)	(24.4-30.0)	(20.9-26.5)	(20.7-27.8)	(22.3-27.7)	(21.7-31.5)	(21.7-27.1)
Smoking[2] n (%)	4 (19.0)	70 (39.8)	1 (5.3)	23 (14.0)	5 (12.5)	93 (27.4)
Age at IBD diagnosis[3]	20.0	32.0	41.0	40.5	31.0	37.0
median (IQR)	(17.0-31.4)	(23.0-49.0)	(29.9-45.1)	(30.0-51.0)	(18.0-41.5)	(25.5-50.0)
Medication[4] n (%)						
Anti-TNF-α therapy	6 (50.0)	66 (33.2)	5 (35.7)	15 (8.1)	11 (42.3)	81 (21.0)
Immunosuppressant	9 (75.0)	116 (58.3)	14 (100)	(46 (24.7)	23 (88.5)	162 (42.1)
Mesalazine	0 (0)	141 (70.9)	9 (64.3)	184 (98.9)	9 (34.6)	325 (84.4)
Steroids	7 (58.3)	(157 (78.9)	9 (64.3)	112 (60.2)	16 (61.5)	269 (69.9)
Location CD[5] n (%)						
Ileum	3 (15.0)	243 (22.4)	NA	NA	NA	NA
Colon	5 (25.0)	72 (37.5)	NA	NA	NA	NA
Ileum + colon	12 (60.0)	77 (40.1)	NA	NA	NA	NA
CD behavior n (%)						
Inflammatory	8 (42.1)	108 (54.3)	NA	NA	NA	NA
Stricturing	1 (5.3)	38 (19.1)	NA	NA	NA	NA
Penetrating[6]	10 (52.6)	53 (26.6)	NA	NA	NA	NA
Perianal[7]	5 (25.0)	19 (9.5)	NA	NA	NA	NA
Location UC[8] n (%)						
Proctitis	NA	NA	2 (11.1)	55 (29.7)	NA	NA
Left-sided	NA	NA	10 (55.6)	95 (51.4)	NA	NA
Pancolitis	NA	NA	6 (33.3)	35 (18.9)	NA	NA

Abbreviations: Psor, psoriasis; CD, Crohn's disease; UC, ulcerative colitis; IBD, inflammatory bowel disease; BMI, body mass index

[1] Missing Psor-CD 6, Psor-UC 14; CD-only 72, UC-only 94

[2] Missing Psor-IBD UC 1, CD 2, CD-only 23, UC-only 22

[3] p=0.001 Age at diagnosis Psor-CD vs CD-only; p=0.536 Psor-UC vs UC-only; p=0.001 Psor-CD vs Psor-UC.

[4] Location CD missing Psor-CD 1; 7 CD-only patients had only upper GI tract involvement

[5] History of systemic medication Psor-IBD vs IBD-only: p=0.023 anti-TNF-α therapy; p<0.001 immunosuppressant; p<0.001 mesalazine; missing Psor-CD 9, Psor-UC 5

[6] p<0.001 penetrating disease Psor-CD vs CD-only; patients with both stricturing and penetrating disease were included in penetrating group

[7] p=0.001 perianal disease Psor-CD vs CD-only

[8] Location Psor-UC vs UC-only; missing Psor-UC 1, UC-only 1

Order of diagnosis

The subsequent order of diagnosis of psoriasis-CD and psoriasis-UC patients are displayed in respectively Table 3 and Table 4. CD was the first diagnosis in the vast majority of the psoriasis-CD patients (85.7%). Psoriasis-UC patients were almost as often first diagnosed with UC (52.6%) as with psoriasis (47.4%). The age of onset of patients first diagnosed with CD was median 20.0 years at CD diagnosis, while in UC this was median 35.5 years. Moreover, the median time between first and second diagnosis when a patient was first diagnosed with UC, was profoundly longer (16.7 years) than with CD (7.4 years). When psoriasis was diagnosed first, psoriasis-UC patients were older at psoriasis onset than psoriasis-CD patients (median 37.3 vs 25.8 years).

Table 3. Order of diagnosis Psor-CD

	1st diagnosis: Psor	**1st diagnosis: CD**
Number of patients (%)	3 (14.3%)	18 (85.7%)
Age (yrs) 1st diagnosis median (IQR)	25.8 (16.9-27.3)	20.0 (17.1-31.2)
Age (yrs) 2nd diagnosis median (IQR)	27.2 (21.1-34.2)	30.1 (25.3-37.0)
Time (yrs) 1st-2nd diagnosis median (IQR)	7.0 (4.2-9.7)	7.4 (4.2-10.8)

Table 4. Order of diagnosis Psoriasis-UC

	1st diagnosis: Psor	**1st diagnosis: UC**
Number of patients (%)	9 (47.4%)	10 (52.6%)
Age (yrs) 1st diagnosis median (IQR)	37.3 (12-41.3)	35.5 (28.6-44.2)
Age (yrs) 2nd diagnosis median (IQR)	44.0 (40.0-45.2)	54.8 (38.7-58.5)
Time (yrs) 1st-2nd diagnosis median (IQR)	6.3 (3.9-20)	16.7 (6.1-22.0)

DISCUSSION

In this study, the prevalence of IBD in psoriasis was four times higher than in the general population[5]. Psoriasis-CD patients had an earlier disease onset of psoriasis and CD than patients with only psoriasis or CD. Psoriasis-PsA patients were more likely to have concomitant IBD, especially CD, compared to psoriasis-only patients. Besides PsA, other auto-immune diseases were also more frequently observed in psoriasis-IBD patients compared to psoriasis-only patients.

The prevalence of PsA in psoriasis in our cohort was 12.2%. Data of previous studies vary between 3-30%[19-21]. The prevalence of IBD in psoriasis patients in our cohort was 1.6%, which is higher than the 1.0% reported in a previous study[6], and 4 times higher than

the prevalence reported in the general population (0.4%). Other studies have confirmed that psoriasis patients are at increased risk for IBD[22, 23], however they published no data about order of diagnosis and clinical characteristics of these patients. Our study showed that psoriasis-CD patients are most often diagnosed first with CD, while psoriasis-UC patients are as often first diagnosed with UC as psoriasis.

Whether psoriasis is has a stronger association with CD or UC remains a debate[6, 23, 24]. In our psoriasis-IBD cohort the ratio CD: UC was almost equal (1:1.07). However, for CD, more distinct characteristics of patients with both psoriasis and IBD were observed compared to patients with UC.

It can be hypothesized that the younger the age of onset, the more genetic factors contribute to the disease. Since psoriasis has a substantial overlap in genetic components with CD[4, 8, 9], this might imply that genetic factors play a larger role in patients with both psoriasis and CD than in patients with one disease, since psoriasis-CD patients were diagnosed at a significantly younger age with psoriasis and CD. This finding was not demonstrated in psoriasis-UC patients, potentially caused by a weaker genetic overlap[6]. It is known from previous studies that UC has a higher age of onset than CD[25, 26], which was confirmed by the cohorts of our study. Therefore in CD a stronger genetic component might be involved than in UC, as studies of monozygotic twins demonstrated an estimated concordance rate of 50% in CD and 10% in UC[27, 28]. In addition, time between diagnoses when first diagnosed with IBD, was notably longer in psoriasis-UC patients UC than in psoriasis-CD patients (16.7 vs 7.4 years). This was even more remarkable given the fact that psoriasis-IBD patients were already older when first diagnosed with UC in comparison with CD (35.5 vs 20.0 years).

Data on the phenotype of psoriasis-IBD patients is scarce, but one study of Lollie et al. found that psoriasis-IBD patients more often had mild psoriasis compared to psoriasis-only patients[7]. Mild psoriasis was also most frequently seen in our psoriasis-IBD cohort, however not significantly different from the psoriasis-only patients. The difference might be explained by different methods, as this study included solely psoriasis-IBD patients first diagnosed with IBD, and thus patients with a higher age of psoriasis onset and possible another (even milder) phenotype. Nevertheless, the psoriasis phenotype was mild in both studies, which might be partially explained by use of immunosuppressive medications for IBD, which are also effective for psoriasis. In contrast, a recent Danish study showed an increased risk for IBD in severe psoriasis[24]. However, in this study, severe psoriasis was classified as "psoriasis diagnosis made in the hospital by a dermatologist" which does not adequately mark disease severity. The second classification for psoriasis severity this study used was "the use of systemic (specific) antipsoriatic therapy" thereby excluding the medication prescribed for both IBD and psoriasis, which might bias their results since psoriasis-IBD patients are often prescribed systemic medicaments effective for both IBD and psoriasis to treat two diseases at once.

In our psoriasis-CD cohort, the CD-phenotype was severe, in contrast to the psoriasis phenotype. Patients with both psoriasis and CD had significantly more penetrating and perianal disease than CD-only patients. Furthermore, although not significantly due to insufficient power, psoriasis-IBD patients had more often an extensive disease location than IBD-only patients. In addition, systemic medication such as steroids, and anti-TNF-α treatment were significantly more prescribed to psoriasis-IBD patients in comparison with IBD-only and psoriasis-only patients. This seems to indicate also a more severe IBD and/or psoriasis in the psoriasis-IBD group, but it should be noted that this observation is difficult to interpret due to possible combined treatments for psoriasis and IBD. Together, these data suggest that a separate IBD entity exists with a more severe, earlier onset CD-phenotype (Table 2).

A confounder in these results could have been the shorter CD disease duration of the Delta cohort (median 3.3 years) compared to the psoriasis-CD cohort (median 13.0 years). However, when comparing our results to other IBD cohorts in the literature with a longer CD disease duration, such as the IBD-only cohort of Lolli et al. (median 10.0 years), we still observe a more severe CD-phenotype of psoriasis-CD patients in our cohort compared to the CD-only patients described in the study of Lolli et al. Noticeably, the more severe CD course that was observed might be related to the younger age of onset in psoriasis-CD patients, as it is known that age of onset is a contributing factor to CD severity[29].

The data collection from different cohorts is another limitation of the study. While larger patient groups would be preferred to obtain a higher power, this study represents one of the largest psoriasis-IBD cohorts with phenotypic data in comparison with psoriasis-only and IBD-only patients so far. Furthermore, due to the retrospective nature, we anticipate that the prevalence in this study might be an underestimation of the true prevalence of IBD in psoriasis. It is conceivable that patients with mild IBD without a yet confirmed diagnosis were not registered in the patient charts and hence missed. It is plausible that IBD is more easily missed than psoriasis because of the ambiguous nature of symptoms and the common delay of IBD diagnosis[30]. Symptomatic psoriasis patients could be screened by endoscopy to investigate the presence of intestinal inflammation[31] and the potential presence of IBD, however the invasive nature of endoscopies needs to be taken into account. Despite these potential pitfalls, the central position of the general hospital MCL in the northern part of the Netherlands allowed inclusion of a broad range of patients for an accurate estimation of the prevalence, precluding tertiary center bias.

Finally, this study emphasizes the increased prevalence of IBD in psoriasis, which confirms the need of awareness and recognition of IBD symptoms as comorbidity in psoriasis. Although psoriasis might be mild in patients with psoriasis and IBD, they present a severe CD-phenotype and are diagnosed with psoriasis and CD at a younger age. Especially patients with mild psoriasis and concomitant PsA are at risk for IBD,

primarily CD. Furthermore, other auto-immune diseases are more often observed when a patient already is diagnosed with psoriasis and IBD. Thus, while physicians can expect that patients with concomitant psoriasis and IBD have a relatively mild psoriasis course, development of IBD warrants increased attention in these patients. An enhanced multidisciplinary approach between Departments of Dermatology, Gastroenterology and Rheumatology, might lead to a more effective, therapeutic, and integrated approach for these patients.

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Supplementary Table 1. Patients' characteristics: prevalence of IBD and PsA in psoriasis

	Total	**Psor - only**	**Psor - PsA**[1]	**Psor - IBD**[1]
Patients n (%)	1669 (100)	1446 (86.6)	203 (12.2)	26 (1.6)
Gender n female (%)	857 (51.3)	751 (51.9)	94 (46.3)	16 (61.5)
Age at diagnosis Psor[2]	43.5	44.5	40.6	41.8
median (IQR)	(28.5-59.0)	(28.1-60.3)	(29.4-53.8)	(28.7-54.3)
Age at diagnosis PsA	44.4	NA	44.4	NA
median (IQR)	(34.8-57.1)		(34.8-57.1)	
Age at diagnosis IBD	33.4	NA	NA	33.4
median (IQR)	(18.1-45.1)			(18.1-45.1)
Type Psor[3] n (%)				
Plaque	1178 (70.6)	1000 (69.2)	169 (83.3)	13 (50.0)
Guttate	241 (14.4)	226 (15.6)	13 (6.4)	4 (15.4)
PPP	109 (6.5)	95 (6.6)	11 (5.4)	5 (19.2)
Inverse	208 (12.5)	175 (12.1)	31 (15.3)	3 (11.5)
Capitis	382 (22.9)	334 (23.1)	41 (20.2)	8 (30.8)
Unguium	100 (6.0)	77 (5.3)	23 (11.3)	0 (0)
Severity Psor n (%)				
Mild	967 (57.9)	853 (59.0)	102 (50.2)	15 (57.7)
Mild-Moderate	296 (17.7)	257 (17.8)	36 (17.7)	6 (23.1)
Moderate	298 (17.9)	251 (17.4)	43 (21.2)	4 (15.4)
Moderate-Severe	34 (2.0)	28 (1.9)	6 (3.0)	0 (0)
Severe	73 (4.4)	57 (3.9)	16 (7.9)	1 (3.8)
Systemic medication n (%)				
Anti-TNF-α therapy	371 (22.2)	259 (17.9)	104 (51.2)	11 (42.3)
Dimethylfumarate	224 (13.4)	147 (10.2)	75 (36.9)	4 (15.4)
Methotrexate	286 (17.1)	97 (6.7)	181 (89.2)	12 (46.2)
Ustekinumab	53 (3.2)	23 (1.6)	29 (14.3)	3 (11.5)
Ciclosporin	336 (20.1)	192 (13.3)	140 (69.0)	5 (19.2)
Acitretin	104 (6.2)	75 (5.2)	28 (13.8)	1 (3.8)
NSAIDs	111 (6.7)	0 (0)	109 (53.7)	6 (23.1)
Azathioprine	13 (0.8)	0 (0)	3 (1.5)	12 (46.2)
Mesalazine	9 (0.5)	0 (0)	1 (0.5)	9 (34.6)
Sulfasalazine	41 (2.5)	0 (0)	39 (19.2)	4 (15.4)
Steroids	53 (3.2)	2 (0.1)	37 (18.2)	16 (61.5)
Mercaptopurine	4 (0.2)	0 (0)	0 (0)	4 (15.4)
Other[4]	21 (1.3)	1 (0.07)	19 (9.4)	1 (3.8)
Number of comorbidities	0	0	1	1
median (IQR)	(0-1)	(0-1)	(0-3)	(1-3)
Number of co-medication	1	2	2	2
median (IQR)	(0-4)	(0-3)	(0-4)	(1.5-5)

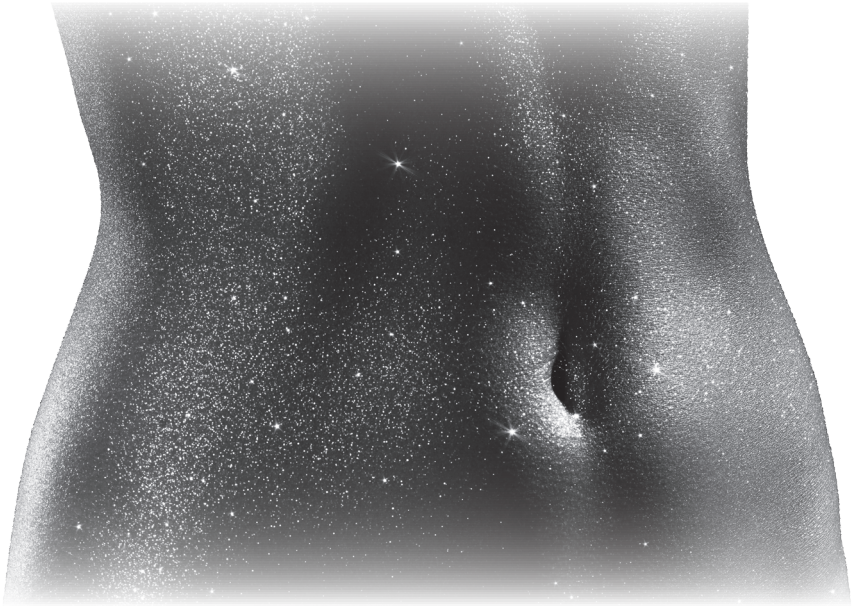
Psor, psoriasis; PsA, psoriatic arthritis; IBD, Inflammatory Bowel Disease; NA, not applicable; PPP, psoriasis pustulosis palmoplantaris

[1] Six patients were diagnosed with psoriasis, IBD and PsA

[2] Missing Psor-only 316

[3] Patients could be diagnosed with more than one type

[4] Other total: hydroxychloroquine 11, leflunomide 6, colchicine 1, cyclofosfamide 1, roaccutane 1, olsalazine 1



CHAPTER 3

Clinical characteristics of patients with hidradenitis suppurativa and inflammatory bowel disease

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H.B. Thio
C.J. van der Woude

Based on: Clinical Gastroenterology and Hepatology 2016

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), and the chronic inflammatory skin disease hidradenitis suppurativa (HS) are chronic inflammatory diseases with substantial overlap in pathogenesis en clinical presentation. HS presents commonly with painful inflammations, nodules, in the axillary, inguinal and perianal region. Fistulas and abscesses are also a common present in HS, which can also be seen in CD, in particular in the perianal region. A recent article by Kamal et al. described the clinical features of patients with CD and HS[1]. The study reported that patients with both CD and HS require increased medical and surgical therapy. Research investigating the dermatologic comorbidities of IBD, including CD and UC, is of importance since these patients might have a more severe IBD disease course and dermatological comorbidity greatly impacts the quality of life[2]. The prevalence of HS in IBD is still unclear[3,4], and we believe that underdiagnosing of HS in IBD is still a pitfall because the symptoms are not recognized by physicians due to similarity with IBD symptoms and/or because of lack of knowledge of the disease HS. Another reason is that HS symptoms are not reported by patients because they are not comfortable with the symptoms occurring in a private area. In our center we have set up a biobank with Dutch IBD patients with skin disorders including 23 patients with IBD and HS. (MEC 2014-371). IBD diagnosis was confirmed by endoscopy and HS diagnosis was confirmed by a dermatologist. Our data was prospectively collected and included disease activity scoring and comorbidities, which adds value in comparison with the study of Kamal et al. The patients were screened at the outpatient clinic at our center by the following procedures: a questionnaire, dermatological examination, faeces collection for calprotectin and HS and IBD disease activity indexes. The patients' clinical characteristics of our cohort are depicted in Table 1 and the disease activity indexes in Table 2.

Several aspects of our cohort with IBD and HS patients are comparable with the cohort of Kamal et al. The most patients are female (70%). Patients with IBD and HS are frequently overweight (n=9) or obese (n=9). In the majority of the cases IBD was diagnosed before HS (74%). The patients in our cohort smoked less (30%) than the patients of the cohort of Kamal et al. (73%). The majority of our cohort had mild-moderate HS while the majority of the cohort Kamal et al. had severe HS. More patients had CD than UC (90%), consistent with literature[4].

Of the UC patients two patients had left-sided UC and one patient extensive UC. Of the CD patients twelve had ileocolonic disease, six had colonic disease and two had ileal disease. The prevalence of perianal CD was not as high in our cohort compared to Kamal et al (22% versus 57% respectively). The CD character was in three patients stricturing and penetrating, in five patients only penetrating, in six patients only stricturing and in the remaining six patients only inflammatory. Six patients underwent an ileocecal resection, one patient an ileum resection and two patients a colectomy. Few patients had first degree family members with HS (n=3), CD (n=2) and UC (n=1).

We could not detect a correlation between the disease activity of HS and the disease activity of IBD. Thus, a more active IBD did not reflect a more active or severe HS. In our

IBD cohort, HS did not appear to be more severe than in the general HS population. However, congruent with the cohort of Kamal et al, IBD patients might have a more severe disease course when concurrent with HS, including a high prevalence of ileocolonic involvement and a high occurrence of stricturing and penetrating disease in CD. These patients frequently use multiple systemic therapies and about 50% require IBD surgery. It is at this point unclear if this also implies for other dermatological IBD comorbidities.

Thus, more emphasis on the recognition of HS in IBD can improve quality of life and is important for adequate IBD monitoring, since these patients are likely to have a more severe IBD disease course.

Table 1. Clinical characteristics of patients with concurrent IBD and HS

Patients (n)	23
Age at diagnosis IBD (median yrs)	25
Age at diagnosis HS (median yrs)	29
Type IBD	
Crohn's disease	19
Ulcerative colitis	3
Undifferentiated IBD	1
Location HS	
Inguinal fold and perianal area	16
Buttocks	5
Axilla	13
Thorax	4
Other (pubis, genitals, legs)	8
Medication[1]	
None	4
Oral antibiotic	5
Steroid	3
Immunosuppressant	12
Biological	9
Comorbidities[1]	
None	6
Dermatologic[2]	2
Respiratory	2
Cardiovascular	2
Hypertension	2
Neurologic	3
Auto immune disease[3]	3
Psychiatric	2
Other[4]	4

Abbreviations: IBD, inflammatory bowel disease; HS, hidradenitis suppurativa; N, number; CD, Crohn's disease; UC, ulcerative colitis; NA, non-applicable

[1] Patients could have used more than one systemic drug or have more than one comorbidity.

[2] Seborrheic dermatitis (n=1), psoriasis (n=1)

[3] Psoriatic arthritis (n=1), morbus Graves (n=1), auto-immune hepatitis (n=1)

[4] Endometriosis (n=1), fibromyalgia (n=1), urolithiasis (n=1), hypogonadotropic hypogonadism (n=1)

Table 2. Disease activity indexes of patients with IBD and HS

Patients (n)	23
Hurley-score	
I	5
II	16
III	1
HS-PGA	
Clear	4
Minimal	8
Mild	8
Moderate	2
Severe	1
HBI-score	
<5	11
5-7	5
8-16	4
SCCAI score	
≤2	1
>2	2
Calprotectin	
<200	14
≥ 200	7
Unknown	2

Abbreviations: HS-PGA, Hidradenitis Suppurativa Physician's Global Assessment Scale; HBI, Harvey-Bradshaw Index for CD (<5, remission; 5-7, mild; 8-16, moderate); SSCAI, Simple Clinical Colitis Index for UC (≤2 remission, >2 active UC); Faecal calprotectin, <200 determined as remission, ≥ 200 as active.

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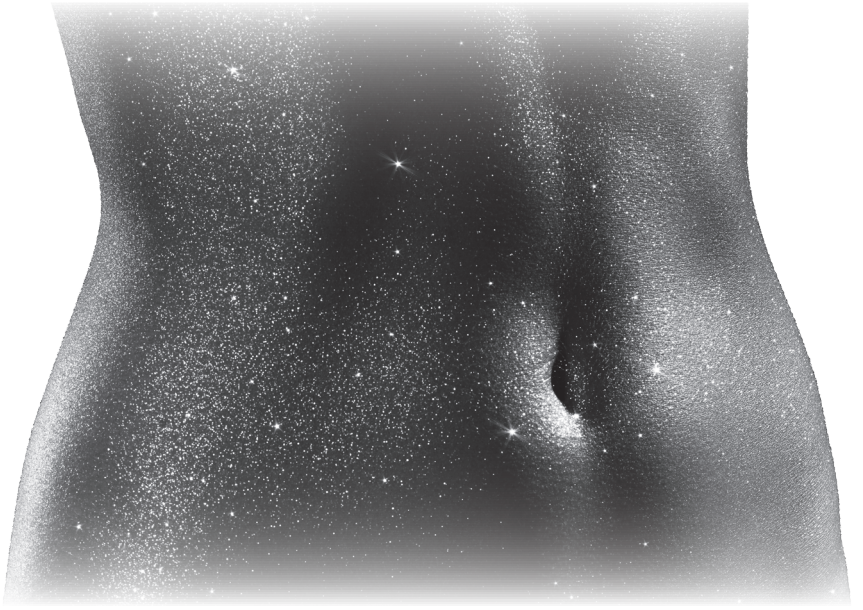

CHAPTER 4

Worsening of bowel symptoms through diet in patients with inflammatory bowel disease

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Based on: Inflammatory Bowel Diseases 2016

The role of diet in inflammatory bowel disease (IBD) is still under debate and underrepresented in IBD research[1]. In clinical practice, one of the most frequently asked questions by patients, "What should I or should I not eat?", has been left unanswered to date. Although recent work, such as the work of Limdi et al.[2] has highlighted diet as important environmental factor in IBD. We all know that everything what we consume comes into direct contact with our intestines, but we still have no clue to what extent food determines the health of the (inflammatory) bowel. Further mapping of IBD symptoms in relation to food is an important step to elucidate this dispute.

In our center data was collected from a prospective questionnaire (MEC-2014-371) after written informed consent from 93 IBD patients with a confirmed diagnosis, including 70 patients with Crohn's disease (CD) (75%), and 23 with ulcerative colitis (UC) (25%). Of all individuals, 50 had an IBD-associated skin disorder (54%), and 10 had one in the past (11%). Patients were asked if they followed a diet and if food/drinks aggravated their bowel and/or dermatologic symptoms. Mean age was 43.7 years (SD 13.4), mean BMI 27.3 (5.9), mean duration of IBD 16.7 years (10.4); mean age at IBD diagnosis 26.9 (11.5); 61% was female, 84% Caucasian, 22% smoking, 18% alcohol consumer. Patients reported to eat an average of 1.4 fruits/day (SD 1.0), 5.7 days vegetables/week (1.7), 4.8 days meat/week (2.0), 1.0 times fish/week (1.1), 5.0 days dairy products/week (2.8) and 0.5 days fast food/week (0.7).

Twelve patients (13%) followed a diet: vegetarian (n=2), lactose-free (n=3), low-carbohydrate (n=4), gluten-free (n=2), sugar-free (n=3), other (n=3). Worsening of bowel symptoms by food was reported in 80% of the patients (Figure 1)[a], compared to 60% of the cohort of Limdi et al. Only 16% of CD, but 35% of UC patients reported that diet had no impact on their bowel symptoms. The following food products that did worsen bowel symptoms were comparable with the cohort of Limdi et al: fatty food (42% vs 29%), spicy food (32% vs 42%), vegetables/fruits (28% vs 19%), dairy products (25%) vs milk products (16%), alcohol (16% vs 21%), and sugary food (6% vs 10%).

Only 7 patients with an IBD-associated skin disorder (12%) reported that food/drinks aggravated their dermatologic symptoms: spicy food (n=2); alcohol (n=3), sugar (n=2)[b]. In addition, dietary habits did not seem to differ between IBD patients with and without a skin disorder.

In conclusion, our cohort confirms the findings of Limdi et al. that certain foods worsen bowel symptoms in the majority of IBD patients, particularly spicy and fatty food. Future research regarding the role of diet in IBD is to be encouraged. Similar to previous studies, different IBD patients respond differently to various foods. Fatty food might aggravate the bowel symptoms in one patient, but this might not be true to another patient. These individual differences complicate the performance of solid dietary research. Dietary intervention trails do often not succeed because of a missing (placebo) control group, low compliance of patients, limited funding, and concomitant use of IBD-medication. But

despite these difficulties, which can be overcome, we should not ignore diet as potential causal factor, or therapeutic option in IBD. More research into the role of diet in IBD is justified.

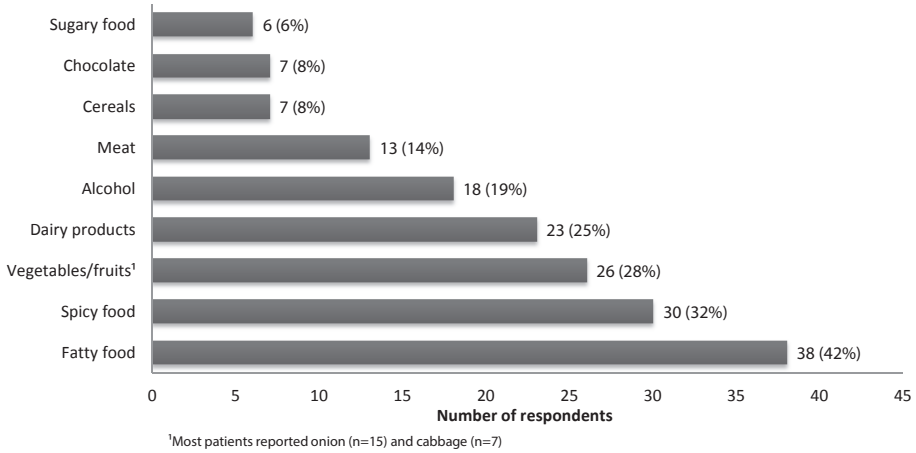

Figure 1. Food/drinks that IBD patients reported to exacerbate their bowel symptoms

[a] Food/drinks reported less than 4 times regarding bowel symptoms were not included in Figure 1.

[b] Food/drinks reported less than 2 times regarding skin symptoms were not included in the text.

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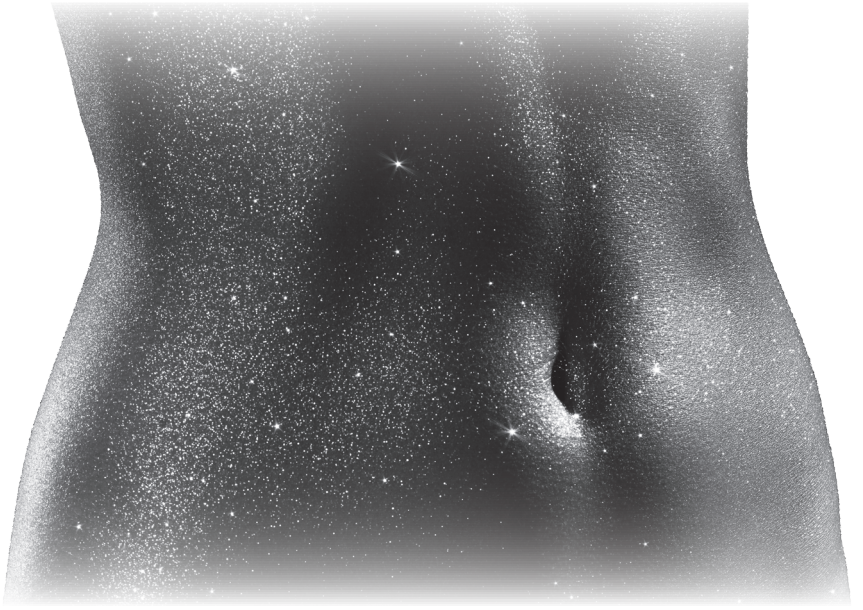
PART II

MICROBIAL SIGNATURES



CHAPTER 5

Similar depletion of the protective gut bacterium *Faecalibacterium prausnitzii* in psoriasis and inflammatory bowel disease, but not in hidradenitis suppurativa

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ABSTRACT

Background and Aims

Psoriasis and hidradenitis suppurativa (HS) co-occur more often with inflammatory bowel disease (IBD) than expected due to shared pathogenic and genetic features. It is known that IBD patients harbour an altered intestinal microbiome characterized by a depletion of *Faecalibacterium prausnitzii* and increase of *Escherichia coli*. At present, it is unclear whether a similar intestinal microbiome trend can be identified in IBD-associated skin disorders. We therefore investigated the *F. prausnitzii* and *E. coli* abundance in psoriasis and HS, with and without concomitant IBD.

Methods

Using quantitative PCR, we compared the *F. prausnitzii* and *E. coli* abundance in the faecal samples from healthy controls (n=33) with samples from patients with psoriasis (n=29), IBD (n=31), and concomitant IBD and psoriasis (n=13). Likewise, we analysed samples from patients with HS (n=17), and concomitant IBD and HS (n=17).

Results

Psoriasis patients harboured a significantly lower abundance of *F. prausnitzii* in their stool than healthy controls ($p<0.001$), which was similar to IBD patients. Together with the reduced *F. prausnitzii* levels, the psoriasis patients had a significantly higher abundance of *E. coli* ($p<0.001$). No significant difference in *F. prausnitzii* or *E. coli* abundance was found in HS. It was apparent that patients with concomitant IBD and associated skin disorder had the greatest decrease of *F. prausnitzii* and increase of *E. coli*.

Conclusions

The study demonstrates, for the first time, an IBD-like decrease of *F. prausnitzii* together with an increase of *E.coli* in psoriasis, supporting the presence of a gut-microbiome-skin axis in psoriasis and IBD.

INTRODUCTION

The resident microbial community in the human intestine, the so-called gut microbiota, is increasingly recognised as an important environmental factor in the development of chronic immune-mediated inflammatory diseases, mainly in inflammatory bowel disease (IBD). Studies concerning IBD, which comprise Crohn's disease (CD) and ulcerative colitis (UC), have indicated that chronic inflammation arises as a result of an inappropriate, ongoing immune response to intestinal microbial antigens in genetically susceptible individuals[1-3]. As a mild gut inflammation has been demonstrated in the chronic inflammatory disease psoriasis, the gut microbiota might also be involved in psoriasis[4]. Various overlapping genetic, immunologic and environmental factors play a role in the increasing incidence of both diseases[5, 6]. The prevalence of psoriasis in patients with IBD, especially in CD, is higher than would be expected if psoriasis and IBD were mutually exclusive diseases[5]. Another recent focus of investigation the past years is the association between IBD and hidradenitis suppurativa (HS), which can be difficult to distinguish due to similar perianal involvement[7, 8]. HS, which is also called acne inversa, is a chronic follicular occlusive disease, affecting the intertriginous skin areas including the axillary, groin and perianal regions.

Currently, research on the relation of the gut microbiota with skin disorders is limited[9] and is mainly focused on the skin microbiota. Although this emphasis has led to some promising results[10], the gut microbiota might be able not only to regulate the local immune system in the gut, but also to affect the systemic immune system, and thereby other organs such as the skin. In the healthy intestinal microbiota there is a balance between differentiations of naïve CD4+T cells into the effector T cells (Th1, Th2 and Th17) and into regulatory T cells (Tregs). Autoimmune diseases like CD and psoriasis[11, 12] are believed to have a dominant Th1- and Th17 arm of adaptive immune system.

Often a term "dysbiosis" is used to ascribe the disturbance in the balance of the pathogenic and beneficial bacteria in the gut[13]. The dysbiosis found in IBD can either be a result of depleted beneficial bacteria as (e.g.) *Lacto-bacilli, Bifidobacteria, Faecalibacterium prausnitzii,* and/or because of the presence of particular pathobionts which include *Escherichia coli, Salmonella, Campylobacter, Mycobacterium, Helicobacter,* and *Alcaligenes*. While beneficial bacteria seem to protect the patient from inflammation, pathobionts promote inflammation. One of the most specific findings in IBD in the past years is a depletion of the abundant and commensal bacterium *F. prausnitzii*, which is thought to be a marker of a healthy gut[14-16]. *F. prausnitzii* is one of the main producers of butyrate, a short-chain fatty acid, which is an energy source for colonocytes, plays a role in reducing oxidative stress and has anti-inflammatory properties[17]. Studies have shown several implications regarding *F. prausnitzii* and CD: the depletion of this bacterium is a cause, not a consequence, of CD[14]; decreased levels of *F. prausnitzii* are associated with

a higher risk of postoperative recurrence in patients with ileal CD[18]; and *F. prausnitzii* monitoring in stool could be a predictor for CD relapse[19]. Importantly, as an inducer of Tregs, *F. prausnitzii* is involved in the balance between the T effector cells and the Tregs[20, 21]. The depletion of the commensal bacterium *F. prausnitzii* may therefore exert systemic effects and affect not only the gut but also the skin.

Using intestinal dysbiosis quantitative PCR-based screening, we therefore investigated whether the abundance of the protective bacterium *F. prausnitzii* was similarly shifted in psoriasis and HS compared to IBD. The abundance of *E. coli, a*nother important member of the gut microbiota, was also determined. Furthermore, we investigated whether IBD patients with the associated skin disorders psoriasis and HS, exhibited a more severe dysbiosis than patients with only IBD.

MATERIALS AND METHODS

Study population

This study was conducted in accordance with the Declaration of Helsinki Principles and approved by the ethical committee of the Erasmus MC-Erasmus University Medical Center, Rotterdam (MEC-2014-371). The patients and healthy controls of this study were all inhabitants of the Netherlands and prospectively included. IBD patients, psoriasis patients and HS patients were included at the outpatient clinic of the department of Dermatology and the department of Gastroenterology and Hepatology. Patients with both IBD and psoriasis/HS were also collected through an advertisement on the website of the patients' organisations. These patients also visited the outpatient clinic of the department Dermatology or the department of Gastroenterology and Hepatology of the Erasmus MC to undergo a dermatological examination and to collect the questionnaires and disease activity indexes of psoriasis (PASI), HS (Hurley stage and HS-PGA) and IBD (SCCAI for UC and HBI for CD). Patients who had a previous colectomy did not receive a HBI or SCCAI score, due to unreliable results. All participants signed written informed consent before start of the study. Methods and criteria for inclusion and exclusion in the study were equally for the three patient groups and healthy controls. Patients were excluded if they were younger than 18 years or older than 75 years, had used antibiotics 8 weeks prior to inclusion. Other exclusion criteria were the presence of cancer, active infection, pregnancy or inflammatory bowel syndrome (IBS). IBD diagnosis and elevated faecal calprotectin (>200µg/g) were exclusion criteria in the group with skin disorders (psoriasis or HS) without IBD. Patients included in the IBD only group were not included if they had a skin manifestation of any sort. All IBD patients had the diagnosis IBD confirmed by endoscopy (this was checked in the electronic chart of the patients) and the diagnosis psoriasis and HS were clinically confirmed by a dermatologist. Of each of the

participants, faecal samples were collected by post (arrival within 48 hours) and then immediately stored at -80ºC.

Patients' characteristics

The patients' characteristics of the psoriasis cohort (psoriasis (n=29), IBD (n=31), concomitant IBD and psoriasis (n=13), healthy controls (n=33) are displayed in Table 1.

Table 1. Patients' characteristics cohort: psoriasis, IBD & psoriasis, IBD, healthy controls

	Psoriasis	IBD & Psoriasis	IBD	Healthy	p-value[1]
N (patients)	29	13	31	33	
Age (mean yrs, SD)	46 (14.0)	50(11.4)	41 (11.7)	41 (14.9)	0.29
Gender (% female)	59	53	58	70	0.96
Smoking (%)	21	20	13	6	0.56
BMI (mean, SD)	27,7 (4.4)	24.6 (4.1)	26.0 (5.7)	24.6 (4.9)	0.30
Caucasian (%)	76	100	77	76	0.38
Age at diagnosis[2]	32 (11.4)	26 (14.2)	25 (9.4)	NA	0.20
(yrs, SD)		27 (9.8)			0.35
Disease duration[2]	15 (11.9)	24 (15.9)	16 (10.0)	NA	0.28
(yrs, SD)		23 (11.3)			0.36
Medication[3]					
None	27	5	11	NA	
Immunosuppressant	2	7	12	NA	
Anti-TNFα therapy	0	3	11	NA	
Dimethylfumarate	0	2	0	NA	

Abbreviations: IBD, inflammatory bowel disease; BMI, body mass index, NA, non-applicable

[1] P-value calculated by one-way ANOVA for age, BMI; unpaired t-test for age at diagnosis, disease duration; chi-squared test for gender, smoking, ethnicity

[2] Column IBD & Psoriasis: first (top) number psoriasis, second number IBD; column p-value: first value psoriasis compared to IBD & Psoriasis, second p-value IBD compared to IBD & Psoriasis

[3] Patients could have been on concomitant drugs

A small proportion of the participants followed a specific diet: in the psoriasis group one patient had a low carbohydrate and sugar free diet, one patient a cow-milk and wheat-free diet and one patient a dairy-free diet. In the IBD group one patient had a lactose free diet and one patient ate mainly liquid food due to bowel complaints. In the group with concomitant IBD and psoriasis one patient used a low carbohydrate, gluten free and sugar free diet, but had a normal diet 5 days prior to sampling and one patient had a lactose free and gluten reduced diet. In the healthy control group two people followed a pescatarian diet and three a vegetarian diet. The other participants followed a regular diet. The majority of the psoriasis group was diagnosed with chronic plaque type psoriasis (86%, n=25) (Table 2). In 86% (n=25) of the psoriasis patients the disease was located on multiple sites, including extremities and thorax, in 7% (n=2) of the patients the psoriasis was located only on the scalp

and in 7% (n=2) only on the feet (palmoplantar pustulosis psoriasis: PPP). The majority of the IBD group was diagnosed with CD (77%, n=24), compared to UC (23% n=7). In the group with concomitant IBD and psoriasis most had chronic plaque type psoriasis (92%). One of the patients, diagnosed with CD and psoriasis, had possibly anti-TNF induced psoriasis. This patient had used infliximab for three years before developing psoriasis and continued the infliximab medication after the diagnosis. In 77% (n=10) of the psoriasis patients the disease was located on multiple sites, including extremities and thorax, 23% (n=3) of the patients had no active psoriasis plaques at time of the study. Of the IBD patients 8 patients (62%) were diagnosed with CD and 5 patients (38%) were diagnosed with UC. In this patient group 69% (n=9) patients first developed IBD and then psoriasis, 31% (n=4) developed psoriasis first and then IBD. A notable observation in this group was that 54% (n=7) of these patients had previous IBD surgery, of which six a colectomy and one an ileal resection.

Psoriasis type and PASI is depicted in Table 2. The two patients with guttate psoriasis did not receive a PASI: one of them had active erythematosquamous plaques on the head, trunk, upper limbs and lower limbs, and one had active erythematosquamous plaques on trunk, upper limbs and lower limbs. The two patients with PPP had active erythematosquamous plaques on their feet. The disease characteristics of IBD are presented in Table 3. The patients' characteristics of the HS cohort (HS (n=17), IBD (n=31), concomitant IBD and HS (n=17), healthy controls (n=33) are presented in Table 4. The IBD and healthy control group for the HS cohort is identical to the cohort described above for the psoriasis cohort. One of the HS patients followed a lactose free diet, and two of the patients with both IBD and HS followed a diet: a non-strict sugar-free, low carbohydrate diet and a cow-milk free diet.

In the HS group, 94% had HS lesions in the inguinal fold and perianal area, 82% in the axilla, 76% thorax, 71% buttock and 71% on other locations, including legs, pubis, genitals and scalp. In patients with both IBD and HS the most common locations of HS lesions were the inguinal fold and perianal area (65%) and the axilla (65%). Lesions on the buttock (12%), thorax (18%) and other locations (18%) were seen less frequently in this group. In the HS group 13 patients previously underwent a deroofing procedure. In the group both IBD and HS 2 patients had a deroofing procedure.

In the group of both IBD and HS the majority was diagnosed with CD (n=14, 82%), compared to UC (n=2, 12%). One (6%) had unclassified IBD. HS was diagnosed before IBD in three patients (18%). Thirteen patients (76%) first developed IBD and then HS, one patient (6%) developed the diseases at the same time.

Table 2. Skin disorder characteristics

	Psoriasis	IBD & Psoriasis	IBD & HS	HS
Psoriasis type (%)				
Plaque	25 (86)	12 (92)	NA	NA
Guttate	2 (7)	1 (8)	NA	NA
Palmoplantaris pustulosis	2 (7)	0 (0)	NA	NA
PASI (%)[1]				
<10	15 (60	11 (92)	NA	NA
>10 en ≤ 20	9 (36)	1 (8)	NA	NA
>20	1 (4)	0 (0)	NA	NA
Hurley (%)[2]				
I	NA	NA	5 (29)	2 (12)
II	NA	NA	12 (71)	14 (82)
III	NA	NA	0 (0)	1 (6)
HS PGA (%)[3]				
Clear	NA	NA	2 (12)	1 (6)
Minimal	NA	NA	7 (41)	3 (18)
Mild	NA	NA	7 (41)	9 (53)
Moderate	NA	NA	1 (6)	4 (24)
Severe	NA	NA	0 (0)	0 (0)

Abbreviations: IBD, inflammatory bowel disease, NA, non-applicable

[1] PASI, Psoriasis Area Severity Index (<10 mild, >10, ≤ 20 moderate, >20 severe). Only applicable for plaque psoriasis.

[2] Hurley (I clear-mild, II mild-moderate, III moderate-severe)

[3] HS PGA, Physician's Global Assessment (clear, minimal, mild, moderate, severe)

Table 3. IBD Disease characteristics

	IBD & Psoriasis	IBD & HS	IBD
IBD type (%)			
CD	8 (62)	14 (82)	24 (77)
UC	5 (38)	2 (12)	7 (23)
IBD undifferentiated[1]	0	1 (6)	0
CD Montreal disease location (%)[2]			
L1	1 (13)	2 (13)	6 (25)
L2	1 (13)	5 (33)	5 (21)
L3	6 (75)	8 (53)	13 (54)
perianal involvement	2 (25)	4 (29)	6 (25)
UC disease location (%)			
Left-sided	3 (60)	1 (50)	3 (43)
Extensive	2 (40)	1 (50)	3 (43)
Proctitis	0 (0)	0 (0)	1 (14)
CD Montreal disease behavior (%)[3]			
B1	4 (50)	5 (33)	8 (33)
B2	1 (13)	4 (27)	10 (42)
B3	3 (38)	6 (40)	6 (25)
Previous surgery[4]			
Ileal/Ileocecal resection	3	5	5
Colectomy	6	0	0
No previous surgery	6	12	26
HBI[5]			
<5	4	9	12
5-7	1	4	7
8-16	0	2	5
SCCAI[6]			
≤2	0	0	6
3	1	2	1
8	1	0	0
Calprotectin[7]			
< 200	12	11	18
> 200	1	6	13

Abbreviations: IBD, inflammatory bowel disease, CD, Crohn's disease; UC, ulcerative colitis

[1] CD was most likely diagnosis, included in this table as CD patient for characteristics

[2] L1, ileum; L2, colon; L3, ileum and colon

[3] B1, inflammatory; B2, stricturing; B3, penetrating

[4] Patients could have had more than one surgery; no SCCAI/HBI score if patients had a colectomy

[5] HBI, Harvey-Bradshaw Index CD (<5 remission, 5-7 mild, 8-16 moderate, >16 severe)

[6] SCCAI, Simple Clinical Colitis Index UC (≤2 remission, >2 active)

[7] Faecal calprotectin (<200 remission, >200 active)

Table 4. Patients' characteristics cohort: HS, IBD & HS, IBD, Healthy controls

	HS	IBD & HS	IBD	Healthy	p-value[1]
N (patients)	17	17	31	33	
Age (mean yrs, SD)	45 (12.4)	39 (11.8)	41 (11.7)	41 (14.9)	0.65
Gender (%female)	65	82	58	70	0.38
Smoking (%)	65	29	13	6	**<0.001**
BMI (mean, SD)	28.0 (5.0)	30.3 (7.5)	26.0 (5.7)	24.6 (4.9)	**0.008**
Caucasian (%)	76	82	77	76	0.96
Age at diagnosis[2]	32.5 (14.6)	31 (11.2)	25 (9.4)	NA	0.06
(yrs, SD)		26 (10.1)			0.97
Disease duration[2]	12 (8.1)	8 (10.2)	16 (10.0)	NA	0.75
(yrs, SD)		13.8 (8.1)			0.54
Medication[3]					
None	16	4	11	NA	
Immunosuppressant	0	11	12	NA	
Anti-TNFα therapy	1	5	11	NA	

Abbreviations: IBD, inflammatory bowel disease; BMI, body mass index, NA, non-applicable

[1] P-value calculated by one-way ANOVA for age, BMI; unpaired t-test for age at diagnosis, disease duration; chi-squared test for gender, smoking, ethnicity

[2] Column IBD & HS: first (top) number HS, second number IBD; column p-value: first value HS compared to IBD & HS, second p-value IBD compared to IBD & HS

[3] Patients could have been on concomitant drugs

Faecal DNA extraction

DNA was isolated from 20 mg of faeces. Briefly, after 1mL of Cell Lysis Buffer was added and vortexed, which was followed by an incubation period of 15 minutes, cell lysis was achieved by bead-beating (three times 30 seconds). The samples were centrifuged whereupon 3:1 protein precipitation buffer was added. 100% isopropanol (1:1) and 100 µl 70% ethanol was added after centrifugation and supernatants were decanted. At last, 50 µl TE buffer was added. The DNA concentration was measured by a Nanodrop spectrophotometer (Isogen Life Science BV, De Meern, the Netherlands) and diluted to a DNA concentration of 10 ng/µl.

Measurement of faecal calprotectin

Calprotectin was measured in the faeces using the Calprotectin Elisa kit (EK-CAL) of Bühlmann, to assess disease activity of IBD[22]. IBD patients with a faecal calprotectin level above 200µg/g were considered to have active disease, below this level they were considered in remission.

Quantitative (q) PCR

qPCR was performed for the bacteria (in duplicate) using the following primers:

F. prausnitzii
F. pra 428 F TGTAAACTCCTGTTGTTGAGGAAGATAA
F. pra 583 R GCGCTCCCTTTACACCCA[23]
E. coli
E. coli 395 F CATGCCGCGTGTATGAAGAA
E. coli 490 R CGGGTAACGTCAATGAGCAAA[23]
Total bacterial DNA (16S rRNA gene copies)
F Bact 1369 CGGTGAATACGTTCCCGG
R Prok 1492 TACGGCTACCTTGTTACGACTT[24]

For each qPCR, 20ng DNA (2µl of 10ng/ml) with mastermix containing 9 µl 2x SYBR® Green Dye, 7µl dH20, 1µl 10µM forward primer and 1µl 10µM reverse primer, was used. Thermocycle conditions: denaturation step 10min at 95°C; 40 cycles of 95°C denaturation for 15 s, 56°C primer annealing for 30s and 72°C extension for 30s followed by a standard meting curve program. The abundance of *F. prausnitzii* and *E. coli* was expressed in log10 copies product per gram wet weight of faeces. For validation of our results, we determined total bacteria DNA as additional information and the relative abundance of *F. prausnitzii* and *E. coli* was measured as the percent of total bacteria DNA copy numbers.

Sample size calculation

In this study, the mean relative abundance of *F. prausnitzii* in the faeces (log10 copies/gram) in healthy controls is compared to the abundance in patients with a disease (IBD, psoriasis and HS). For this power calculation, we used data from the paper of Joossens et al.[15] because it was the most comparable study regarding their methods and patient group. With a power of 0.80 and a two-sided alpha of 0.05, we used the following numbers for the sample size calculation: mean of the healthy controls (11.3), mean of IBD patients (9.70) and a sigma of 2.3. This power calculation demonstrated that at least 20 patients have to be included in each group. This included an extra of 15% because the distribution of the amount of *F. prausnitzii* might be non-parametric. Therefore the groups containing patients with HS, and both IBD and HS, have a sufficient sample size (n=17), since the abundance was parametric. However in the group with both IBD and psoriasis it was not feasible to include enough patients (n=13), therefore those results are preliminary pilot results.

Statistical analysis

To compare the clinical characteristics between the groups, the one-way ANOVA, unpaired t-test and chi-squared test were used. Analyses of the abundance data for *F. prausnitzii* and *E. coli* were analysed with a one-way ANOVA for comparing the difference between the four groups (with the condition that the abundance of the bacterium was normally distributed). If the one-way ANOVA showed a p-value < 0.05, unpaired t-tests were performed to assess a difference within the groups. For non-parametric variables the Kruskall-Wallis test and the independent samples Mann-Whitney U-test were used. Subgroup analysis was performed by excluding patients which had previous IBD surgery. We used the False Discovery Rate (FDR) for correction of multiple testing according to Benjamini and Hochberg Y[25]. Only FDR-corrected p-values below 0.05 were considered to be significant. All analyses were performed by using IBM SPSS 21.0 statistical software, Armonk, NY, USA.

RESULTS

Clinical differences within the psoriasis and HS cohort

The summary of the clinical data of the participants of the four study groups of the psoriasis cohort (psoriasis (n=29), IBD (n=31), concomitant IBD and psoriasis (n=13) and healthy controls (n=33) is depicted in Table 1. The four groups were comparable with respect to age, ethnic background, gender, BMI and smoking status. Disease characteristics and disease activity indexes of psoriasis and/or IBD are depicted in respectively Table 2 and Table 3.

The clinical data of the participants of the four study groups within the HS cohort (HS (n=17), IBD (n=31), concomitant IBD and HS (n=17) and healthy controls (n=33) are depicted in Table 4. The four groups showed good comparison for age, gender, ethnicity, age at diagnosis of HS and/or IBD and for the duration of HS and/or IBD. However, a significant difference was found between the groups for BMI and smoking behavior; patients with both IBD and HS had a significant higher BMI than healthy controls (p=0.048), in the other groups no significant difference was observed for BMI. The smoking behavior in the HS and concomitant IBD and HS group was significantly higher compared to healthy controls (respectively p<0.001 and p=0.024). More smoking was observed in HS patients compared to IBD patients (p<0.001) and patients with both diseases (p=0.039). Patients with both IBD and HS did not smoke more often than patients with only IBD (p=0.161). The disease activity scores of HS (Hurley stage and HS-PGA score) are depicted in Table 2.

Depletion of *F. prausnitzii* in psoriasis and in concomitant IBD and psoriasis

The one-way ANOVA showed a significant difference in the abundance *of F. prausnitzii* (log10copies/gram) between the four groups ($p<0.001$). The FDR-corrected q-value was 0.042 after adjusting for multiple testing.

The *F. prausnitzii* abundance in the psoriasis group was significantly lower than in healthy controls (mean log10copies/g, SD: psoriasis 8.07, 0.85; healthy 8.95, 0.80, $p<0.001$, Figure 1); after excluding the two psoriasis patients who had systemic medication the result remained significant ($p<0.001$). Including solely the patients with plaque type psoriasis (n=25) the finding remained significant ($p<0.001$). There was no correlation between the PASI score and the *F. prausnitzii* abundance (Pearson correlation 0.310; $p=0.131$).

Consistent with existent literature, in IBD (8.21 log10copies/g, 0.81) the abundance of *F. prausnitzii* was significantly lower than in healthy controls ($p<0.001$). Within the IBD group no significant difference in the abundance of *F. prausnitzii* was found between CD and UC ($p=0.294$), see also Figure 1. The results stayed the same after exclusion of UC ($p=0.004$). Also the results were the same for only UC versus healthy controls ($p=0.005$). There was no significant difference between the *F. prausnitzii* abundance in IBD compared to psoriasis ($p=0.532$). No correlation was found between the *F. prausnitzii* abundance and faecal calprotectin in IBD (Pearson correlation -0.233; $p=0.206$). When we excluded the five IBD patients who had an ileocecal resection the difference remained significant ($p=0.003$).

In the group with concomitant IBD and psoriasis (n=13, 6.10 log10copies/g, 1.83), the *F. prausnitzii* abundance was also significantly decreased ($p<0.001$). The average *F. prausnitzii* abundance in the UC patients with psoriasis (n=5, 6.25 log10copies/g, 2.10) psoriasis was comparable to that in CD patients with psoriasis (n=8, 6.00 log10copies/g, 1.82). When we excluded the patients which had previous extensive IBD surgery (colectomy, n=6), the *F. prausnitzii* abundance remained significantly lower than healthy controls ($p=0.002$). Of the 7 residual patients in this group one had an ileal resection, after excluding this patient the result remained significant ($p=0.030$).

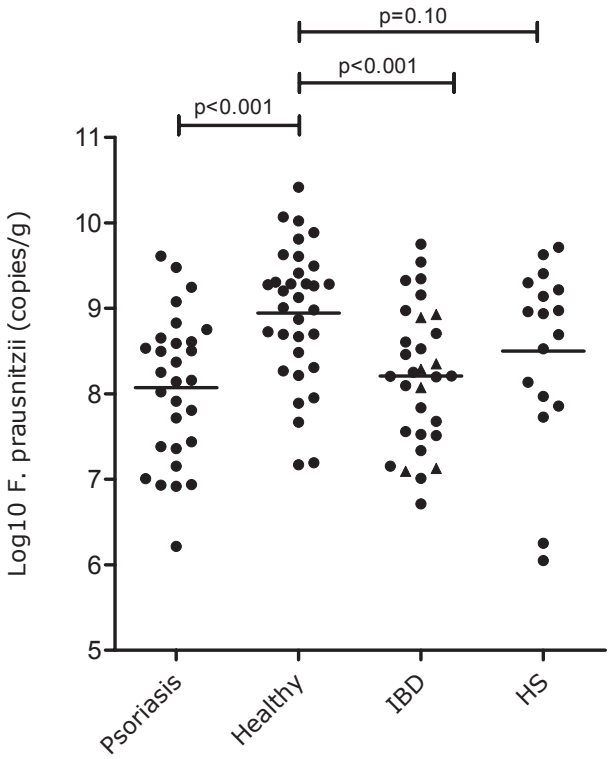

Figure 1. Quantification of *Faecalibacterium prausnitzii* in the faecal microbiota. Compared to healthy controls, patients with psoriasis and patients with IBD had significantly less *F. prausnitzii* in their stool (p≤0.001), while patients with HS did not (p=0.010). (middle line=mean; IBD group: circle=CD; triangle=UC)

Depletion of *F. prausnitzii* in concomitant IBD and HS, but not in solely HS

One-way ANOVA showed a significant difference in the abundance of *F. prausnitzii* between the four groups: HS, HS & IBD, IBD, healthy controls (p<0.001). A significant depletion was found in the abundance of *F. prausnitzii* of patients with concomitant IBD and HS (n=17, 7.46 log10copies/g, 1.55, p=0.001) compared to healthy controls, however this was not found in patients with solely HS (8.50 log10copies/g, 1.07, p=0.10, Figure 1). When we only included the patients with CD and concomitant HS (n=14) the average *F. prausnitzii* abundance was 7.17 log10copies/g, 1.54.

Profound depletion of *F. prausnitzii* in concomitant IBD and psoriasis

It was apparent that the *F. prausnitzii* count in patients with both IBD and psoriasis was even lower than in patients with only IBD (p=0.001) or only psoriasis (p=0.002). However, after excluding the patients who had extensive IBD surgery (n=6), the study was not enough powered to conclude a significant difference for this observation after FDR correction (p=0.048 and p=0.065 respectively).

In the HS cohort, patients with both IBD and HS had a significantly lower *F. prausnitzii* count than patients with only HS (p=0.030). Compared to patients with only IBD this did not reach significance (p=0.075).

Total bacterial DNA (16S rRNA gene copies)

For validation of our results we also analysed total bacterial DNA (16S rRNA gene copies) in the groups. One-way ANOVA for total bacterial DNA (16S rRNA gene copies) was significantly different between the four groups in the psoriasis cohort (p<0.001), but not between the groups in the HS cohort (p=0.102). Healthy controls, psoriasis patients and IBD patients had significantly more total bacterial DNA than patients with both IBD and psoriasis (respectively p=0.003, p<0.001 and p=0.017). Healthy controls and psoriasis patients had significantly more total bacterial DNA than IBD patients (p=0.012 and p=0.003). Psoriasis patients had similar 16S abundance as healthy controls (p=0.384).

The Kruskall – Wallis test showed a significant difference between the relative abundance of *F. prausnitzii* compared to total bacterial DNA of the four groups in the psoriasis cohort (p<0.001), and of the HS cohort (p=0.003). The relative *F. prausnitzii* abundance compared to the total bacterial DNA was also significantly lower in the psoriasis compared to healthy controls (Mann-Whitney U test, p<0.001). In the IBD group, the relative abundance of *F. prausnitzii* was not decreased compared to healthy controls (p=0.209). When excluding the 5 patients with IBD surgery this finding remained not significant (p=0.119). When we calculated the p-value for solely CD patients (n=24) the relative *F. prausnitzii* abundance was also not significant decreased (p=0.410).

In patients with concomitant IBD and the skin disorder psoriasis or HS, the decreased relative abundance of *F. prausnitzii* was significantly lower than in healthy controls (p<0.001 and p<0.001). The relative abundance of *F. prausnitzii* was significant lower in concomitant IBD and psoriasis versus only IBD (p=0.001), but not versus only psoriasis (p=0.200).

In concomitant IBD and HS the relative abundance was significantly lower than that in only IBD or only HS (respectively p=0.009 vs p=0.005). In HS the relative abundance of *F. prausnitzii* was not decreased compared to healthy controls (p=0.975).

Escherichia coli

In addition to *F. prausnitzii,* the abundance of *E. coli* was analysed in both cohorts (log10 copies/gram, psoriasis n=27, concomitant IBD and psoriasis n=13, IBD n=31, healthy controls n=33, HS n=17, concomitant IBD and HS n=17). One-way ANOVA showed a significant difference between the groups in the psoriasis cohort (p<0.001), but not of the HS cohort (p=0.591). Further analysis within the psoriasis cohort showed that psoriasis patients had a significant higher *E. coli* abundance than healthy controls (mean log10copies/g, SD: psoriasis 7.85, 0.94; healthy 6.98, 1.09, p=0.002, Figure 2). In IBD (7.25

log10copies/g, 1.26) no significant increased abundance of *E. coli* was demonstrated (p=0.360) compared to healthy controls. However, the *E. coli* abundance in psoriasis was not significantly different from that in IBD (p=0.051). Patients with both IBD and psoriasis (8.57 log10copies/g, 1.05) had a significant higher *E. coli* abundance than healthy controls (p<0.001), also after excluding the 6 patients with extensive IBD surgery (p=0.007). Patients with concomitant IBD and psoriasis had a significant higher abundance than only psoriasis (p=0.039) or only IBD (p=0.002). However, when excluding the 6 patients with extensive IBD surgery (colectomy) these findings were not significant (p=0.312 and p=0.055 respectively). The results of the relative *E.coli* abundance confirmed the findings above, however not for the IBD group. Now, in the IBD group, the relative *E. coli* abundance was increased compared to healthy controls (p=0.016). No correlation was found between the PASI and *E. coli* abundance (Pearson correlation -0.175; p=0.424)

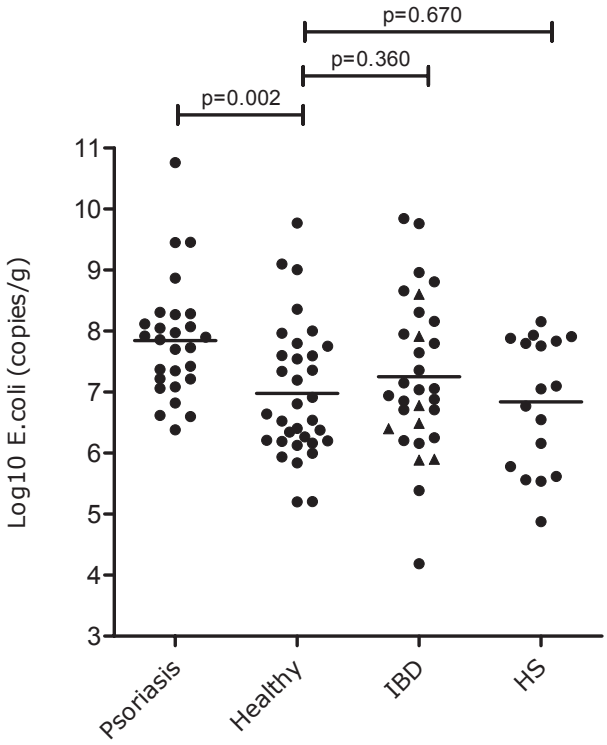

Figure 2. Quantification of *Escherichia coli* in the faecal microbiota. Compared to healthy controls, patients with psoriasis had significantly higher levels of *E. coli* in their stool (p=0.002), while patients with IBD and patients with HS did not (respectively p=0.360 and p=0.670). (middle line=mean; IBD group: circle=CD; triangle=UC)

DISCUSSION

To our knowledge, this is the first study to demonstrate that the anti-inflammatory bacterium *F. prausnitzii* is significantly decreased and *E.coli* is significantly increased in the gut of psoriasis patients with and without concomitant IBD. A strength of this study is its direct comparison of psoriasis with IBD patients, in whom this findings are repeatedly demonstrated[2, 23, 26-28], combined with the comparison to healthy controls. Previously the study of Scher et al. described they found a similar dysbiosis in patients with psoriasis and psoriatic arthritis to that in IBD, however they did not describe the abundance of *F. prausnitzii* and not included IBD patients in their cohort[9]. Interestingly, a decreased abundance of *F. prausnitzii* was not found in HS, and might be thus specific for psoriasis, and not apply for IBD-associated skin disorders in general.

In the normal healthy gut, *F. prausnitzii*, which belongs to the phylum class Clostridium IV, accounts for 5 - 15% of the bacterial microbiota and is one of the most abundant commensal species[29]. *F. prausnitzii* is able to secrete anti-inflammatory molecules that modulate the host immune system[18, 29, 30], and is one of the main producers of butyrate in the colon, which is an energy source for colonocytes and has important anti-inflammatory effects[17]. Systemic delivery of *F. prausnitzii* and, above all, of its supernatant, could exert an anti-inflammatory effect that is unrelated to the presence of butyrate[18]. The depletion of this bacterium could therefore be linked to systemic inflammation in both skin and intestine, a link that clearly awaits further study.

Although some studies show that the abundance of *F. prausnitzii* varies with the disease activity of IBD, the abundance is also lower in IBD patients who are in remission[14]. In our study, however, we found no correlation between active disease in psoriasis and/or IBD and a lower abundance of *F. prausnitzii*.

The trend that we observed that patients with concomitant IBD and psoriasis had even a lower count of *F. prausnitzii* than patients with only psoriasis or IBD, needs further confirmation in a larger study in which patients who had previous IBD surgery should be excluded. Our observation of the high surgery rate in this group suggests that the course of disease is more severe in patients who have both diseases characterized by a lower *F. prausnitzii* abundance and a reduction in its anti-inflammatory compounds. Although the observation is limited due to the small sample size in this group, we do comment that surgery has a profound effect on the ecophysiology of the gut that could affect *F. prausnitzii* growth as this bacteria is extremely oxygen sensitive and requires antioxidants for its growth[31]. Previous bowel surgery is a major microbiota composition confounder that should be taken into account and is often neglected in ongoing studies.

With the IBD group as an exception, our results (log10 copies/gram) were confirmed by the percentage of total 16S rRNA gene copies for all groups. For the reason that in our cohort IBD patients had a lower total bacteria DNA count compared to psoriasis and

healthy controls, the relative abundance of *F. prausnitzii* and *E. coli* in IBD was different from the primary used method. Although we do know that in IBD total bacteria DNA is composed of an increase of pathobionts and decrease of commensals (to varying degrees), this does necessarily help to interpret which method is most accurate for this group, and remains a question mark for future research.

Our study groups within the psoriasis cohort were comparable on confounding factors such as age, gender, diet, BMI, smoking (Table 1). Within the HS cohort smoking behaviour and BMI differed between the groups, which is consistent with literature[32]. We do not expect that a higher prevalence of smoking is the reason for the normal levels of *F. prausnitzii* that we found in HS[33]. In our psoriasis and HS group, drug use was no confounder, as the vast majority had no medication and excluding the patients using medication did not change the results. In IBD medication use can be a confounder, which is difficult to exclude since IBD patients often require these. In our study we did not find a relationship between the use of specific medication, such as biologicals, and the abundance of *F. prausnitzii*.

As stated earlier, *F. prausnitzii* is an inducer of Tregs and hence involved in the balance between the T effector cells and the Tregs[20, 21]. A recent study has identified a unique and original 15 kDa protein (ZP05614546.1), called MAM, which has anti-inflammatory properties and is produced by *F. prausnitzii*. This protein and/or its derived peptides are able to inhibit the NF-κB pathway in several intestinal epithelial cells lines and are likely to have an extra- intestinal effect when released in the host systemic circulation[30]. In addition bacterium-derived short chain fatty acids are the cognate ligands for GPRC 41 and 43, two G protein-coupled receptors that mediate anti-inflammatory responses[34]. GPCR43 knockout mice showed exacerbated inflammation in models of inflammatory diseases and thus the production of short chain fatty acids provides another possible direct link between *F. prausnitzii* and inflammatory responses in the skin. It is therefore likely that such an abundant member of the intestinal microbiota can affect the local mucosal immunity in the intestine, and that this can in turn affect the immune skin status. Reduced abundance of a particular bacterium such as *F. prausnitzii* might therefore be causally related to the presence of (concomitant) psoriasis. After this initial study, further research is now required to obtain more evidence. Although *F. prausnitzii* and *E. coli* are important members of the microbiota, we acknowledge this study is limited to these bacteria and other members of the microbiota are a justified focus of future research. Further studies are required to further validation of our results. The study might provide novel opportunities for investigating the gut microbiota in IBD-associated skin disorders. Unlike the human genome, the composition of the human microbiota can be readily manipulated. Further studies could explore the administration of *F. prausnitzii* as probiotic for IBD and psoriasis patients and modulation of the intestinal microbiota by

diet. In addition to therapy, further research in this field may lead to new insights into the mechanisms of the development of psoriasis and IBD.

In conclusion, depletion of the beneficial gut bacterium *F. prausnitzii*, which is often reported in IBD, is also present in patients with psoriasis but not in patients with HS. Interestingly, the presence of *E.coli* was increased, providing more evidence for the presence of a gut dysbiosis in psoriasis patients. The disturbance of the gut microbiota may be greater in IBD patients with dermatologic comorbidity than in patients with only IBD. This might be due to a more severe type of IBD or due to more systemic inflammation including the skin. The next step in future research should advance this result from correlation towards causation. Nevertheless, our results support the presence of a gut-microbiome-skin axis in IBD, which could provide new patient-friendly therapeutic venues for dermatological comorbidity, such as psoriasis, in IBD.

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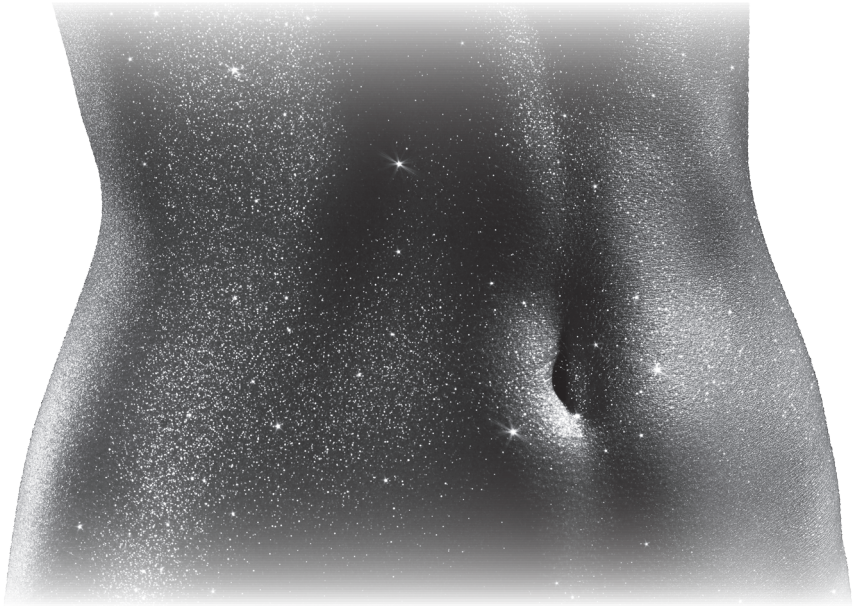

CHAPTER 6

The microbiome is not the missing pathogenic link between hidradenitis suppurativa and Crohn's disease: an exploratory study

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ABSTRACT

Background

Hidradenitis suppurativa (HS) is a chronic inflammatory skin disease characterized by nodules, abscesses and fistulas in axillary, inguinal and perianal areas. Because of its high response to antibiotic treatment and its strong association with Crohn's disease (CD), where the gut microbiome is known to be disrupted, we hypothesized that the skin and/or gut microbiome in HS might be altered in comparison with healthy subjects.

Findings

The study included faecal samples of 17 HS patients without oral antibiotic treatment for at least 2 months, and 20 healthy controls. In addition, axillary skin swabs were collected from 6 patients and 7 healthy controls. 16SrRNA sequencing was performed on all skin and faecal samples. No significant deviation in the composition of the skin and/or faecal microbiome composition of HS patients versus healthy controls were observed. However, one patient, the only patient diagnosed with severe HS, harbored a distinct skin and faecal microbiome colonization.

Conclusions

These initial data indicate that there is no evidence for skin or gut dysbiosis in HS etiology similar to that observed in CD. However, but most likely secondarily, severe disease activity might change the skin and faecal microbiome composition in HS.

INTRODUCTION

Hidradenitis suppurativa (HS), also known as acne inversa, is a chronic inflammatory immune-mediated skin disease with unclear and complex etiology. The most frequently affected areas in HS are the axillary, inguinal and perianal regions. An association between HS and the inflammatory bowel disease Crohn's disease (CD) has been suggested[1-5] and was recently confirmed in a study by Shalom et al[6]. CD and HS share common inflammatory immunopathogenic pathways, and both can present with sterile abscesses as well as fistulas in the perianal region, which can make them difficult to distinguish from each other. The missing pathogenic link between CD and HS might relate to the bacterial skin and/or gut microbiome as we have proposed earlier [7]. For CD, microbial involvement in interaction with an aberrant immune system has been widely recognized, but in HS no such data is available to date. Research on this topic is timely for HS, as acknowledgment of the disabling disease expands and its incidence keeps rising in the modern society. The current prevalence of HS is estimated to be up to 4% in the general population[8, 9] with a higher prevalence of HS in CD (6.8-23%)[10, 11]. Besides the strong association with CD, the high response of HS to antibiotic treatment together with the clinical presentation suggests that microbes are involved in the pathogenesis of HS. Not only the skin microbiome might be of interest in HS, the gut microbiome as well, as gut bacteria can modulate immune responses beyond the intestine[12]. In CD, one of the most specific and validated microbiotic aberration is a depletion of the anti-inflammatory intestinal bacterium *F. prausnitzii*, one of the main butyrate producers in the gut. Recently, we have demonstrated a similar depletion of *F. prausnitzii* in psoriasis and CD patients, however this depletion was not observed in HS patients[13]. Therefore, we hypothesized that other CD-associated microbial changes might be involved in HS. Hence, in an exploratory study, we compared the overall bacterial skin and gut microbiome composition of HS patients versus healthy controls by 16SrRNA sequencing.

METHODS

Medical ethical approval was obtained from the ethical committee at the Erasmus University Medical Center - Erasmus MC, Rotterdam, The Netherlands (MEC-2014-371) and all participants (18-65 years) signed informed consent. No oral antibiotics were allowed 8 weeks prior to inclusion and no topical antibiotics/steroids were allowed 7 days before sampling. A total of 17 HS patients were included at the outpatient clinic of the Department of Dermatology of the Erasmus MC. All patients underwent a physical (dermatological) examination and the HS diagnosis was clinically confirmed by a dermatologist. The Hurley stage and HS-PGA (HS-Physician Global Assessment) were used as

disease-activity tools. Additional exclusion criteria were the presence of cancer, active infections, pregnancy, and inflammatory bowel diseases (IBD). From each participant, a faecal sample was collected by post (arrival within 48 hours) and then immediately stored at -80°C. Six of these patients also provided an axillary lesional skin swab, of which 3 additionally provided an axillary skin swab of the non-lesional side (the remaining patients had symmetrical skin lesions in both axilla's). After the collection of the skin samples, using sterile cottons swabs, they were immediately stored at -20°C in a medium mixture of phosphate buffered saline (PBS) and Cell lysis buffer (Ambion, Life Technologies) (1:1). The samples of the HS patients were compared with healthy controls (n=20), of whom we collected a faecal sample and, of some HS patients, an axillary skin sample (n=7). Characteristics of the participants are depicted in Table 1 (for detailed (individual) characteristics see Supplementary Table 1 and 2).

Table 1. Characteristics of HS patients and healthy controls

	HS	Healthy controls	P-value
Number of patients	17	20	
Age (mean yrs, SD)	45.9 (11.8)	37.9 (13.6)	0.065
Gender (%female)	64.7	65.0	1.000
Smoking (%)	64.7	5.0	<0.001
BMI (mean, SD)	28.3 (4.6)	24.9 (4.2)	0.023
Caucasian (%)	76.5	75.0	1.000
Age at diagnosis (mean years, SD)	31.9 (14.3)	NA	
Disease duration (mean years, SD)	14.1 (8.9)	NA	
Local HS medication[1] n (%)	9 (52.9)	NA	
Anti-TNFα therapy n (%)	1 (5.9)	NA	
Hurley score n (%)[2]			
I	2 (11.8)	NA	
II	14 (82.4)	NA	
III	1 (5.9)	NA	
HS-PGA score n (%)[3]			
Clear	1 (5.9)	NA	
Minimal	3 (17.6)	NA	
Mild	10 (58.8)	NA	
Moderate	3 (17.6)	NA	
Severe	0 (0)	NA	

HS, hidradenitis suppurativa, BMI, body mass index

[1] None of the patients used local HS medication 7 days before collection of the samples

[2] Hurley (I clear-mild, II mild-moderate, III moderate-severe)

[3] HS-PGA, Physician's Global Assessment (clear, minimal, mild, moderate, severe)

DNA isolation

DNA was isolated from 20 mg of faeces as previously described[13]. Briefly, 1 ml of cell lysis buffer was added, samples were vortexed and incubated for 15 minutes at room

temperature. Cell lysis was achieved by bead-beating (three times for 30 seconds). The samples were centrifuged, whereupon protein precipitation buffer was added at a 3:1 ratio. After centrifugation, DNA was precipitated from the supernatant using [1:1] 100% isopropanol, and the DNA pellet washed with 100 µl 70% ethanol. DNA was dissolved in 50 µl Tris-EDTA (TE) buffer.

16SrRNA sequencing

Isolated DNA was sequenced at the Macrogen Institute, Korea, using Illumina MiSeq paired-ends. The hyper variable region V3-V4 was amplified using the 16S Amplicon PCR Forward Primer [5' TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG] and 16S Amplicon PCR Reverse Primer [5'GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC]. Low quality reads were filtered out and extra-long tails were trimmed. The paired reads were then merged using FLASH[14]. The trimmed reads were clustered into Operational Taxonomic Units (OTU) using CD-HIT-DUP algorithm[15]. Assigning bacterial taxonomy to OTUs was done using primer-specific version of the Green Genes 13.5 reference database[16] and QIIME building software UCLUST[17]. QIIME analysis was used[18] for gathering basic statistics on the microbiome dataset. All analyses were performed following illumina's guidelines for 16S metagenomic library preparation for Miseq system (www.macrogen.com).

Statistical analysis: The Shannon diversities and species abundances of the samples were analyzed using paired and unpaired t-tests. False Discovery Rate was used to adjust for multiple testing[19].

RESULTS

Gut microbiome

The composition of the faecal microbiome of HS patients was not significantly different from that of healthy controls (Figure 1A and 1B phylum level per individual HS patient, Figure 1C average main phyla abundances and p-values). In addition, no specific microorganisms significantly associated with HS were found at genus level. Furthermore, the faecal α-diversity was not significantly different between the HS patients and healthy controls (Figure 1D). Notably, at all levels, and most clear at family level (Figure 2), the only HS patient with severe HS (see supplementary Table 1 patient ID #15, Hurley III stage) showed a prominent deviation of the faecal microbiome composition compared to the other HS patients and healthy individuals.

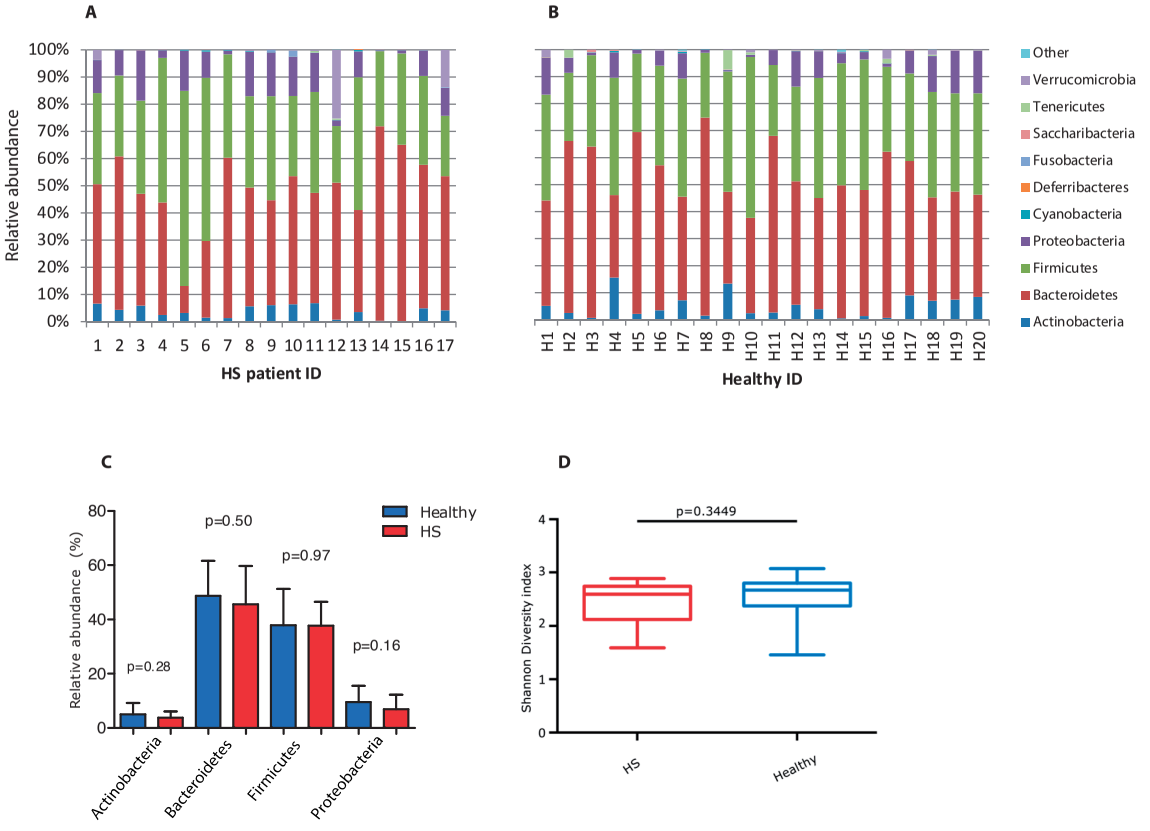

Figure 1. Faecal microbiome composition of HS patients (A) and healthy controls (B) at phylum-level. The composition of the faecal microbiome of the main phyla was not significantly different between HS patients and healthy controls (C; mean, SD). The Shannon Diversity Index was not significantly different between HS patients and healthy controls (D).

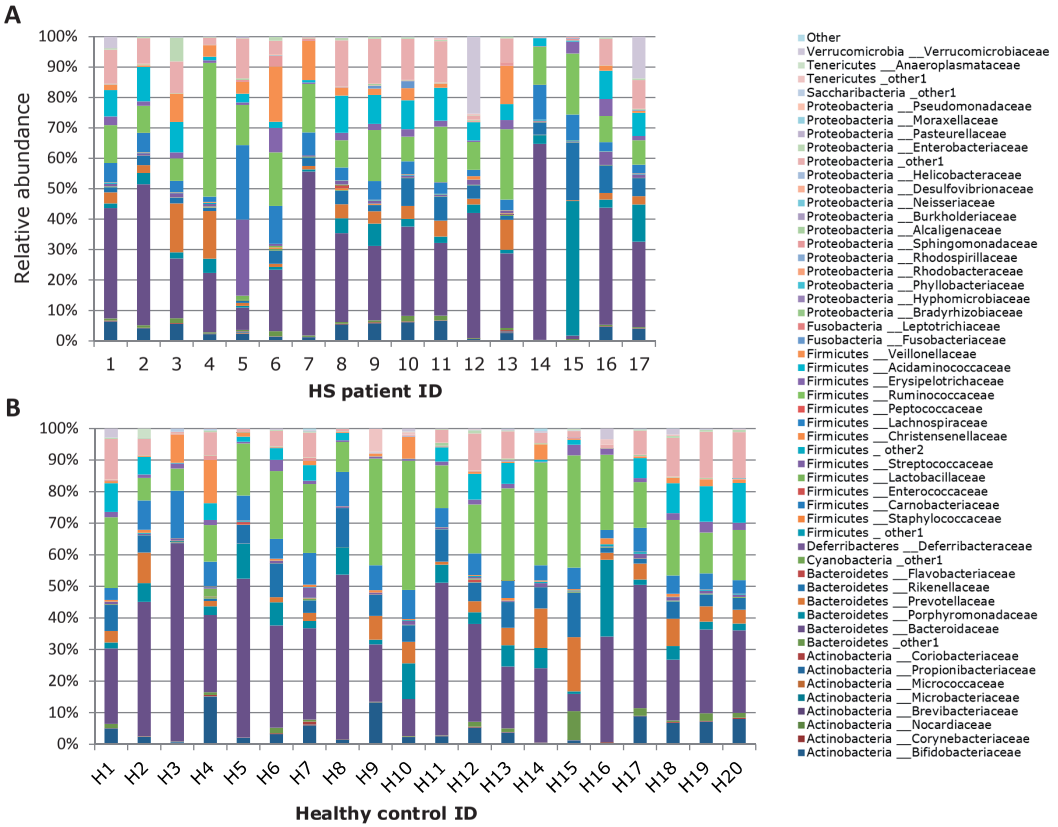

Figure 2. Faecal microbiome composition of HS patients (A) and healthy controls (B) at family-level. HS patient #15 showed a great deviation of the faecal microbiome composition compared to the other subjects.

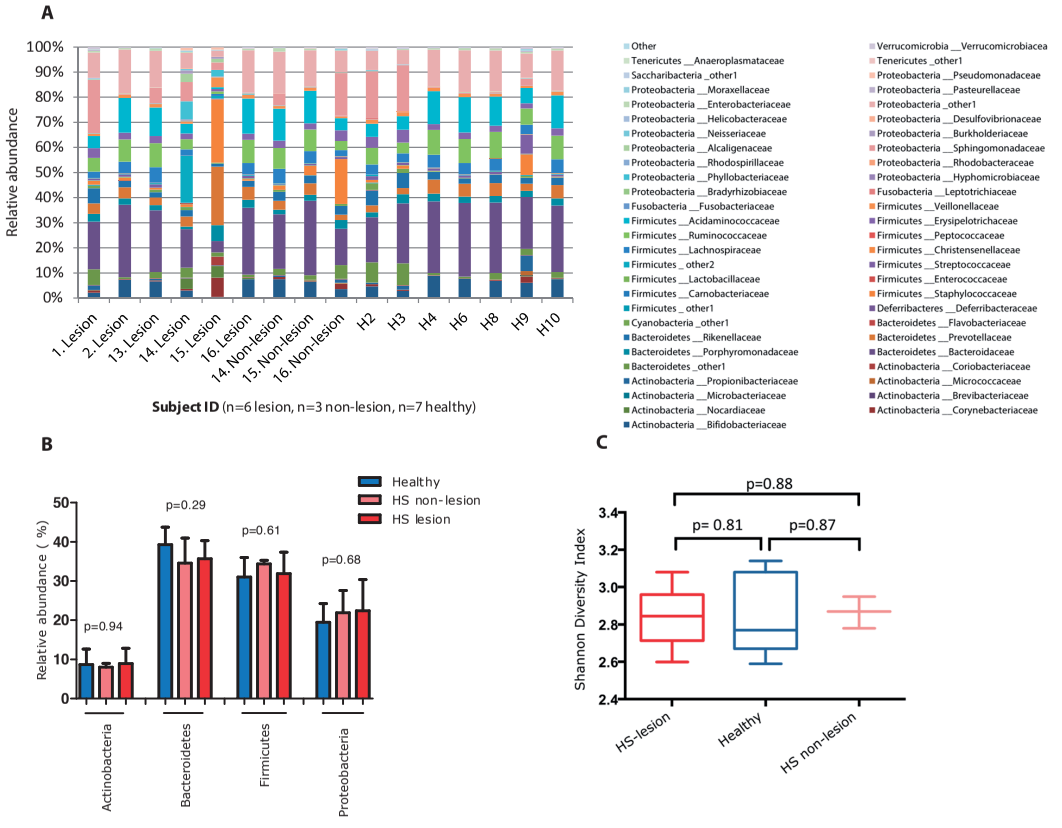

Figure 3. Skin microbiome composition of HS patients and healthy controls. At family-level the lesional skin sample of HS patient #15 showed a deviation in comparison to the other samples of HS patients and healthy controls (A). The composition of the skin microbiome of the main phyla was not significantly different between HS lesional skin, HS non-lesional skin, and healthy controls (B; mean, SD). The Shannon Diversity Index was not significantly different between the groups (C).

Skin microbiome

Of the 3 HS patients of whom both a lesional and non-lesional axillary skin sample was collected (patient ID #14, 15, 16), the lesion sample of HS patient #15, but not the non-lesional skin sample, showed a great deviation of the skin microbiome composition from the other samples (Figure 3A family level). This was the same patient of which the faecal microbiome composition was altered and the only patient with severe HS in the cohort (see supplementary Table 1 and 2). In the other paired samples (patient ID #14 and #16), no distinct differences in skin microbiome composition were observed.

One-way ANOVA showed no significant differences in the skin microbiome composition between the lesional skin of HS patients (n=6), non-lesional skin of HS patients (n=3) and healthy controls (n=7) (Figure 3B phylum-level). The Shannon-diversity was not significantly different between the HS patients (lesional or non-lesional) versus healthy controls (Figure 3C).

DISCUSSION

In this study the composition of the skin and gut microbiome of HS patients was found not to be altered in comparison with healthy controls, which is in line with our previous work[13]. However one HS patient with severe disease (Hurley III stage) showed a prominently altered composition of the lesional skin microbiome, and also of the faecal microbiome. Severe HS may therefore be associated with a specific skin and faecal microbiome profile, which would need confirmation in a larger cohort of severe HS patients. However, we hypothesize that a dysbiosis limited to severe HS is rather secondary to the inflammation. Especially since the bacterial composition of the non-lesional skin sample of this patient did not show a prominent change. While it is speculated that intestinal microflora dysbiosis may contribute to the etiology of autoinflammatory diseases in other organs, the current study suggests this is not the case for HS. No previous studies on the gut microbiome in HS have been reported, and for the published studies on the skin flora almost all studies used culture-based methods and no healthy control group[20-22]. Metagenomics was performed in one study on six HS samples, but this study did not include a control group[23].

So if bacterial dysbiosis is unlikely to contribute to the etiology of HS, how is the efficacy of antibiotics in HS explained? That might not be due to their anti-bacterial effects, but rather to other immunomodulatory effects of antibiotics. Furthermore, characterization of the skin microbiome, in comparison with the gut microbiome, is still in its infancy and the best and most valid method has yet to be determined due to the topographical diversity and confounding factors[24, 25]. In addition, it may be that while the superficial

skin microbiome is not disturbed in HS, the skin microbiome at a deep tissue-level is. However invasive biopsies would be required to prove this.

In addition, fungal dysbiosis, which has also been demonstrated in CD[26], is not excluded and might be a future focus. Inflammatory reaction to pathogenic fungi, such as *Candida albicans* can drive inflammatory immune responses, whereas a beneficial yeast such as *Saccharomyces cerevisiae* can stimulate anti-inflammatory triggers, such as IL-10 production.

Smoking and high BMI are well-known profound risk factors for HS, which was reflected in our study group[27]. While smoking is also a risk factor for CD to a smaller extent, it is conceivable that in HS external "life-style" factors such as smoking and obesity may play a larger etiologic role than microbial dysbiosis.

This is the first study analyzing the skin and gut microbiome in HS patients using 16SrRNA sequencing. The results demonstrated no evidence for involvement of the skin or gut microbiome dysbiosis in HS' etiology similar to that in CD. However, probably as a consequence, severe disease activity might alter the gut and lesional skin microbiome composition of HS patients. Therefore, in subpopulations of HS a distinct microbiome composition might be the result. Future studies are needed to validate these findings, and should also investigate the deeper parts of the hair follicle, abscesses, sinus tracts and the fungal microbiome in HS.

FUNDING

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Supplementary Table 1. HS patient characteristics

Patient ID	Gender/ Age	Smoker	BMI	Ethnicity	Age at Diagnosis	Disease duration	Hurley/ HS-PGA	Skin swab[1] n	Lesion/ non-lesion
1	F/25	Yes	36.4	Cau	16	9	1/min	1	Lesion
2	F/41	Yes	25.9	Cau	19	22	2/mild	1	Lesion
3	F/55	Yes	31.1	Cau	29	26	2/mod	0	
4	M/45	No	27.4	Arab-berber	17	28	2/min	0	
5	F/51	Yes	26.0	Cau	38	13	1/min	0	
6	M/59	Yes	36.0	Cau	48	11	2/mild	0	
7	M/45	No	25.8	Indian descent	23	22	2/mod	0	
8	F/56	No	35.3	Cau	53	3	2/mild	0	
9	F/44	No	22.9	Indian descent	39	5	2/clea	0	
10	F/30	Yes	32.0	Cau	20	10	2/mild	0	
11	F/47	Yes	23.2	Cau	15	32	2/mild	0	
12	F/58	Yes	27.1	Cau	48	10	2/mild	0	
13	M/60	Yes	31.7	Cau	55	5	2/mild	1	Lesion
14	F/53	No	26.0	Afro-caribbean	38	15	2/mild	2	Both
15	M/32	Yes	22.7	Cau	18	14	3/mod	2	Both
16	M/55	Yes	23.9	Cau	45	10	2/mild	2	Both
17	F/25	No	28.0	Cau	21	4	2/mild	0	

M, male; F, female; Cau, caucasian.

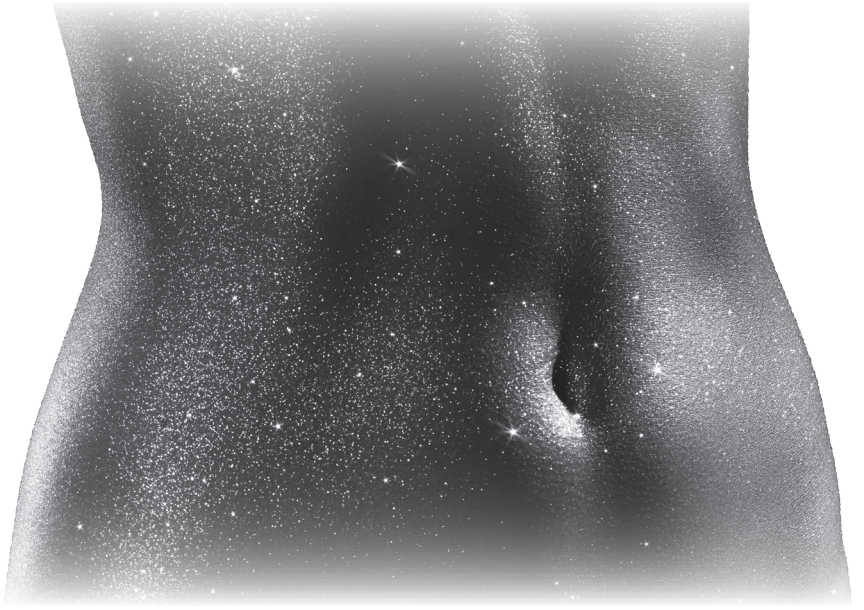
[1]Of every patient a faecal sample was collected. 0, no skin sample was collected; 1, a lesional skin sample was collected; 2, both a lesional and non-lesional skin sample were collected.

Supplementary Table 2. Healthy control characteristics

Patient ID	Gender/Age	Smoker	BMI	Ethnicity	Skin swab[1] n
H1	F/25	No	22.6	Cau	0
H2	F/29	No	22.4	Cau	1
H3	F/29	No	22.4	Asian	1
H4	M/24	No	30.1	Mixed	1
H5	F/29	No	20.4	Cau	0
H6	M/30	No	23.1	Mixed	1
H7	F/22	No	22.3	Asian	0
H8	M/23	No	20.5	Cau	1
H9	F/27	No	37.2	Mixed	1
H10	F/27	No	22.8	Cau	1
H11	F/26	No	21.5	Cau	0
H12	F/53	No	25.8	Cau	0
H13	F/52	No	22.4	Cau	0
H14	M/52	No	28.1	Cau	0
H15	M/54	Yes	28.4	Cau	0
H16	F/53	No	29.1	Cau	0
H17	M/63	No	25.1	Cau	0
H18	F/51	No	23.6	Cau	0
H19	F/45	No	28.7	Cau	0
H20	M/44	No	20.8	Cau	0

M, male; F, female; Cau, caucasian

[1]Of every healthy subject a faecal sample was collected



CHAPTER 7

The effects of UVB light on the skin and gut microbiome

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ABSTRACT

Ultraviolet (UV) light treatment is used in the dermatological clinic to treat conditions such as psoriasis. While it is known that UV light has direct anti-bacterial effects *in vitro*, to date no studies have investigated the effect of UV light on the skin and gut microbiome. We therefore investigated the effect of UV light on the skin and gut microbiome in dermatological patients (n=35), most of whom suffer from psoriasis (n=20), undergoing UVB therapy at the Department of Dermatology. We collected skin and faecal samples before start and after 8 weeks of UVB therapy for patients' skin disease. On the skin, *Fusobacteria* were significantly decreased after UVB therapy, including the genus *Leptotrichia*. *Actinobacteria* were significantly increased after UVB therapy, of which *Micrococcus* showed a significant higher abundance and *Propionibacterium* showed a trend towards increased abundance. In contrast, in the faecal samples, *Actinobacteria*, in particular the genus *Collinsella,Cyanobacteria* and *Verrucomicrobia/*genus *Akkermansia* were significantly decreased after UVB therapy in the faecal samples. In conclusion, UVB light alters the composition of both the human skin and gut microbiome in dermatological patients undergoing UVB therapy. Future research should elucidate the role of UV-exposure in interaction with these taxa in health and disease.

INTRODUCTION

In the past decades, the field of microbiome research has been expanding tremendously. Most research has focused on the largest bacterial reservoir in our body, the gut, which contributes to health and disease into a large extent. However, microbes are not only present in the gut, and other organs such as the skin, including gut-skin interactions, are also of interest. The majority of microbiome studies focus on disease related microbial signatures. However, environmental factors that can influence the microbiome composition, and can therefore potentially influence the findings of disease focused-studies, are often disregarded. Age[1], gender[2], obesity[3], alcohol use[4], smoking[5], diet[6] and medication use, in particular antibiotics[7], are all factors known to influence the microbiota composition. A previous study showed that the gut microbiome even varies within the different seasons[8], although it is not clear whether this variation was due to variations in, for example, diet or sun-exposure. Of bacteria living outside our body, it is known that they are highly susceptible to UVB-light, as a consequence of a lack of UV-protective pigmentation, their minuscule size and short reproduction time[9]. It has long been established that symptoms of several skin diseases improve in summer time, and nowadays UVB treatment has been implemented in standard care for skin diseases such as psoriasis and atopic dermatitis, but also for other indications such as pruritus. Interestingly, in psoriasis and atopic dermatitis microbial alterations have been recently demonstrated[10-12]. The exact mechanism of this beneficial UV-effect on dermatological symptoms is unclear but might involve beneficial alterations of the microbiome. However, we also know that UV light induces DNA damage and has immunosuppressive effects, and therefore it is also accompanied by an increased risk of skin cancer. Taken together, UVB light might be expected to either have beneficial or harmful effects to commensal or pathogenic bacteria. This study investigated the effects of UVB light on the human skin and gut microbiome composition, by comparing bacterial abundance in skin and faecal samples of 35 dermatological patients before and after UVB therapy in a paired set-up, using 16SrRNA sequencing.

MATERIALS AND METHODS

The study had a multicenter set-up: patients were included in the Erasmus University Medical Center (Erasmus MC) in Rotterdam, Reinier de Graaf Gasthuis location Delft and location Voorburg, all in the province of South-Holland in the Netherlands. Before start of the study medical ethical approval was obtained for both centers (MEC-2015-705). All participants in the study signed written informed consent. The inclusion of patients took place from January 2016 till April 2016. The study period was 8 weeks. After this period the inclusion was discontinued to exclude seasonal confounders. Inclusion criteria of the study were: all

patients starting with UVB therapy in the inclusion months and age between 18-75 years. Exclusion criteria were: oral antibiotic use < 4 weeks for start of the study, active infection/fever, treatment with UV therapy < 3 months before start of the study, inflammatory bowel disease (IBD), pregnancy, history of bowel resection, holiday plans during the study (>10 days to a western country (Europe/USA) but not in week 7-8, all other countries always exclusion).

At every center, each patient received whole body UVB therapy 3 times per week (Monday, Wednesday and Friday). As in regular practice, the received UVB-dosage was dependent on skin type (Fitzpatrick classification)[13]. Of every participant 4 samples were collected: 1. faecal sample before start of UVB therapy (t=0), 2. faecal sample after 8 weeks of UVB therapy (t=8), 3. skin sample before start of UVB therapy (t=0), 4. skin sample after 8 weeks of UVB therapy (t=8). In addition to the paired set-up of the study, partners of the patients who were not receiving UVB therapy were asked if they were also willing to participate in the study to serve as an extra control group, based on the assumption that partners are exposed to similar environmental factor and dietary factors as the patients. Also the same inclusion- and exclusion criteria were applied for the partners (except for the UVB therapy criteria).

The faecal samples were immediately stored at -80°C after arrival in the laboratory (within 48 hours, of two patients within 72 hours). Skin samples were taken from non-affected skin and were immediately stored after collection in the hospital at -20°C. A skin sample (swab) was taken using a sterile cotton swab on the middle of the right fore-arm (dorsal side), a UVB-exposed area of the body. Whether a patient used topical steroids/antibiotics on the sampling site or had a lesion on the swab-site was registered. For psoriasis patients PASI (Psoriasis Area and Severity Index), and for atopic dermatitis SCORAD (Scoring Atopic Dermatitis) was recorded at the different times points of sample collection to assess the disease-activity and response on UVB therapy. A patient was classified as a responder when the PASI-score improved more than 50%[14], or de SCORAD-score improved more than 25%[15].

Sequencing

Bacterial genomic DNA from faecal and skin samples before and after exposure to UV treatment (total 4 samples per subject), was isolated using NorDiag-Arrow Stool DNA extraction kit and NorDiag-Arrow DNA extraction kit (Autogen, Hill road, Holliston, USA). The V3-V4 hypervariable regions of the 16S rRNA gene were amplified using Hot-start HiFidelity PCR kit (QIAGEN) and the forward and reverse primers 5'-ACTCCTACGGGAGGCAGCAG-3' and 5'-ACTACHVGGGTWTCTAAT-3' respectively. PCR product was normalized using SequalPrep™ Normalization kit (ThermoFisher Scientific) and sequenced using Illumina MiSeq platform (2X 300PE, v3).

Preprocessing

Low quality reads were filtered out. Sample's barcodes (12 bp) were trimmed off the forward and reverse reads and concatenated in order to demultiplex reads to samples.

The paired reads were then merged using PEAR[16]. The merged reads were then demultiplexed using QIIME [17]. The primers and the other non-biological sequences were trimmed using TagCleaner[18]. A total number of 87 and 84 samples were successfully sequenced (sufficient amount of reads), after rarefaction analysis for skin and faecal samples respectively. The faecal samples were rarefied to a total number of 10000 reads per samples and skin samples to 5000 reads per sample, since at this point both maximum number of diversity and maximum number of samples were achieved.

OTU picking

In order to produce the Operational Taxonomic Units (OTUs), the demultiplexed reads were clustered using UPARSE (USEARCH, v8)[19]. An OTU "radius" of 3 (minimum cluster identity of 97%) was used at this step. The reads that had a chimeric model built from more abundant reads were discarded using UCHIME algorithm (USEARCH v8). The reads were mapped back to OTUs and subsequently aligned to pre-aligned SILVA 16S rRNA gene database (version 119) using Ribosomal Database Project (RDP) Naïve Bayesian Classifier v.2.2[20] with a confidence threshold of 0.90. Singletons (reads with a sequence that were present exactly once) were discarded. Finally, OTUs were further filtered in QIIME with the recommended conservative threshold of c=0.005%[21]. At this point a total number of 607 and 737 OTUs were obtained for skin and stool samples, respectively.

Statistical analysis

The primary endpoint of the study was the difference in relative phylum abundance before UVB therapy and after UVB therapy in skin and faeces of the patients. Only the phyla detected in a minimum of 20% of patients were included to exclude for non-relevant phyla (see Figure 1A and B below for which phyla for skin and faeces were excluded, respectively). To further minimize the effect of multiple testing, further sublevels (class, order, family, genus) were only analyzed when a significant difference in its upstream taxon was found. In addition to taxa abundance, diversity (Shannon en richness [number of OTUs] were determined and compared between the groups. When the taxa were normally distributed, a parametric paired t-test was performed, and when not normally disturbed, a Wilcoxon signed rank paired test was performed. Of the phyla that were significantly altered in the patients undergoing UVB therapy, the abundance in partners was also analyzed between the two time-points to check whether a similar trend was observed in subjects in similar environments, but without receiving UVB therapy.
Phylogenetic trees for all samples were generated using the FastTree pipeline (v2.1.3)[22]. Each tree was subjected to weighted and unweighted UniFrac analysis. In order to not only rely the result on phylogenetic-based distance metrices, dissimilarity matrix Bray-Curtis (abundance) was also generated. The clusteranalysis was performed according UPGMA clustering. UPGMA (Unweighted Pair Group Method with Arithmetic mean, also

known as average linkage). The statistical comparisons were carried out in R and R package vegan. We used taxon and phylogenetic-based analyses to compare 16S rRNA gene sequences within all samples. Only samples that have a pair (before and after treatment) were compared at this step. A p-value of less than 0.05 was considered significant.

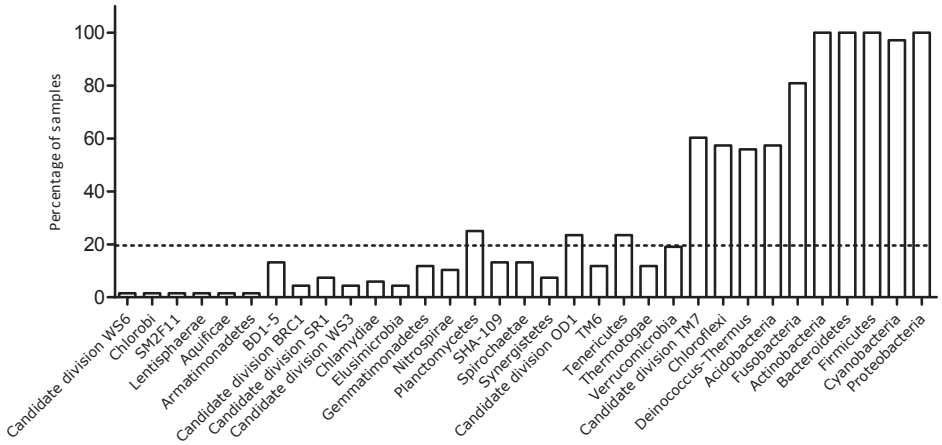

Figure 1A. Percentage of patient samples showing presence of skin phyla

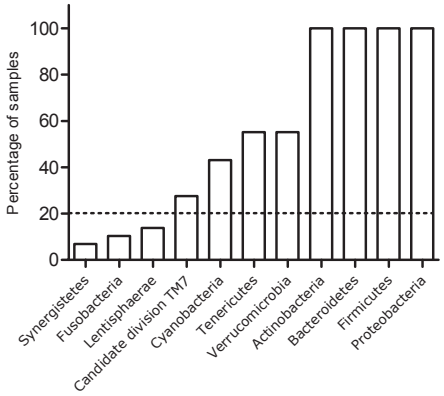

Figure 1B. Percentage of patient samples showing presence of gut phyla

RESULTS

Study population

In total 35 patients and 12 partners were included in the study, at the Erasmus MC (4 patients, 3 partners), Reinier de Graaf Gasthuis location Delft (18 patients, 4 partners) and location Voorburg (13 patients, 5 partners). General characteristics of patients and partners are depicted in Table 1, and additional skin and UVB characteristics of patients in Table 2. Supplementary Table 1 displays additional patient characteristics for each individual. Most of the patients were diagnosed with psoriasis (n=20, 57.1%), all of which were plaque-type psoriasis, 7 (20%) had atopic dermatitis and the remaining had different skin disorders (Table 1). Skin type II was the most common skin type (68.6%). One patient went for 4 days to Italy, and one for 5 days to France, the other patients did not go on holiday during the study (Supplementary Table 1). None of the patients used oral antibiotics 3 months preceding the study inclusion. Some patients received additional UVB-doses on specific body parts, such as the lower legs. Some patients were after inclusion excluded due to (unplanned) antibiotic use/holiday etc. and sequence drop-outs (see supplementary Figure 1 for a flow-chart of the study). Due to drop-outs due to a low number of reads (<5000 for skin and <10.000 for faecal samples, 29 of 35 patients were included for paired analysis of faecal samples, and 34 out of 35 patients for skin analysis. Of the partners this was 8 out of 12 for faecal analysis and 7 out of 12 for skin analysis (Supplementary Table 1 and 2).

Table 1. General characteristics of patients and partners

	Patients	**Partners**
Number	35	12
Gender n (% female)	12 (34.3)	7 (58.3)
Age (median yrs, IQR)	56.0 (42.5-65.0)	52.0 (41.8-66.3)
Caucasian n (%)	29 (82.9)	12 (100)
BMI (median, IQR)	26.4 (23.4-28.0)	26.3 (23.4-28.1)
Smoking n (%)	7 (20.0)	0 (0)

Table 2. Skin characteristics of patients

Number (patients)	35
Age at diagnosis (median yrs, IQR)	43.0 (27.5-57.5)
Disease duration (median yrs, IQR)	6.0 (1.5-16.5)
Skin disease n (%)	
Psoriasis	20 (57.1)
Atopic dermatitis	7 (20.0)
Pruritus	2 (5.7)
Nummular eczema	1 (2.9)
Asteotic / Seborrheic dermatitis	1 (2.9)
Polymorphous light eruption	1 (2.9)
Pityriasis lichenoides chronica	1 (2.9)
Lichen planus	1 (2.9)
Mycosis fungoides	1 (2.9)
Skin type n (%)	
I	1 (2.9)
II	24 (68.6)
III	7 (20.0)
IV, V, VI	3 (8.6)
Medication n (%)	
None	11 (31.4)
Local immunosuppressant	22 (63.0)
Local + systemic immunosuppressant	2 (5.7)
Cumulative UVB dosage (median, IQR, J/cm^2)	18.1 (12.0-24.3)
Weeks in study (median, IQR)	8.0 (7.5-8.0)
Total number of UV-sessions (median, IQR)	22.0 (22.0-24.0)
Previous UVB therapy n (%)	20 (57.1)

Clusteranalysis

A clusteranalysis of the skin and faecal samples (Figure 2), based on genus bacterial abundance, demonstrated that for the gut, the paired samples of the same subject (before and after UVB therapy) cluster together suggesting a good internal validity of the measurement and methodology. Therefore the inter-individual variability seems to be larger than the intra-individual variability in the faeces, emphasizing the need for paired testing of patients before and after treatment regimes. In contrast, on the skin, the clustering of samples of the same subject was less evident. Furthermore, neither skin nor faecal samples showed evidence of clustering of patients or healthy subjects, indicating that the constitutive microbiome does not show a disease-specific pattern.

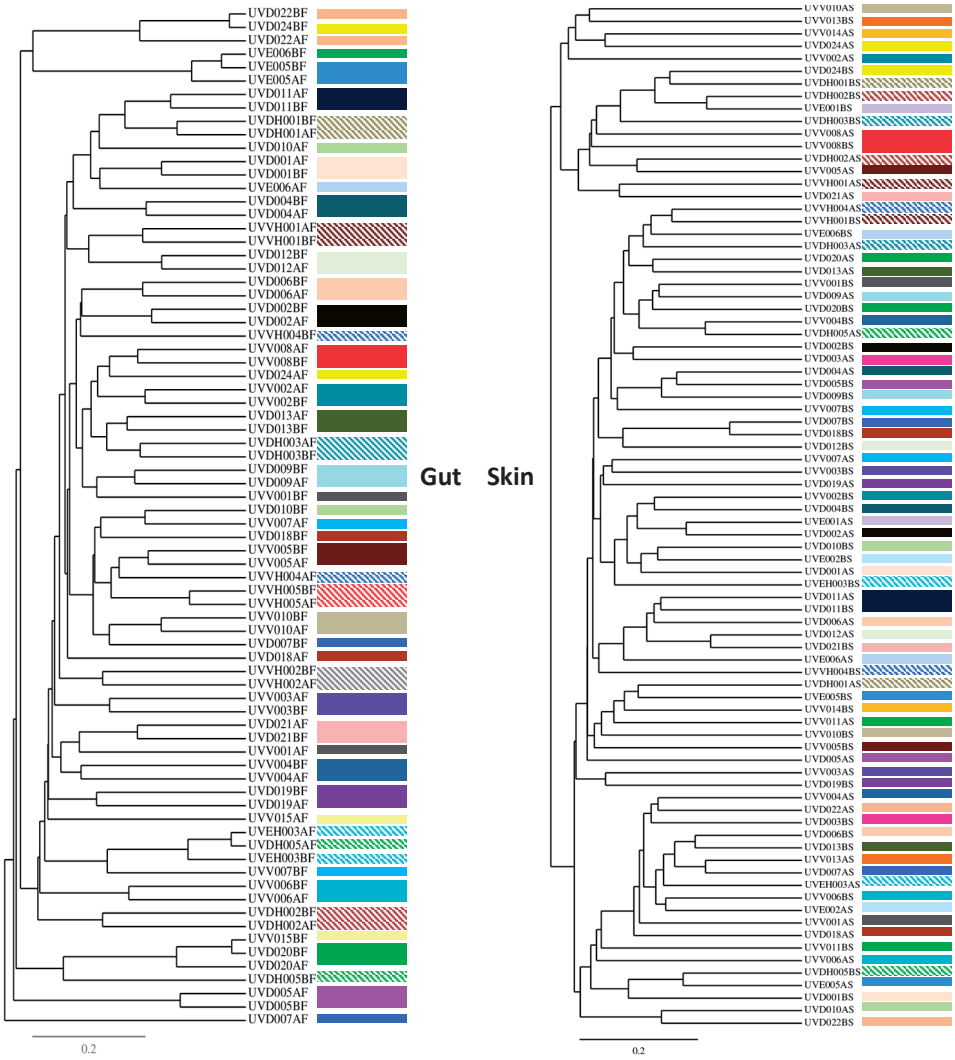

Figure 2. Clusteranalysis of gut and skin. The different colors indicate the different subjects (striped are the healthy partner controls). Within the faecal samples, the two samples of one subject often cluster together (before and after UVB therapy), while on the skin no such pattern was observed.

Skin microbiome

Several differences in the composition of the skin microbiome before and after UVB therapy were observed (Figure 3). Of the 13 phyla tested, four showed significant changes upon UVB treatment. On the skin, the phylum *Actinobacteria* had a significantly higher abundance after UVB therapy (p=0.028, Figure 3A), which was also true at class level (p=0.024). Within this class, the order *Micrococcales* (p=0.041) was significantly higher after UVB therapy, and the order *Propionibacteriales* reached near-significance (p=0.051). At the next level, the family level *Micrococcaceae* also showed a significant increase (p=0.022), and the family level *Propionibacteriaciae* showed a higher abundance after UVB therapy, which almost reached significance (p=0.052). At genus level, M*icrococcus* presence was significantly higher after UVB therapy (p=0.015, Figure 3B), with *Propionibacterium* on the margin of significance (p=0.052, Figure 3B).

Tenericutes, a less abundant phylum, and its class *Mollicutes* were significantly increased after UVB therapy (p=0.017 and p=0.017 respectively, Figure 3A). Within the *Mollicutes*, the order *RF9* (p=0.011), and at family level- and genus-level *uncultured bacteria* (p=0.014), were also significant increased after UVB therapy.

In contrast, the *Fusobacteria* phylum had a significantly lower abundance after UVB therapy (p=0.029, Figure 3A). Of the sublevels, class and order *Fusobacteriia* and *Fusobacteriales*, respectively, were also significantly lower after UVB (p=0.029). Within the *Fusobacteriales,* the *Leptotrichiaciae* family showed also a significant lower abundance after UVB therapy (p=0.032). At genus level, *Leptotrichia* was significantly decreased after UVB therapy (p=0.044, Figure 3B).

For the phylum *Firmicutes* there was a trend towards a lower abundance on the skin after UVB therapy (p=0.0556, Figure 3A). The above changes were not observed between the time-points within the healthy control subjects (partners).

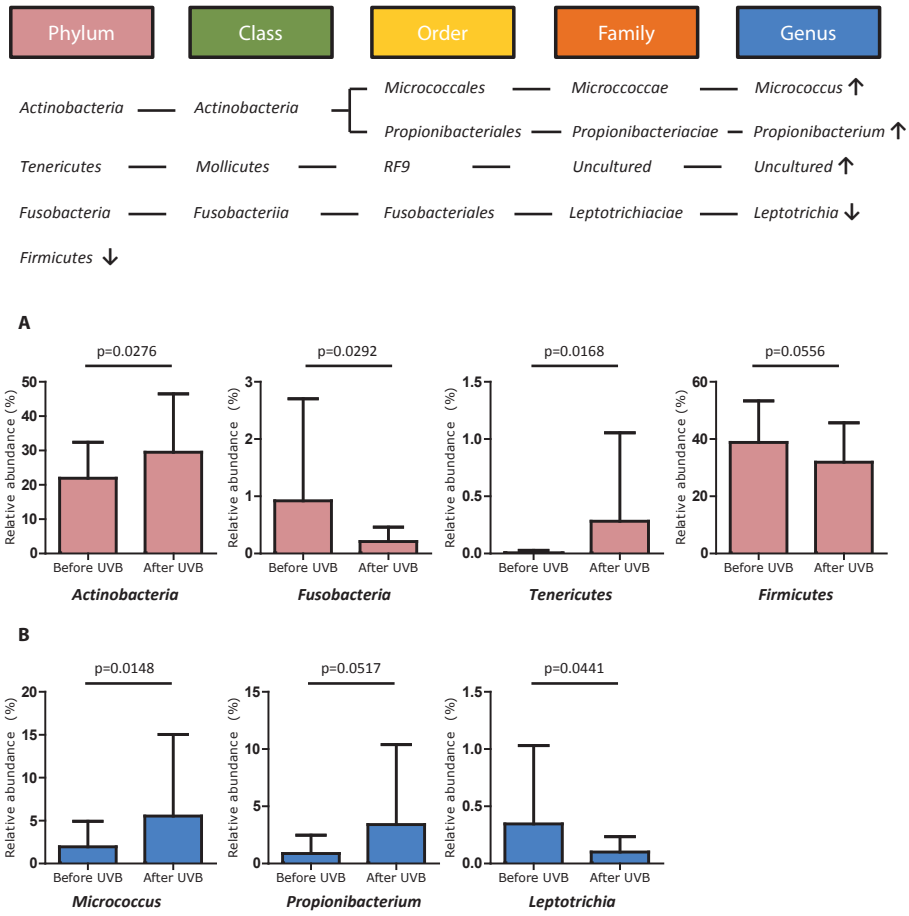

Figure 3. Differences in skin microbiome composition in patients before and after UVB therapy.
At phylum level (A) *Actinobacteria* and *Tenericutes* had a significant higher abundance after UVB therapy (p=0.0276 and p=0.0168 respectively).
Fusobacteria were significant decreased (p=0.0292), and *Firmicutes* showed a trend towards lower abundance after UVB therapy (p=0.0556).
At genus level (B) *Micrococcus* and *Propionibacterium*, belonging to the *Actinobacteria* phylum, were significant increased after UVB therapy (p=0.0517).
Leptotrichia had a significant lower abundance after UVB therapy (p=0.0441).

Gut microbiome

Of the 8 phyla tested in faecal samples, *Verrucomicrobia, Actinobacteria* and *Cyanobacteria* showed significant changes upon UVB treatment of patients. Of the intestinal *Verrumicrobia* phylum, only one genus, *Akkermansia*, was detected. Faecal *Akkermansia* abundance was significantly lower after UVB therapy (p=0.001, Figure 4). Faecal *Actinobacteria* were also significantly lower after UVB therapy (p=0.047, Figure 4A), in

contrast to the skin findings where the abundance of *Actinobacteria* was higher after UVB therapy. Within the *Actinobacteria*, the class of *Coriobacteriia*, order *Coriobacteriales* and family *Coriobacteriacia*e were also significantly lower after UVB therapy (p=0.003). At genus level, *Collinsella* was significantly decreased after UVB therapy (p=0.025, Figure 4B), whereas for *Enterorhabdus*, belonging to the same family, there was a trend toward significance (p=0.055, Figure 4B). At genus level, *uncultured bacterium* was also significantly lower after UVB (p=0.010).

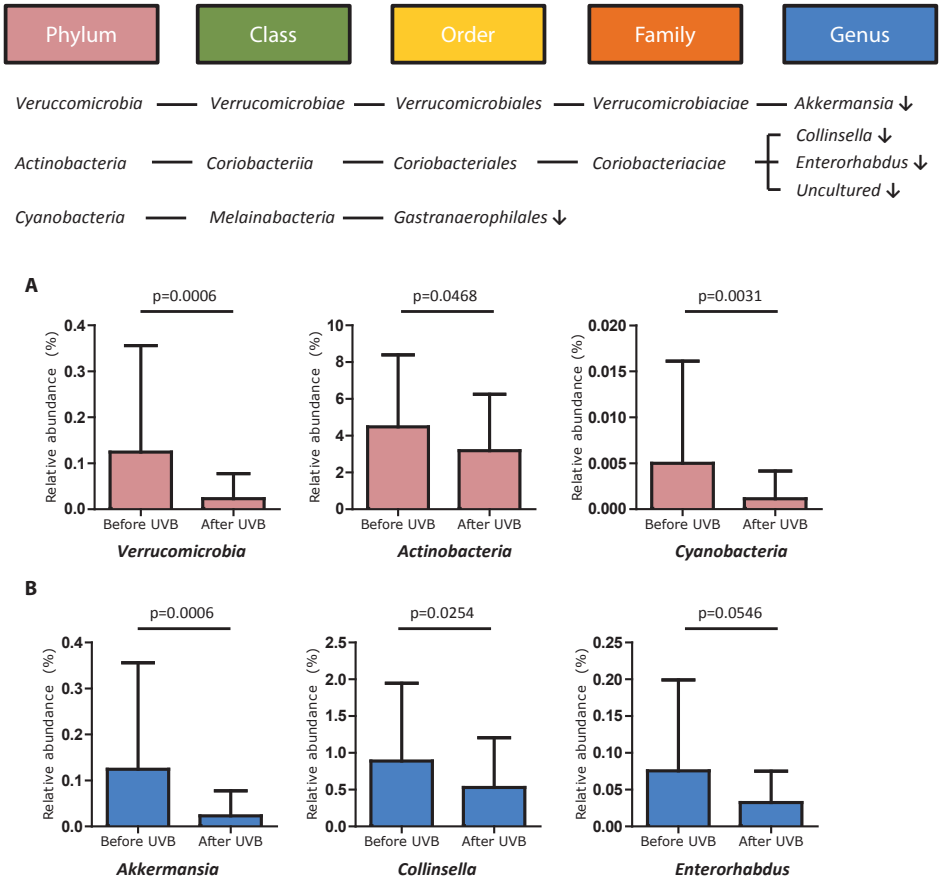

Figure 4. Differences in faecal microbiome composition in patients before and after UVB therapy.
At phylum level (A), *Verrucomicrobia, Actinobacteria* and *Cyanobacteria* were significant decreased after UVB therapy (p=0.0006, p=0.0468, and p=0.0031 respectively).
At genus level (B), *Akkermansia* (the only genus detected of the phylum *Verrucomicrobia*), and *Collinsella* had a significant lower abundance after UVB therapy (p=0.0006, p=0.0254 respectively).
Enterorhabdus showed a trend towards a decreased abundance after UVB therapy (p=0.0546).

Cyanobacteria were also significantly decreased after UVB therapy (p=0.003, Figure 4A). Subclass *Melainabacteria* and order *Gastranaerophilales* were also significantly lower after UVB therapy (both p=0.003), however at family and genus level no reliable results could be obtained due to the limited number of patients in which genus and family level were demonstrated.
When comparing the above significant phyla in faecal samples from healthy partner controls, taken at different time points, no differences in the gut microbiome were observed.

Richness and diversity after UVB therapy

At the skin, microbiome richness (number of OTUs) (p=0.4423) and Shannon-diversity (p=0.8219) were not significantly different before and after UVB therapy. Likewise, the faecal microbiome richness (p=0.4179) and Shannon-diversity (p=0.3482) were not significantly different before and after UVB treatment.

Sub analysis

Although in a small sample size, we performed a sub analysis for responders vs non-responders (see classification into responders, and non-responders, supplementary Table 1). In the sub analysis for the skin (16 responders, and 11 non-responders), no significant differences were found after UVB therapy in either group. In faeces (14 responders, and 9 non-responders), the abundance of *Verrucomicrobia/Akkermansia* was significantly increased in the responders after UVB therapy (p=0.0020). The other differences before and after UVB therapy were not present in the faeces of these subgroups. No clustering of responding or non-responding patients was observed in the cluster analysis (Figure 2).

DISCUSSION

The study showed that 8 weeks of UVB therapy significantly alters the composition of the skin and gut microbiome in dermatological patients. Skin microbiome analyses showed that UVB exposure was related to a significantly higher abundance of *Actinobacteria,* one of the most prevalent phyla on the skin. At the genus level, *Micrococcus*, belonging to the *Actinobacteria* phylum, was found to be significantly increased after UVB therapy. *Micrococcus luteus* is known to possess a UV endonuclease for efficient DNA repair[23], and might therefore be more resistant to UV-induced cell death, potentially explaining its relative increase in abundance during UV treatment of the skin. In addition, there was a trend to significance for an increased abundance of *Propionibacterium*, also belonging to the *Actinobacteria* phylum. *Propionibacterium acnes* has been thought to be involved

in acne vulgaris[24], and an increase of *Propionibacterium* might contribute to this disease. No consensus has been achieved about whether acne vulgaris improves or worsens in summer, although one of the largest studies demonstrated an aggravation, probably by increased humidity and sweating[25]. The effects of UV light on acne are unclear. While an increase of *Propionibacteria* after UV exposure has been demonstrated[26], in accordance with our findings, a decrease of *P. acnes* after UV exposure has also been described[27]. Unfortunately, the used sequencing method does not allow identification of bacteria into species level, precluding definitive statements on *P. acnes* levels in our study.

In contrast to the increase of *Actinobacteria* on the skin, intestinal *Actinobacteria* were significantly decreased after UVB therapy. The relative abundance of *Actinobacteria* is lower in the gut compared to the skin, but still an important phylum of the intestinal community. In addition, *Akkermansia,* an intestinal mucin-degrading bacteria, was significantly decreased after UVB therapy. *Akkermansia*, belonging to the phylum *Verrucomicrobia*, is classified as a beneficial bacterium and is thought to account for 3-5% of the normal intestinal flora[28,29]. It is believed to have a beneficial role in obesity[30]. Interestingly, *Akkermansia municiphila* is severely reduced in mucosa from patients with IBD, chronic afflictions of the gastrointestinal tract, often associated with skin diseases such as psoriasis[31]. While it is apparent that UV light has a direct effect on the microbes on the skin, the mechanisms of effects on the gut are more complex. One hypothesis is that whole body exposure to UV-light cannot only induce immunosuppression of the skin, but of the systemic immune system[32] and therefore influence the intestinal microbial communities. Due to the bidirectional interaction between the microbiome and immune system[33], the abundance of bacteria in the gut might consequently be altered. The effect of UV light on diseases associated with altered gut microbiomes, such as IBD, is unclear[34]. However, while a lower sunlight exposure has been associated with increased rates and severity of IBD[35], this seems at odds with our results indicating that UVB reduces *Akkermansia* abundance. What is clear, is that the intestinal microbiome confers triggers for psoriasis development, which has been attributed to the capacity of the intestinal microflora to activate specialized Th17 lymphocytes[36]. Thus, a cross-talk between gut and skin exists, and may provide clues for the pathology and link between skin and intestinal diseases.

Interestingly, in the intestinal flora only a decrease in the abundance of several taxa was demonstrated, while on the skin both increases and decreases of phyla were observed. Some of this might be explained by an efficient UV-damage repair mechanism of certain microbes (i.e. as *Micrococcus*) or production of anti-oxidants, in comparison to other microbes, growth of which has been shown to inhibited *in vitro* by UV light probably due to DNA damage[9, 37,38]. However, it does not seem likely that UV light directly affects microbial communities in the gut – rather the above described immunological changes induced by UVB might be the cause. Possibly, effects are mediated by a UV-mediated

increase in vitamin D levels, which is described to have immunologic effects[39]. To date, limited *in vivo* data on the effects of UVB light is available. Previous pilot studies on the effect of UVB therapy on the skin microbiome in atopic dermatitis showed a decline of *S. aureus*[40]. Unfortunately, a direct comparison with the current study cannot be made, as *Staphylococcus* had a very low prevalence in our subjects, probably due to the swab site in this study – dorsal side of the fore-arm, as *S. aureus* is mainly present in moist areas of the skin (i.e., skin folds). The topographical variation of the composition of the skin microbiome is important to note because it varies between different body regions, which can be categorized in sebaceous, moist and dry regions[41]. The study swab-site, the fore-arm, can be classified a dry region, a sun-exposed area, and hosts the most diverse, mixed population of the various phyla, *Actinobacteria, Proteobacteria, Firmicutes, Bacteroidetes*[41].

The strengths of this study include the paired set-up. Furthermore, the short inclusion period in the same time of the year precluding seasonal microbial changes. However more studies are required to confirm our results in a larger sample size. Other factors that might have influenced our results are the skin disease itself, and concomitant use of local immunosuppressants, although this use was constant in most patients on minor body parts and not on the swab site. Furthermore, we acknowledge that our study only investigated the effect of UVB-light (290-320 nm) on the microbiome because this is commonly used for therapy in patients with skin diseases, but sunlight reaching the earth also contains UVA (320-400nm). The study included patients with different skin diseases, therefore, the results might be different in individuals without skin diseases or in specific skin diseases.

In conclusion, this is the first study that demonstrated that UVB light alters the composition of the human skin and gut microbiome at different taxonomic levels in dermatological patients undergoing 8 weeks of UVB therapy. Future studies should further investigate the beneficial and harmful effects of UV light on human microbes in interaction with health and disease.

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Supplementary Table 1. Characteristics per patient

ID nr	UV Diag	Age (yr)	Sex	BMI	Smoking	Ethnicity	Age D (yr)	DD (yr)	Skin type	Med	Swab site	Sub type psor	DAS 1	DAS 2	Respons	Ab past	Study Wks	Times UV	Cum UV J/cm²	Add. UV J/cm²	Vacation	Faeces reads Before/after	Skin reads Before/after
D1	PS	62	M	31.0	ES	Cau	47	15	3	LI	No	C, U	6.1	2.4	Yes	>1yr	8	26	34.1	LL35.6	No	54150/37492	48651/46282
D3	PS	75	M	27.9	ES	Cau	60	15	2	LI	No	C	7.9	2.6	Yes	>1yr	8	23	20.5	LL29.3	No	59832/0*	20233/32636
D5	PS	62	M	27.1	NS	Cau	57	5	2	No	No	C, U	19	3.8	Yes	>1yr	7	22	18.1	NA	No	61646/67839	28635/34632
D7	PS	59	F	30.1	NS	Cau	58	1	2	LI	Yes	C, U	13.8	4.7	Yes	>1yr	8	24	24.3	NA	No	71284/64896	134401/22989
D9	PS	65	F	22.9	EX	Cau	35	30	2	No	No	I, C	6.8	3.9	No	>1yr	8	24	26.8	NA	No	72846/71548	29503/19106
D10	PS	22	M	21.1	S	Cau	21	0.7	2	LI	Yes	C	1.9	0.6	Yes	>1yr	8	23	9.8	LL27.8	No	77571/62689	94124/51242
D11	PS	63	M	25.3	NS	Cau	29	34	2	LSI	No	C	7.3	3.3	Yes	>1yr	7	22	16.8	NA	No	59123/33274	31904/68527
D13	PS	66	F	25.7	ES	Cau	50	16	2	LSI	No	I,C,U	11.6	6.6	No	>1yr	8	23	18.4	H/LL26.3	No	74849/77336	21416/83698
D19	PS	56	M	28.7	ES	Cau	43	13	2	No	No	C,U	10	6.1	No	>3m	8	23	22.2	NA	No	103591/68528	32161/32923
D20	PS	67	M	27.7	ES	Cau	64	3	2	LI	No	I	5.4	3.1	No	>3m	7	20	8.8	NA	No	94391/34175	15566/42371
E1	PS	65	F	21.1	NS	Cau	55	10	2	LI	No	I, C	11.5	3.1	Yes	>1yr	7	22	32.1	NA	No	64625/7185*	14231/80933
E2	PS	69	M	29.3	NS	Cau	67	2	2	LI	No	C	3.5	1.6	No	>1yr	7	22	18.0	NA	No	66324/228*	33542/45380
V1	PS	53	M	27.1	NS	Cau	43	10	2	No	No	I,C	9.6	1.6	Yes	>1yr	8	24	43.4	NA	No	102985/81423	27772/19952
V2	PS	27	M	27.7	ES	Mix	27	0.5	4	No	No	I,C	7.2	0.9	Yes	>1yr	10	22	18.7	NA	No	69772/61586	11279/24727
V3	PS	72	F	22.9	ES	Cau	45	27	2	No	No	U	5.3	2.5	Yes	>1yr	7	22	16.2	LL0.7	No	47656/85100	8972/13786
V5	PS	35	M	26.6	S	Cau	18	17	2	LI	Yes	I,C,U	8.6	5,1	No	>1yr	8	23	16.2	H/LL32.3	No	60897/26444	27639/5693
V7	PS	30	M	28.3	NS	Cau	24	6	2	No	No	C	7.9	4,2	No	>1yr	7	22	12.3	NA	4d	59772/59241	13632/10918
V11	PS	49	M	24.7	NS	Cau	28	21	3	LI	No	I,C,U	8.9	7.4	No	>1yr	8	21	11.3	LL5.8	5d	8/36770*	9656/14754
V13	PS	45	F	26.2	NS	Cau	18	27	2	LI	No	I,C	4.8	2.7	No	>1yr	8	23	19.5	NA	No	8/208*	6126/10257
V14	PS	42	M	29.1	NS	Cau	36	6	3	LI	No	C	10	0,9	Yes	>1yr	8	23	24.4	LL24.5	No	69706/109*	10862/8085
D4	AD	52	M	19.0	S	Mix	51	0.8	3	No	No	NA	29.7	12.8	Yes	>1yr	8	20	12.0	LL12.0	No	76337/69184	30596/56798
D21	AD	73	F	26.4	NS	Cau	41	32	2	LI	No	NA	21.4	39	No	>3m	8	21	6.8	NA	No	76181/76843	14665/29975
D24	AD	20	F	26.4	NS	Cau	4	16	2	LI	No	NA	32.2	16.9	Yes	>1yr	8	22	11.8	H/F18.1	No	77993/36067	44584/23499
E6	AD	25	F	18.4	NS	Cau	0	25	3	LI	Yes	NA	8[1]	4	Yes?	>1yr	7	18	17.9	NA	No	56072/57328	18941/27711
V4	AD	51	F	26.0	S	Cau	4	47	2	LI	Yes	NA	36	5,5	Yes	>3m	8	24	16.8	NA	No	61665/58726	23866/11114

Supplementary Table 1. Characteristics per patient (continued)

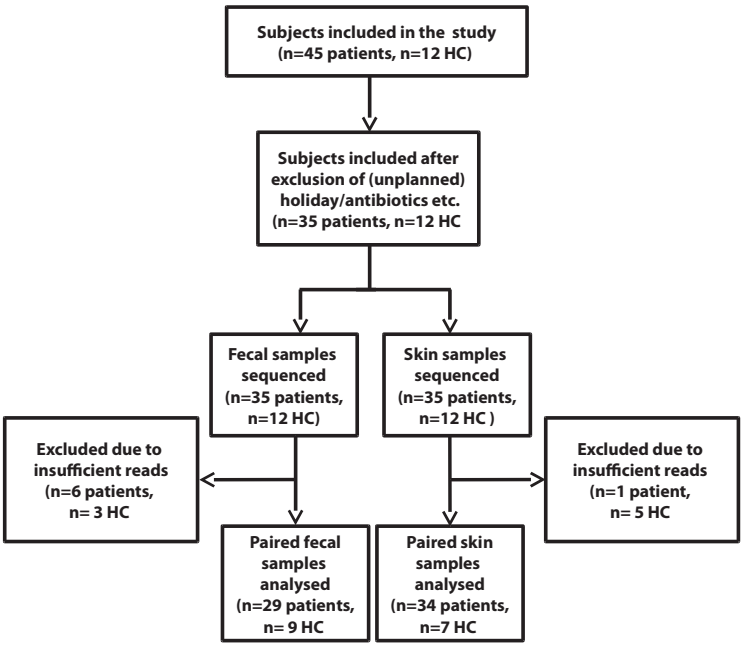
ID nr	UV Diag	Age (yr)	Sex	BMI	Smoking	Ethnicity	Age D (yr)	DD (yr)	Skin type	Med	Swab site	Sub type psor	DAS 1	DAS 2	Respons	Ab past	Study Wks	Times UV	Cum UV J/ cm^2	Add. UV J/ cm^2	Vacation	Faeces reads Before/after	Skin reads Before/after
V6	AD	65	M	28.0	NS	Indian	63	2	3	No	No	NA	17.9	11.7	Yes	>1yr	8	25	33.2	NA	No	65302/44936	5503/16307
V10	AD	29	M	24.8	NS	Cau	24	5	2	LI	No	NA	52	38.7	Yes	>3m	8	24	18.3	NA	No	67917/45730	24334/14917
V15	PR	59	M	23.5	ES	Cau	58	0.9	2	LI	No	NA	NA	NA	NA	>3m	9	21	11.2	LL12.9	No	54379/38321	20625/106*
D2	MF	67	M	25.4	ES	Cau	67	0.1	3	LI	No	NA	NA	NA	NA	>1yr	7	21	20.3	LL35.3	No	76922/60597	43158/16788
D6	NE	66	M	23.4	ES	Cau	65	1	2	LI	No	NA	NA	NA	NA	>1yr	8	24	24.3	LL32.3	No	58517/61840	19280/64978
D12	PLC	32	M	27.8	NS	Mix	32	0.4	5	LI	No	NA	NA	NA	NA	>1yr	8	24	34.8	H/LL28.3	No	81012/73822	40353/40755
D18	LP	45	F	23.1	S	Cau	44	1	2	LI	No	NA	NA	NA	NA	>3m	8	22	17.2	H/F15.3	No	15226/30347	30050/28726
D22	ASD	65	M	32.1	ES	Cau	59	6	2	No	No	NA	NA	NA	NA	>1yr	8	21	10.4	NA	No	64495/28290	56023/247125
V8	PLE	43	F	22.9	S	Indian	35	8	1	No	No	NA	NA	NA	NA	>1yr	9	25	6.3	NA	No	65918/76120	8576/39777
E5	PR	53	M	35.0	S	Iranian	48	5.5	4	LI	No	NA	NA	NA	NA	>1yr	8	22	34.3	NA	No	79561/76932	44446/17939

Abbreviations: UV Diag, skin diagnoses for which UV therapy was given; PS, psoriasis; AD, atopic dermatitis, PR, pruritus; MF, mycosis fungoides; NE, nummular eczema; PLC, pityriasis lichenoides chronica; LP, lichen planus; ASD, asteatotic and seborrhoic dermatitis; PLE, polymorphous light eruption; M, male; F, female; BMI, body mass index; ES, ex-smoker; NS, never-smoker; S, smoker; Cau, caucasian; Age D, Age at skin diagnosis; DD, disease duration; Med, immunosuppressive medication; LI, local immunosuppressant; LSI, local and systemic immunosuppressant; subtype psoriasis, I, inverse; C, capitis; U, unguium; DAS, disease activity score (PASI for PS and SCORAD for AD), 1 (before UVB), 2 (after UVB); Ab past, oral antibiotics in the past year; Cum UV, cumulative dosis UV therapy; Add UV, additional UV therapy other parts of the body; LL, lower legs; H, hands, F, feet; [1]No SCORAD of this patient was collected, a VAS score instead; *dropout skin < 5.000 reads, faeces < 10.000 reads

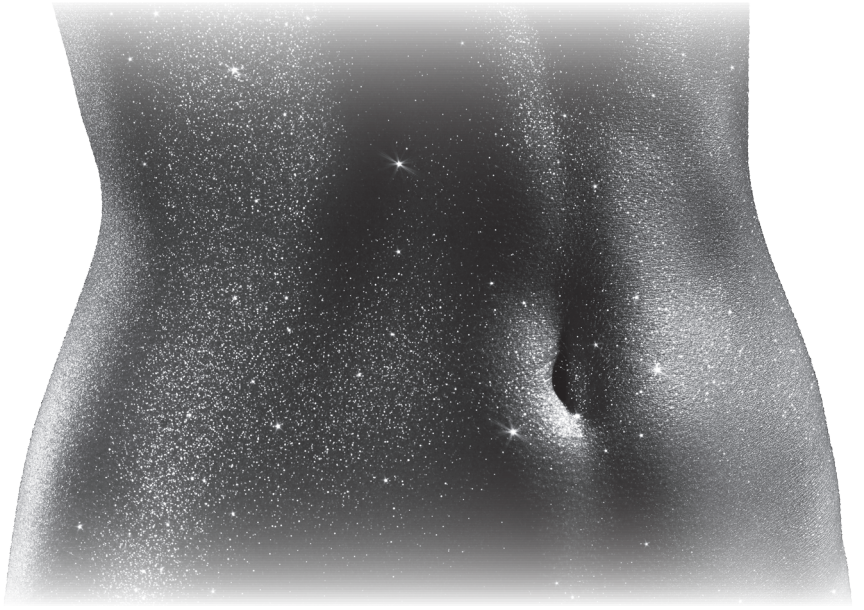
Supplementary Table 2. Characteristics of healthy control partners

ID nr	Match to	Age (yr)	Sex	BMI	Smoking	Ethnicity	Skin type	Med	Swab site	Ab past	Vacation	Faeces reads Before/after	Skin reads Before/ after
DH1	D1	53	F	25.2	ES	Cau	2	No	Yes	2-3m	No	59456/31001	7611/606477
DH2	D6	57	F	29.4	ES	Cau	2	LI	No	>1yr	No	62850/76319	18139/13450
DH3	D2	67	F	22.1	ES	Cau	2	No	Yes	>1yr	No	72399/89433	13500/30672
DH5	D18	51	M	23.5	NS	Cau	3	No	No	>3m	No	37470/68238	18156/7385
EH1	E1	66	M	26.0	NS	Cau	3	No	No	>1yr	No	37488/11*	3948/14837*
EH2	E2	67	F	26.6	NS	Cau	2	No	Yes	>1yr	No	54603/11*	19387/4838*
EH3	E6	28	M	23.2	NS	Cau	2	No	No	>1yr	No	29958/60775	19563/21930
VH1	V3	73	M	23.1	NS	Cau	3	No	Yes	>1yr	No	60852/24480	8580/25232
VH2	V7	29	F	27.7	NS	Cau	2	No	No	>1yr	No	61616/45161	6675/100*
VH3	V8	48	M	26.8	ES	Cau	3	SI	No	>1yr	No	42535/5835*	2/21995*
VH4	V10	28	F	29.8	NS	Cau	2	No	No	>1yr	No	25928/39837	6285/6967
VH5	V11	46	F	29.3	NS	Cau	2	No	No	>1yr	No	59735/38803	10/2966*

Abbreviations: F, female; M, male; ES, ex-smoker; NS, never-smoker; Med, medication, LI, local immunosuppressant;
SI, systemic immunosuppressant; Ab past, oral antibiotics in the past year; *dropout skin < 5.000 reads, faeces < 10.000 reads



Supplementary Figure 1. Flowchart of patients and healthy control partners that were excluded for paired analysis during the study



CHAPTER 8

Depletion of *Saccharomyces cerevisiae* in psoriasis patients, restored by dimethylfumarate therapy

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ABSTRACT

Background

Psoriasis and inflammatory bowel disease (IBD) are chronic inflammatory diseases sharing similar pathogenic pathways. Intestinal microbial changes such as a decrease of bakers' yeast *Saccharomyces cerevisiae* have been reported in IBD, suggesting the presence of a gut-skin axis.

Objective

To investigate whether the *S. cerevisiae* abundance was altered in psoriasis patients versus healthy controls, and whether dimethylfumarate (DMF) interacted with this yeast.

Methods

Using qPCR, faecal samples were compared between psoriasis patients without DMF (n=30), psoriasis patients with DMF (n=28), and healthy controls (n=32).

Results

Faecal *S. cerevisiae* abundance was decreased in psoriasis compared to healthy controls ($p<0.001$). Interestingly, DMF use raised *S. cerevisiae* levels ($p<0.001$). Gastrointestinal adverse-effects of DMF were correlated with a higher *S. cerevisiae* abundance ($p=0.010$). *In vitro*, a direct effect of DMF on *S. cerevisiae* growth was observed. In addition, anti-*Saccharomyces cerevisiae* antibodies were not elevated in psoriasis.

Conclusions

The abundance of baker's yeast *S. cerevisiae* is decreased in psoriasis patients, but appears to be restored upon DMF use. *S. cerevisiae* is generally classified as a yeast with beneficial immunomodulatory properties, but may also be involved in the occurrence of DMF's gastrointestinal adverse-effects.

INTRODUCTION

Psoriasis and inflammatory bowel disease (IBD) are chronic inflammatory diseases sharing similar pathogenic pathways[1]. Both diseases are characterized by an increased inflammatory response at the epithelial barrier. Secondly, similar genetic susceptibility plays an important role in the development of both disease entities[2, 3]. A third contributing factor to IBD development is an altered intestinal microbial composition, with a decreased abundance of several commensal microbes (e.g. *Faecalibacterium prausnitzii*) and an increase of pathogens (e.g. adherent invasive *Escherichia coli*)[4]. Changes in the fungal microbial community are also common, with a decreased *Saccharomyces cerevisiae* abundance as one of the most prominent observations[5]. *S. cerevisiae*, also called "baker's yeast" or "brewer's yeast" because of its usage in fermentative production of bread, beer or wine, is the most intensively studied eukaryotic organism in literature and one of the most abundant yeasts in our gut. *S. cerevisiae* is known to possess anti-inflammatory properties by being able to stimulate IL-10 and inhibit TNF-α[5, 6]. Whether the fungal microbiome also plays a role in psoriasis is as yet unknown.

Dimethylfumarate (DMF) is an effective therapy for psoriasis, and an emerging therapy for multiple sclerosis (MS)[7, 8]. There is growing interest for the implementation of DMF for other chronic diseases such as IBD[9], although the exact mechanisms of action, besides immunomodulation, have not yet been fully elucidated[10]. DMF has been also applied as a biocide in shoe soles, clothes and furniture for prevention of mold growth. However, due to allergic eczematous reactions, its use in these applications has been discontinued[11, 12]. As IBD and psoriasis share similar pathogenic pathways, including similar bacterial disturbances in the gut as we have recently shown[13], we speculated that, as in IBD, *S. cerevisiae* abundance might be decreased in psoriasis. Interestingly, in Crohn's disease, anti-*Saccharomyces cerevisiae* antibodies (ASCAs) are significantly elevated compared to healthy controls[14, 15]. Recently, elevated ASCA levels have also been demonstrated in patients with spondyloarthritis[16], but in psoriasis this has not been studied.

In this study we investigated whether psoriasis patients harbor an altered faecal *S. cerevisiae* abundance compared to healthy controls. Furthermore, in *vivo* and in *vitro*, we investigated whether DMF had impact on the *S. cerevisiae* abundance. In addition, ASCA levels were measured.

MATERIAL AND METHODS

Patients

All patients were included at the outpatient clinic of the Department of Dermatology, Erasmus MC in Rotterdam, the Netherlands. The study was approved by the medical ethical committee of the Erasmus MC (MEC-2014-371). Written informed consent was obtained for all participants. Inclusion criteria were a confirmed psoriasis diagnosis by a dermatologist, and age between 18-75 years. Exclusion criteria were oral antibiotic use 8 weeks prior to inclusion, IBD-comorbidity, history of bowel resection, pregnancy and active infection. Clinical data was collected regarding medical history, comorbidities, medication, and disease characteristics. In the electronic charts, none of the included patients were reported to have used oral antifungal or antibiotic medication 8 weeks before the study or during the study. Duration of DMF use and presence of adverse-effects were recorded. Psoriasis Area and Severity Index (PASI) was used to assess disease activity (<10 mild, 10-20 moderate, >20 severe). From every participant, the faecal sample was sent by mail and stored at -80° Celsius within 48 hours.

A total of 49 psoriasis patients were included, of which the characteristics are depicted in Table 1. In total 30 samples were collected from psoriasis patients without DMF. A total of 28 samples were collected from psoriasis patients with DMF. Of nine of these patients, two samples were collected, one sample before and one sample after (6-9 weeks) start of DMF.

DNA isolation and quantitative polymerase chain reaction (qPCR)

DNA was extracted from 20 mg faeces per sample as described previously[13]. Briefly, 1 ml of cell lysis buffer (Ambion, Life Technologies) was added, followed by 15 min incubation period. Full cell lysis was achieved by bead-beating [three times 30s]. The samples were centrifuged, whereupon 3:1 protein precipitation buffer (Promega) was added; 100% isopropanol [1:1] was used to precipitate DNA from the supernatant; 100µl 70% ethanol was used to wash the DNA pellet. Finally, DNA was resuspended in 50µl TE-buffer, DNA concentration was measured on a Nanodrop spectrophotometer [Isogen Life Science BV, De Meern, The Netherlands] and diluted to 10 ng/µl. QPCR was performed two times as described[13]. QPCRs were performed in duplicate for *S. cerevisiae* using the following primer: F-AGGAGTGCGGTTCTTTG; R-TACTTACCGAGGCAAGCTACA[5].

Bacterial abundance analysis was performed by SybrGreen based qPCR reaction containing 20ng DNA [2µl of 10ng/µl], 9µL SYBR®Select MasterMix for CFX (ThermoFisher Scientific), 7µl dH20, 1µl 10µM forward primer, and 1µl 10µM reverse primer.

Thermocycle conditions comprised: denaturation step 10 min at 95°C; 40 cycles of 95°C denaturation for 15s; 56°C primer annealing for 30s; and 72°C extension for 30s followed by a standard melting curve analysis. The mean was calculated from the two qPCRs and the *S. cerevisiae* abundance was expressed in log10copies/gram faeces.

Table 1. Patients' characteristics

	Psoriasis - DMF	Psoriasis + DMF	Healthy controls	P-value[1]
N (patients)	30	28	32	
Age (mean yrs, SD)	46.1 (13.9)	42.7 (14.1)	42.6 (14.1)	P=0.54
Gender (% female)	60.0	50.0	62.5	P=0.52
Smoking (%)	20.0	28.6	6.3	P=0.07
BMI (mean, SD)	27.8 (5.3)	27.2 (4.5)	25.3 (4.8)	P=0.11
Caucasian (%)	80.0	82.1	81.3	P=0.98
Age at diagnosis (mean yrs, SD)	30.8 (12.2)	25.4 (11.8)	NA	P=0.09
Disease duration (mean yrs, SD)	15.7 (11.6)	17.0 (11.1)	NA	P=0.67
Psoriasis type n (%)				
Vulgaris	25 (83.3)	25 (89.3)	NA	
Guttate	3 (10.0)	2 (7.1)	NA	
Palmoplantaris	2 (6.7)	1 (3.6)	NA	
Psoriasis therapy[2]				
Immunosuppressant	2	1	NA	
Local therapy	15	14	NA	
UVB therapy	2	0	NA	
Duration DMF (wks, SD)	NA	66.8 (94.0)	NA	
PASI[3]				
< 10	16 (64.0)	20 (80.0)	NA	
>10 en ≤ 20	8 (32.0)	5 (20.0)	NA	
>20	1 (5.0)	0 (0)	NA	

DMF, dimethylfumarate; BMI, body mass index; NA, non-applicable

[1] P-value calculated by one-way ANOVA for age, BMI; unpaired-t-test for age at diagnosis, disease duration; chi-square test for gender, smoking, ethnicity

[2] Patient could have been on concomitant drugs

[3] PASI, Psoriasis Area Severity Index for plaque type (<10 mild, 10 - 20 moderate, >20 severe)

XTT assay

In vitro analysis was performed to establish whether the change in *S. cerevisiae* abundance was directly or indirectly induced by DMF. The yeast *S. cerevisiae* (MycoBank#:163963) was obtained from CBS-KNAW Fungal Biodiversity Centre, institute of the Royal Netherlands Academy of Arts and Sciences. The yeast was cultured at 30°C for 48 hours and 100µl of a 0.1McFarland yeast suspension was pipetted into a 96-well plate. Serial dilutions of DMF, fumaric acid and an antimycotic (amphotericine-B-deoxycholate) were added to the cells in triplicate. H_20 and ethanol were used as vehicle control. XTT/PMS was added to the suspension as per manufacturers' directions (Molecular Probes/Thermo Scientific) and the plate was read after 24h at OD415 nm. Six independent experiments were performed.

ASCA measurement

Serum was collected of 30 psoriasis patients, including 18 without DMF and 12 with DMF, and 17 healthy controls, for the determination of anti-*Saccharomyces cerevisiae* antibodies (ASCAs), IgA and IgG. Results were categorized as following: ASCAs (units) <20 negative; 20-25 borderline positive; >25 positive. ASCAs were determined using QUANTALite ASCA IgA/IgG ELISA (Inova Diagnostics, San Diego, CA). ELISA was performed according to the manufacturer's instructions, without modifications.

Statistical analysis

Patients' characteristics were compared between psoriasis patients without DMF use, psoriasis patients with DMF use, and healthy controls by using the one-way analysis of variance (ANOVA), unpaired t-test and chi-square test, depending of the presence of a normal distribution. IBM SPSS 21.0 statistical software, Armonk, NY, USA was used for the statistical analyses. *In vitro* data were analyzed using Graphpad Prism (version 5.1), performing paired-t-tests.

RESULTS

Patients' characteristics

There were no significant clinical differences between the groups (Table 1). Nine participants (10%) followed a specific diet. In the psoriasis patients without DMF, one followed a sugar-free and one a cow-milk- and wheat-free diet. In the psoriasis patients with DMF, one followed a gluten-free, one a low-carbohydrate, one a both vegetarian and low-carbohydrate diet and one a dairy-free diet. Of the healthy controls three were vegetarian. In the electronic charts, none of the included patients were reported to have used oral antifungal or antibiotic medications 8 weeks before the study or during the study. In the psoriasis patients without DMF, the majority had plaques on multiple body sites (diffuse) (n=28, 88%). Two had solely plaques on their feet, and two solely on their head. In the psoriasis patients with DMF, plaques were located diffusely in 26 patients (93%). One had solely psoriasis plaques on the neck and scalp, and one on the feet.

Significant reduction of *S. cerevisiae* abundance in psoriasis, corrected by DMF treatment

A significant difference in *S. cerevisiae* abundance was demonstrated by one-way ANOVA of the *S. cerevisiae* abundance between the three groups (1. psoriasis patients without DMF, 2. psoriasis patients with DMF, 3. healthy controls; p<0.001). Psoriasis patients without DMF had a significantly lower *S. cerevisiae* abundance than healthy controls (mean log10 copies/g± SD: 5.20±0.64 vs 6.25±0.63, p<0.001). Psoriasis patients using DMF had a significant higher *S. cerevisiae* abundance than patients without DMF (6.04±0.72, p<0.001), reaching levels that were similar to those of healthy controls (p=0.233, Figure 1). Excluding the psoriasis patients using systemic immunosuppressant's (n=2 without DMF, n=1 with DMF) did not affect the results. A subgroup analysis was performed for the patients for whom paired samples were available, before and after DMF (n=9). Within this group there was also a trend observed towards an increased *S. cerevisiae* abundance upon DMF treatment (before DMF, 4.99±0.64; after DMF 5.62±0.60; p=0.086). All patients showed clinical response to DMF, and PASI scores did not correlate to the *S. cerevisiae* abundance.

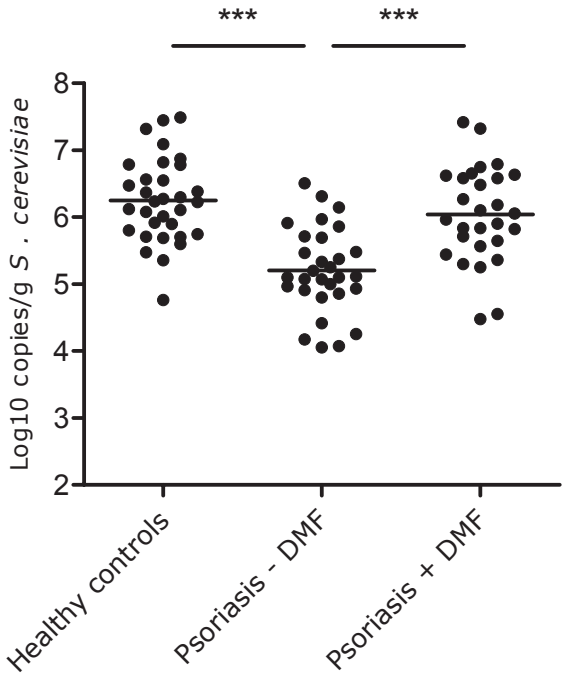

Figure 1. Psoriasis patients without DMF had a significantly lower faecal *Saccharomyces cerevisiae* abundance than healthy controls and psoriasis patients on DMF (both p<0.001). Psoriasis patients using DMF had similar *S. cerevisiae* abundance compared to healthy controls (p=0.233). The middle line represents the average abundance.

The presence of side-effects of DMF correlate with a higher *S. cerevisiae* abundance

The majority (20 of 25 patients (80%), n=3 missing) of the psoriasis patients who used DMF reported adverse effects of DMF use. The most reported adverse effects were flushing (42.9%), diarrhea (32.1%), abdominal pain (25.0%) and nausea (14.3%). Gastrointestinal side-effects (abdominal pain, diarrhea and/or nausea), were reported in 17 patients on DMF, while 8 patients had no side-effects. Patients with gastrointestinal side-effects had a significant higher *S. cerevisiae* abundance than patients without these side-effects (6.33±0.63 vs 5.55±0.72; p=0.010, Figure 2). When we included all adverse-effects, including flushing, similar results were observed (6.26±0.63 vs 5.37±0.81; p=0.013).

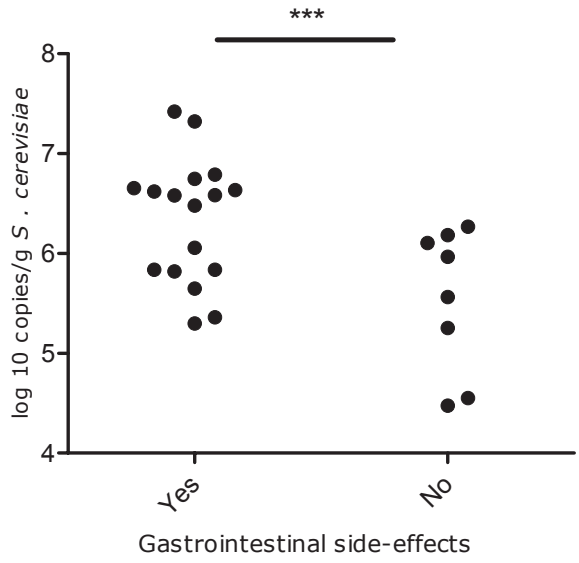

Figure 2. Psoriasis patients on DMF who had gastrointestinal side-effects had a significantly higher *S. cerevisiae* abundance than psoriasis patients without the side-effects (p=0.010).

DMF directly stimulates *S. cerevisiae* growth *in vitro*

To investigate whether the increase in *S. cerevisiae* in faeces from patients treated with DMF was a direct effect of DMF on the yeast itself, we treated S. cerevisiae *in vitro* with DMF. Results show that DMF significantly induces *S. cerevisiae* growth after 24 hours in comparison to untreated cells (p=0.0128; Figure 3). DMF is a dimethyl ester of fumaric acid, and addition of fumaric acid directly to *S.cerevisiae* also resulted in a significant increase in yeast growth (p=0.010). Administration of the antimycotic amphotericine-B-deoxycholate resulted, as expected, in a significant decrease of cultured *S. cerevisiae* (p<0.001). In addition, to exclude the possibility that the depletion of *S. cerevisiae* in

psoriasis was due to lower faecal fumaric acid levels in these patients, fumaric acid measurement in faeces was performed in 16 paired psoriasis samples, without DMF use and with DMF, and in 8 healthy controls. No significant differences in fumaric acid levels between these groups were found (see supplementary data, in particular supplementary Figure 2).

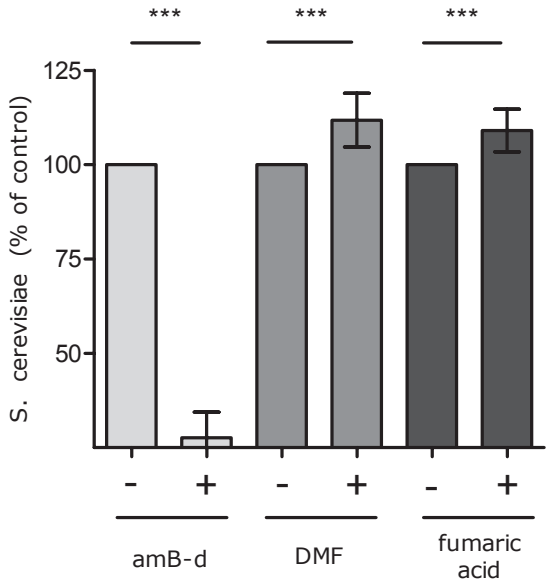

Figure 3. DMF (1.6 mg/ml) and fumaric acid (0.46 mg/ml) significantly stimulate *S. cerevisiae* growth (p=0.01), amphotericine-B-deoxycholate (amB-d) inhibits *S. cerevisiae* as expected. Maximal concentration fumaric acid used (0.46 mg/ml) was limited by the dissolvent, ethanol. Data are expressed as mean, SD.

Anti-*Saccharomyces cerevisiae* antibodies (ASCAs) are not elevated in psoriasis

The ASCA IgA level was elevated in one (3.3%) of the 30 psoriasis patients. This patient used DMF. Two patients (6.7%) (both not on DMF) had a borderline positive ASCA IgA, compared to one (5.9%) healthy subject (total n=17).

The ASCA IgG level was elevated in 3 out of 30 patients (10%), of which two with and one without DMF. One of them had also a positive IgA level. Of the healthy subjects two (11.8%) had a positive IgG ASCA, which were different subjects than the healthy control with a positive IgA. No correlation between a positive ASCA and faecal *S. cerevisiae* abundance was found.

DISCUSSION

This study demonstrates a significant depletion of *S. cerevisiae* in psoriasis, which appears to be restored in psoriasis patients on DMF. Additionally, our study confirmed that, *in vitro*, DMF can directly stimulate *S. cerevisiae* growth.

To date, information on the impact of medication on the (bacterial or fungal) microbiome is still scarce, and this study shows we should not underestimate the consequences of medication use, whether they are beneficial or harmful to our microbiome. It is unclear whether microbes and medication act synergistically or whether microbial changes could be a result of the disease remission and the anti-inflammatory milieu caused by the medication. Our *in vitro* data showing that DMF directly stimulates the growth of *S. cerevisiae* suggests that the increased colonisation is not only the result of an anti-inflammatory environment. Recent evidence shows that *S. cerevisiae* itself exhibits several immunomodulatory properties. The cell wall of *S. cerevisiae* consists mainly of β-glucans, which has immunomodulatory effects[17], and TNF-α reduction and IL10 stimulation by *S. cerevisiae* have recently been shown[5, 6]. TNF-α (pro-inflammatory) and IL10 (anti-inflammatory) are important cytokines in the pathogenesis of chronic inflammatory immune-mediated diseases such as psoriasis and IBD. Thus, a higher *S. cerevisiae* abundance might contribute to the anti-inflammatory effect of DMF. In addition to the immunomodulatory effects of DMF itself, a DMF-induced increase of *S. cerevisiae* might contribute to achievement (and maintenance) of stable remission in patients, providing a beneficial milieu for colonization. The finding that DMF seems to restore a (fungal) disturbance in psoriasis, could also be extrapolated to the *S. cerevisiae* depletion in IBD[5, 9], or potentially, MS. *S. cerevisiae* is one of the most abundant members of the fungal microbiota and might exert favorable immunologic effects in these diseases.

It is of interest to note that the benefit of *S. cerevisiae* when used as skin-conditioning agent has been demonstrated earlier[18]. Our study outlines the potential beneficial role of *S. cerevisiae* in skin (and gut) homeostasis. At present, *S. cerevisiae* is also used as dietary supplement because of its nutrients (rich in amino acids) and functions. It is unknown whether *S. cerevisiae* supplemented in food colonizes the intestine similarly as the stimulation of (probably resident) *S. cerevisiae* by DMF. Interestingly, a variant of *S. cerevisiae, Saccharomyces boulardii*, is already used as probiotic for diarrhea. Besides *S. cerevisiae*, other members of the fungal microbiome could be of interest, and moreover, bacteria could be influenced by DMF as well.

However, while the potential use of *S. cerevisiae* (and DMF) as probiotic is interesting, it should, in the light of our study, be carefully implemented after further investigations into its effect and safety[19]. Our study showed that a higher *S. cerevisiae* abundance might also have less favorable effects: the presence of gastrointestinal side-effects, such as diarrhea, nausea and abdominal pain, which are often reported side-effects of DMF

therapy, were correlated with a significant higher *S. cerevisiae* abundance. Nevertheless, although in the past decades an increasing incidence of (opportunistic) infections involving *S. cerevisiae* have been described[20], *S. cerevisiae*, which is used in baking and brewing, is still considered to be safe and non-pathogenic.

A strength of this patient cohort was that 93% did not use additional systemic medication, excluding other medication bias. Future studies should include a complete paired-sample set, and also investigate other members of the fungal microbiome in psoriasis.

In conclusion, *S. cerevisiae* is depleted in psoriasis patients. In *in vitro* and *in vivo* experiments, DMF is able to stimulate the growth of *S. cerevisiae,* which might play a role in the etiopathogenesis of psoriasis and possible also other diseases such as MS and IBD. It is also conceivable that *S. cerevisiae* acts as a bystander, however not innocently, as it might be the cause the gastrointestinal adverse-effects of DMF. Further investigations should look into the immunologic and therapeutic functions of *S. cerevisiae* and the mechanistic effect of DMF and other medications on the inhabitants of our gut. *S. cerevisiae* as probiotic might be a potential candidate for novel treatments in psoriasis patients.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY DATA

Faecal fumaric acid measurement

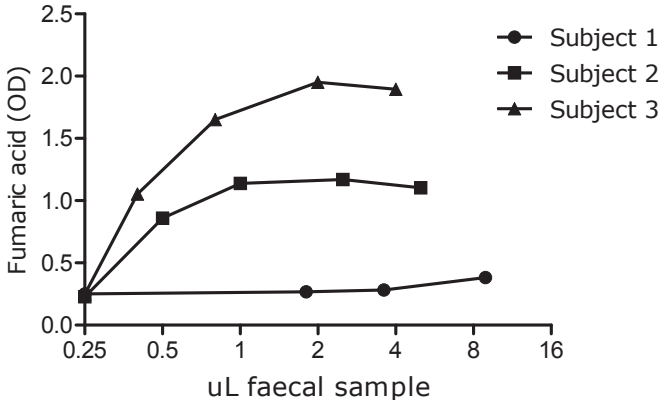
A Fumarate Detection Kit (Abcam 102516) was used for fumaric acid measurement in faeces. Forty mg of faeces was weighed, and 100 µl Assay Buffer was added to the samples. Bead-beating (2 times 30 seconds) was performed for homogenization of the samples, followed by centrifugation for 10 minutes (13.000 rpm). The protein concentration of the supernatant was measured with the DC protein quantification kit Bradford (Hercules, CA). To the supernatant of the faeces and the Assay buffer (total 50 µl), 100 µl reaction mix was added, which consisted of 90 µl Fumarate Assay Buffer, 8 µl Fumarate Developer and 2 µl Fumarate enzyme mix per sample. This was followed by 60 minutes of incubation at 37°C. The absorbance was measured at 450 nm in a microplate reader. OD measurements of samples were converted to µg/ml fumaric acid by means of standard curves included in each assay.

Psoriasis patients show normal levels of fumaric acid in faeces

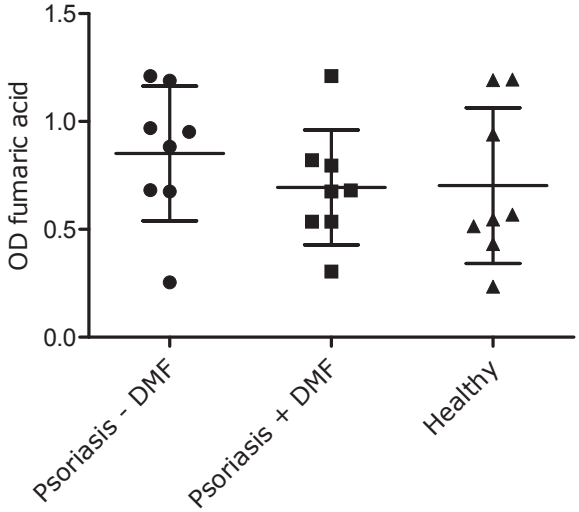
As *in vitro* both fumaric acid and its ester can stimulate growth of *S. cerevisiae*, and abundance of this organism was decreased in stool from psoriasis patients, we next wondered whether fumaric acid levels in stool from psoriasis patients were affected (and would be lower than normal). We used a commercial kit, which detects only fumaric acid and not its esters (this was tested in the assay). As fumaric acid measurement in stool using this assay has not been described before, we first performed a pilot experiment on stool samples from three individual donors, showing variable amounts of fumaric acid (Supplementary Figure 1) in these samples, using a range of faecal sample input.

Supplementary Figure 1 demonstrates that differences of fumaric acid in individual stool samples can be quantitatively measured using this assay.

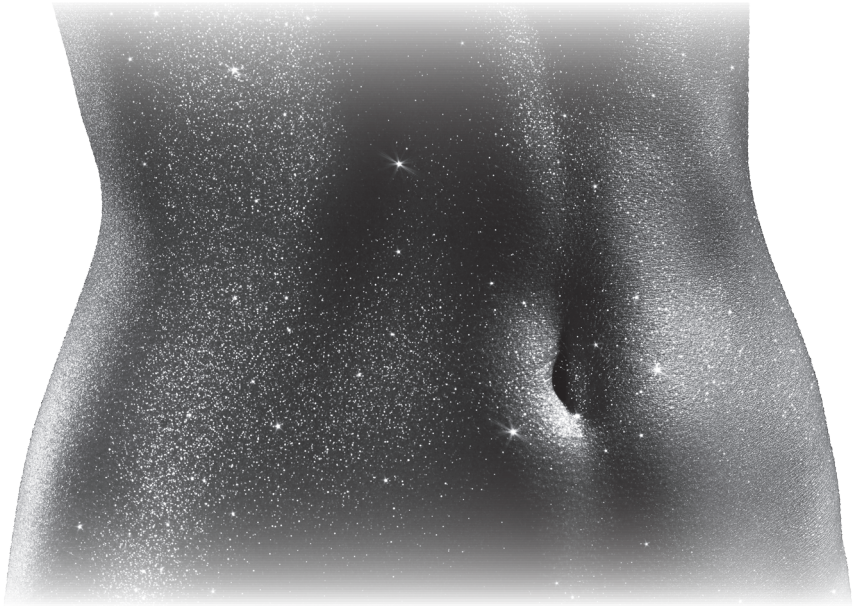
We subsequently measured the presence of fumaric acid in 8 faecal samples of psoriasis patients without DMF and in 8 faecal samples of psoriasis patients on DMF treatment, as well as in faecal samples of 8 healthy controls. A wide variety in fumaric acid levels in stool was observed, but no significant differences between the groups were demonstrated (Supplementary Figure 2).



Supplementary Figure 1. Different concentrations of faecal sample demonstrate different levels of fumaric acid (OD) in three (test) subjects.



Supplementary Figure 2. Measurement of faecal fumaric acid levels did not demonstrate a significant difference between the groups (p=0.540) (mean, SD)



CHAPTER 9

Gut microbiota developments with emphasis on inflammatory bowel disease: report from the Gut Microbiota for Health World Summit 2016

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Gastroenterology 2016

ABSTRACT

The rapidly expanding knowledge on the involvement of the gut microbiota in inflammatory bowel disease (IBD) is as exciting as it is complicated. Although the development of standardized and safe microbiota modulation therapies for IBD has just begun, initial success of such approaches has been mixed, leaving gastroenterologists in doubt as to its potential for therapeutic management for IBD. Encouragingly, as discussed by world leading experts at the Gut Microbiota for Health World Summit 2016, the most recent data indicate that a new era of personalized medicine for IBD and other western diseases is at hand. It appears that the impact of western lifestyle on our microbes has been underestimated, and might be part of both the cause and the solution for IBD. There is reason to believe that different IBD-subsets exist and further classification of these, possibly by microbes or their metabolites, will support an individualized approach. Future microbiota modulation approaches are evolving from faecal microbiota transplantation towards defined gut microbiota consortia, supported by dietary and lifestyle modifications. Thus the meeting painted a highly exiting picture as how personalized intervention in the microbiome field may yet change IBD medicine.

In the past decades the view of the gut microbiota has transformed from fighting the bad bugs with antibiotics to protecting the good bugs that function as a defense barrier and contribute to overall health. The gut microbiota is now commonly recognized as a separate "organ" which, together with the human genome and the immune system, constitutes one of the three pillars that support human health. The complexity of interactions of the microbiota with not only the host genome and the immune system, but also with the endocrine system, brain, and nutrition, makes it a challenge to grasp the overall picture regarding the role of the microbiota in human health. However, in recent years the Gut Microbiota for Health organization (GMFH) has made considerable progress toward this goal. GMFH is an organization that aims to extract relevant available knowledge from the rapidly accumulating body of basic scientific data and extrapolate it to clinical settings. The GMFH organization represents a collaborative effort between the American Gastroenterological Association (AGA) and the European Society of Neurogastroenterology & Motility (ESNM) and is steered by a joint scientific committee comprised of members from both societies. At the Gut Microbiota for Health World Summit held on March 5 and 6, 2016 in Miami, leading microbiome experts from all over the world presented state-of-the-art gut microbiota science to an audience of scientists, clinicians, nutritionists, students, representatives from food and pharmaceutical industry, as well as journalists. This meeting summary highlights the updates presented at this summit, including the pitfalls and future promises regarding the microbiome, with special emphasis on inflammatory bowel disease (IBD), an entity in which the involvement of the microbiota is now widely accepted.

Diet: what has changed in the Environment?

With an increased global spread of Western lifestyle, the incidence of Western diseases, in particular cancer and autoimmune diseases such as IBD, continues to rise. Modern lifestyle, in particular the increased consumption of fast foods, sugar, and decreased consumption of fiber, fruits and vegetables may contribute to this increase in diseases by disrupting the microbiome from an early age. Andrew Gewirtz showed that emulsifiers, commonly added to Western foods to improve shelf-life, change the location (i.e. increase the mucosal-adherent bacteria) and composition of gut bacteria and thereby promote chronic inflammation. In addition, Bernd Schnabl presented data demonstrating that chronic alcohol abuse disrupts the intestinal barrier and can modify microbiota composition. He showed that such a disturbance of the gut microbiota composition can extend to effects beyond the intestine, and affect distant organs such as the liver, for example by bacterial translocation of pathogens or their products through the blood stream. Gut microbes (and dietary habits) might therefore also be involved in the extra-intestinal comorbidity of IBD.

Important work presented by Kishore Vipperla, on behalf of Stephen J. O'Keefe's group, suggests a connection between diet and colon cancer. Their work showed that switching from a Western African-American diet to a rural African diet for two weeks distinctly changes gut microbiota composition, and lowers risk parameters for colorectal cancer, demonstrated by increased saccharolytic fermentation and butyrate production, and decreased secondary bile acid synthesis. A dietary switch from African to western diet showed the opposite effects. Vipperla's advice was to increase the fiber content in our diet to 50 g/day and reduce fat by half in order to establish a low-CRC-risk microbiota composition. This is especially relevant for IBD patients, since they carry an increased risk for inflammation-induced colon cancer.

These studies likely demonstrate only a fraction of the dietary effects on the microbiota and health. In IBD, there is still no consensus on the exact role of diet on disease state, but the link between diet and microbes is clear. Therefore, since microbes are involved in IBD etiology, there is reason to believe diet contributes to some degree as well. As highlighted at the GMFH Summit during the panel discussion, some gastroenterologists have observed substantial success with dietary intervention in IBD patients, such as the specific carbohydrate diet, but current data is too limited to suggest general guidelines[1]. Compounding progress factors of dietary intervention trials are a poor compliance to dietary therapy, absence of appropriate controls, a lack of understanding as to patient-specific responses, as well as limited funding.

Life events that alter Gut Microbiota

Besides dietary habits, life-events can alter the gut microbiota composition. One of these life-events is pregnancy, which was discussed by Omry Koren. He showed that in healthy pregnant women (n=93) the gut microbiota composition differed distinctly between the first (T1) and third (T3) trimester, with microbial diversity in T3 becoming aberrant, characterized by an increase in *Proteobacteria* and *Actinobacteria*. It was discussed that these gestational-age dependent microbiota alterations have also been demonstrated in IBD patients, with flares of colitis remarkably less frequent in T3.

Another major life-event is birth, when maternal and environmental microbes colonize the newly born infant. Jose Clemente emphasized the importance of the first human-microbial contact for the development of a healthy microbial ecosystem. Cesarean-born infants had a persistent alteration of their gut microbiota compared to vaginal-born infants, who showed increased microbiota diversity. A recently published study by this group showed that partial restoration of the gut microbiota can be achieved in cesarean-born infants via vaginal microbial transfer from the mother[2]. Notably, Jose Clemente also referred to a study by Sevelsted et al. demonstrating that IBD patients (among other chronic immune disorders) are more frequently born by cesarean section

as compared to the general population[3]. Although this study was recently contradicted by a meta-analysis that failed to demonstrate higher IBD-risk following cesarean section[4], the children of IBD mothers do carry a higher risk for IBD, probably due to genetic and/or microbial factors. Therefore, hypothetically, children of IBD mothers might benefit at the time of birth from vaginal microbial transfer from healthy mothers.

Dr. Victoria Ruiz then discussed the importance of the colonization of the gut microbiota from early life. Data was presented showing that early-life antibiotics can cause robust and enduring alterations in the gut microbial composition. Broad-spectrum antibiotics can induce dysbiosis and provide a niche for infection by pathogens. In contrast, narrow-spectrum antibiotics targeting specific pathogens may actually protect the commensal strains, resulting in minimal alterations in the overall microbiota composition.

Taken together, this session provided clear consensus regarding the impact of "early-life" events on the microbiota and immune development, which could well be a critical window for disease prevention.

Gut Microbiota dysbiosis in "IBD-subsets"

As was agreed upon in the IBD workshop by Jonathan Braun and R. Balfour Sartor, there is no sole cause for IBD. Multiple factors are involved, including microbial, immunological, genetic and environmental factors, all of which interact. In each patient, these factors might contribute to disease to a different extent and be provoked by a specific trigger. The microbiota perturbations in IBD include reduced diversity and a depletion of beneficial bacteria and/or an increase of pathobionts, a so-called "dysbiosis"[5, 6]. Since not all IBD patients carry similar microbiotic signatures[7], identification of defined IBD-subsets will be imperative for the development of novel therapies, but progress has been limited so far in this respect. Jonathan Braun proposed in the workshop that identification of subsets of IBD based on the faecal metabolites of the bacteria, called "metabotypes", might be superior to defining the bacterial composition. Novel therapies could then target the functional end, that is the metabolites of the microbiota rather than individual bacterial strains. Although patient classification into subsets is required for a refined targeted approach, multiple factors complicate the identification of the subtypes. These factors include genetic background (which may correlate with microbiota composition), lifestyle (including dietary habits), comorbidities, and drugs such as antibiotics. Furthermore, IBD-specific factors, such as the IBD type (CD vs UC), age of onset, disease location and behavior need to be taken into account. Microbiota, genetics and disease-specific characteristics are all believed to be interrelated and therefore the hope is that the number will be limited to tens of designated subsets, rather than thousands, which will constitute an important advance for designing rational novel avenues for microbiome-targeted treatment for IBD.

Gut Microbiota Interventions

Various state-of-the-art approaches for microbiota modulation were also discussed. Eamonn Quigley presented an "old" but still viable and safe approach, in which the target is one (or few) bacteria stimulated by (prebiotics) or added to (probiotics) the diet. *F. prausnitzii* is one of the potential probiotic candidates for IBD, although its anaerobic nature has delayed its therapeutic development. Fortunately, prebiotics also have the capacity to stimulate beneficial bacteria and their functions such as butyrate production, which has anti-carcinogenic and anti-inflammatory properties. Prebiotics that can stimulate probiotic bacteria (such as *F. prausnitzii*) as well as their functional components include inulin, resistant starch, riboflavin and diverse dietary fibers. However, to date, limited data for the use of pre- and probiotics in IBD exists[8] and further research was recommended.

A second method of microbiome modulation is the transplantation of faecal material from donor to patients (faecal microbiota transplantation, FMT). FMT is highly effective for the treatment of recurrent *Clostridium difficile* toxin-induced colitis as discussed by Dale Gerding[9]. As highlighted by R. Balfour Sartor, the usefulness of FMT for IBD is currently under debate. In UC patients, one randomized controlled trial (RCT) compared FMT via enema with placebo and showed superiority for FMT[10]. A second RCT in UC patients, via nasoduodenal tube, did not demonstrate superiority of FMT, however[11]. In both studies, the transplanted microbiota given to responders showed distinct features (most noticeably increased diversity) suggesting that more stringent selection of donors is required. Furthermore, as several speakers emphasized, before FMT is implementable as accepted therapy, several issues need to be clarified. First, is FMT best used as induction or maintenance therapy, in mild, moderate or severe IBD, and in what intervals and dosage; second, the issue of durability of the effects needs to be resolved; third, the optimal mode of application needs to be addressed (antibiotic use/bowel preparation and fresh vs frozen stool); fourth, and related to the previous point, is mode of delivery (capsule, nasoduodenal, enema, colonoscopy); fifth, the potential risks with respect to transmitting feces-derived infections and potentially chronic disease phenotypes require clarification; and finally, distinguishing "good" from "bad" donors. This last issue included consideration of categorical factors (e.g. including or excluding relatedness by family, genetics, or microbial features; and, level of microbial diversity). However, the foregoing presentations highlight the need to incorporate rapidly emerging biologic and pre-clinical model data, and post-hoc analysis of human FMT trials, to define parameters that match recipients with optimal donors and their microbial functions.

Interestingly, in unpublished work, Ted Dinan discussed results that "healthy" rats that received FMT from "depressed" rats developed a similar depressive phenotype with a change in corticosterone release and tryptophan metabolism. It was hypothesized

that bacteria support coping with stress and anxiety reduction, thereby influencing mood and behavior. This gut-brain-microbiota connection, an upcoming field, might also be involved in the psychiatric comorbid symptoms in IBD (including fatigue and depression). There is evidence that microbes have the ability to produce many common neurotransmitters including GABA, serotonin, norepinephrine, dopamine, acetylcholine and histamine, and communicate with the gut via the vagus nerve, in a bidirectional manner. Consequently, "psychobiotics" are now being examined as novel therapeutics. For example, Ted Dinan presented data showing that providing *Bifidobacterium longum* to rats and humans results in a lower stress- and anxiety level compared with placebo. As such, it was commented that the selection of FMT donors should possibly be restricted to those without psychiatric comorbidity, because, intriguingly, the depressive phenotype might transfer to the recipient. This poses an additional risk for FMT, that is the potential transmission of other comorbidities such as obesity and metabolic syndrome.

Gut Microbiota as Therapeutics

According to experts' opinions, the future of FMT will evolve from processed faecal products towards defined microbial consortia. As argued by Gary D. Wu, by using defined microbial communities, cocktails of diverse bacteria, methods can be standardized to obtain reproducible and more sustainable results. Of most importance, safety will improve and adverse outcomes, such as pathogen transmission, will be prevented or significantly reduced.

Initial steps in the use of gut microbiota consortia as therapeutics have already been taken as presented by Matthew Henn from Seres Therapeutics. Consortia of commensal microbes from stool from healthy donors, ecobiotic drugs, (for example SER-109, now in Phase II), show great potential for treating *Clostridium difficile* infection[12]. Results from two exciting ongoing trials in IBD will soon follow (SER-287 Phase I/Ser-301 Preclinical phase). The goal is to establish both direct colonization of the added bacteria and indirect colonization of other commensals repopulating the gut.

Furthermore, ecobiotic drugs inhibit pathogens directly by outcompeting them or indirectly by evoking a directed immune response. Gut microbiota as therapeutics will likely be implemented in the near future for *Clostridium difficile* infection, while for IBD, a more complicated disease, further advancement in our understanding of the disease as well as the technologies is a prerequisite.

Meetings' conclusions and future implications for IBD patients

One of the major messages of the GMFH Summit 2016, which all speakers agreed upon, was that a more targeted and individualized approach is crucial for the future of gut microbiota therapeutics. Especially for IBD, there is an increased need and support for a future therapeutic shift from strictly immunosuppressive approaches to targeted and individualized approaches including microbiota modulation, as became evident during the panel discussion of the meeting. As Figure 1 shows, a prospective personalized treatment including new microbial therapies will most likely belong in the bottom of the treatment pyramid for IBD, because of the presumed low risks of short-term and long-term adverse effects. In personalized medicine, patients' preference should be prominent in the therapy choice, which is expected to contribute to its efficacy and lead to a higher quality of life. Gastroenterologists will advise patients based on their IBD subset type (possibly defined by a combination of microbiological and genetic determinants) including IBD severity. It was commented that combination treatment strategies, such as steroids for induction and gut microbiota consortia and/or diet modification for maintenance, could be a reasonable approach. The safest and lowest cost strategies include lifestyle and dietary modifications from early-life on. Compliance with these forms of therapies can be only achieved by increasing awareness of the consequences of the western lifestyle, supported by sufficient evidence. The role of FMT for IBD in its current form is unsettled at this point, yet a "pill" containing diverse beneficial gut microbiota consortia could provide a safer and more reproducible approach. Essential for moving towards personalized medicine is the identification of specific IBD-subsets based on specific microbial compositions and/or their metabolites that can help guide physicians' decisions.

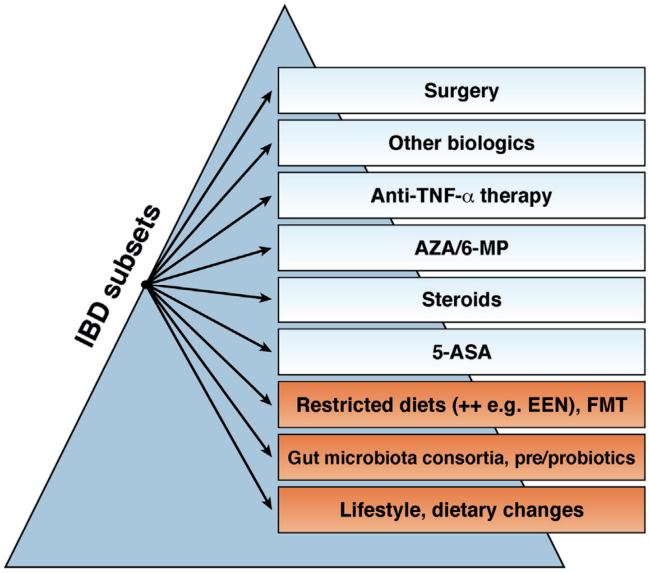

Figure 1. A hypothetical future pyramid of IBD treatment strategies. Categorization of patients into subsets will assist in choosing the best layer of the pyramid for each patient, taking patients' preference into account. (Mixed treatments are probable: e.g. induction with prednisone, maintenance with gut microbiota consortia and dietary changes). Abbreviations: AZA, azathioprine; 6-MP, 6-mercatopurine; 5-ASA, 5-aminosalicylzuur/mesalazine; EEN, exclusive enteral nutrition; FMT, faecal microbiota transplant.

APPENDIX

Speakers of the Gut Microbiota for Health World Summit 2016:

Kishore Vipperla (US); Bernd G. Schnabl (US); Andrew, T. Gewirtz (US); Omry Koren (Israel); Jose Clemente (US); Victoria E. Ruiz (US); Lee M. Kaplan (US); Max Nieuwdorp (Netherlands); Emeran A. Mayer (US); Ted Dinan (Ireland); R. Balfour Sartor (US); Jonathan Braun (US); Eamonn M. Quigley (US); Fernando Azpiroz (Spain); Dale Gerding (US); Gary D. Wu (US); Matthew Henn (US)

Panel discussion:

Matthew Henn (US); Gary D. Wu (US); Joël Doré (France); Fernando Azpiroz (Spain); Dale Gerding (US); R. Balfour Sartor (US); Lee M. Kaplan (US)

Moderators:

Gail A. Hecht (US); Francisco Guarner (Spain); Joel Dore (France); Gary Wu (US); Philippe Marteau (France)

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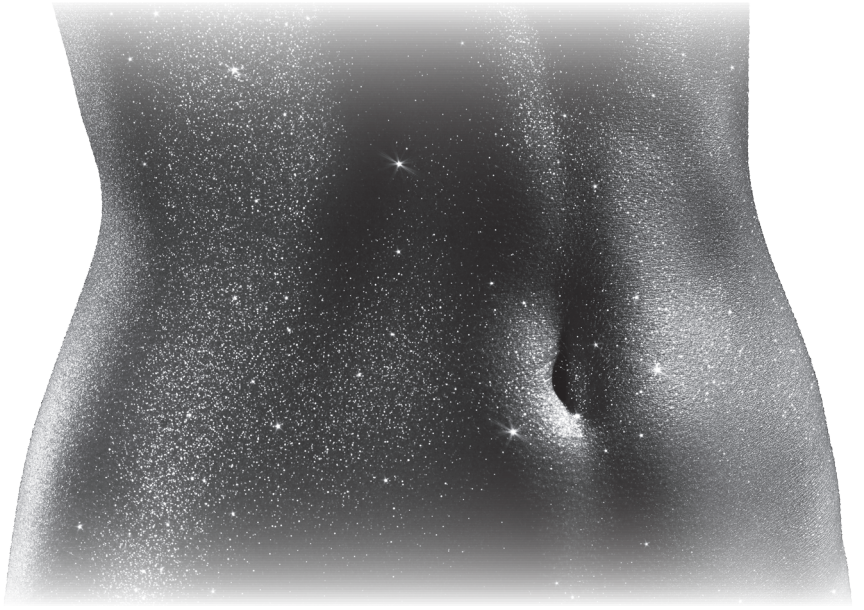

CHAPTER 10

General discussion

The link between gut and skin was the central theme in this thesis. Although the studies have focused on inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), and the skin disease psoriasis and hidradenitis suppurativa (HS), the findings might also apply to other chronic inflammatory diseases of other organs, such as psoriatic arthritis. Clinical and microbial signatures of the diseases were assessed and the chapters presented in this thesis have emphasized the interrelatedness of these diseases. As a consequence, new questions and discussions on this topic have emerged.

Concurrent IBD and skin disease: a separate disease entity?

Why does one patient develop two auto-immune diseases, while the other patient does not? A genetic predisposition in combination with exposure to certain environmental factors or triggers is required for the development of one or more auto-immune diseases. But to what extent do genetic, microbial, immunologic and environmental factors play a part in the etiology of these diseases? And to what extent do these factors overlap between the individual diseases or determine whether patients develop one or the other disease? The interaction between the multiple factors makes the etiology highly complex, and makes elucidation of the interplay of these factors between different diseases even more complicated.

In the first part of this thesis we have described the clinical characteristics of patients with concurrent IBD and either the skin disease psoriasis **(Chapter 2)** or HS **(Chapter 3)**. In these chapters we have observed that the IBD phenotype, in particular that of CD, is more severe when co-occurring with the skin diseases psoriasis or HS. Interestingly, the concomitant skin disease seems to be relatively mild in these patients. This may in part be due to the fact that these patients receive a more aggressive treatment for their IBD, which in turn has a beneficial effect on the concurrent skin disease. Most of the patients with concurrent IBD and a skin disease were first diagnosed with IBD, and consequently psoriasis or HS, which was more apparent for CD, than UC. Patients with concurrent IBD and a skin disease also seem to develop both IBD and the skin disease at a significant younger age, which might be an indication for a stronger genetic involvement in patients developing both a bowel and skin disease.

In clinical practice, it is assumed that skin diseases are more easily diagnosed than bowel diseases because of their visibility on the skin. This does apply for psoriasis, but not for HS. In HS, the symptoms are mainly located in the private areas of the body: patients often feel ashamed of their symptoms and might not present their symptoms to their physician as readily as psoriasis patients. Furthermore, HS can mimic CD in the perianal region (fistulas and abscesses)[1] and may therefore be misdiagnosed as perianal CD. Therefore, HS might still be underdiagnosed in CD and in the general population. Aspecific gastrointestinal and fatigue symptoms in combination with requirement of

more invasive procedures such as endoscopy, make the establishment of a timely IBD diagnosis also a hurdle. Since IBD, especially CD, seems to be more severe in patients with a concurrent skin disease, it is important to be aware of the potential comorbidity in skin diseases. Personal patient experiences during the studies indicated an increased need for recognition of dermatological symptoms in IBD patients. A large delay of skin diagnosis and unanswered patient questions were often reported by patients. Better anamnesis for bowel complaints by dermatologists and skin symptoms by gastroenterologists will benefit these patients. Since similar treatments are effective for both IBD and the skin diseases, increased collaboration might improve the medicamental and non-medicamental care for these patients. The paradoxal fact that anti-TNF-α treatments used in IBD are sometimes associated with the development of skin symptoms makes the necessity for cross-talk between these two disciplines even more urgent[2]. Optimal treatment for IBD should be prioritized over the treatment of skin diseases, because of the severe phenotype of IBD and its higher risks of complications and bowel resection surgery. Of note: we have observed that also other chronic inflammatory immune-mediated diseases are more often prevalent in patients with IBD and concomitant psoriasis, therefore the need for an intensive multi-disciplinary approach might also apply to specialists outside the gut-skin field.

Taken together, the disease course of patients with concurrent IBD and a skin disease shows a distinct disease pattern and might thus be classified as a specific IBD-subset, requiring a multidisciplinary approach, which will be further elucidated upon in the next sections.

The Microbiome: how important is it in Health and Disease?

It is only relatively recent that the general view on how to deal with the microbes in our body has changed from killing the bad bugs to protecting the good inhabitants in our body. New technologies, including 16SrRNA sequencing, have provided a bulk of microbiome data that has helped change this view on microbes. While earlier studies on the characterization of the microbiome were dependent on the identification of cultured bacteria, which was more or less limited to aerobic bacteria and a few anaerobic ones, new sequencing methods have been instrumental in showing the wide variety of bacterial life that inhabits our body. Novel investigations have opened up a new way of thinking about these symbionts we carry with us throughout life. Without the presence of microbes, humans would not exist. Not only from an evolutional viewpoint but also on a day to day basis – host-microbe interactions are required for maintaining nearly all functions of human physiology. Nevertheless, harmful bacteria, the pathogens, are still present, and even some good bacteria can have harmful consequences (pathobionts). But the vast majority of beneficial bacteria residing in a healthy microbiome will prevent

pathogens from invading our body and causing harm. A lot of theories have arisen the past decades about what has changed in our gut microbiome composition, and whether this might have impacted the type of diseases developing in modern society. One of them is the "missing microbes" theory of Martin Blaser, who describes that we have lost several beneficial microbes important to health due to our Western lifestyle, in particular excessive antibiotic use[3]. From birth, we are colonized by thousands of microbes coming from the mother and the environment and it is now even thought that the microbial colonization begins in utero[4]. The microbiome affects physiological function in every stage of life. The development of a more stable adult gut microbiome (entero)type[5], occurs parallel to the development of the immune system, cognitive functions, and physiology of our ecosystem[6].

However, it is good to bear in mind that there is yet no absolute consensus about the definition of a healthy "core" microbiome composition. In the past decades, several association studies have described an association between microbial alterations and diseases such as obesity, diabetes mellitus, rheumatoid arthritis, IBD and even depression[7-10]. In IBD, in contrast to skin diseases, a lot of microbiome data has been gathered to date. The pathogenesis of IBD involves an exaggerated microbial-immunological interaction in a genetically susceptible host, triggered by environmental factors. There is no sole cause for IBD, with these genetic, microbial, immunological and environmental factors contributing to disease to a different extent in each patient. The microbial changes in IBD include a reduced diversity together with a dysbiosis between the beneficial bacteria and pathobionts in the intestines[11,12]. A depletion of the protective gut bacterium *Faecalibacterium prausnitzii* is one of the most recognized and characteristic microbial signatures in IBD, in particular CD. Several anti-inflammatory properties which protect from IBD are attributed to this bacterium, including production of butyrate, inhibition of the NF-kB pathway and stimulation of IL10-producing regulatory T cells[13,14]. But the microbial changes are not similar in all IBD patients[5], and classification of individual patients into IBD-subsets will be required for the development of future microbiota targeted treatments **(Chapter 9)**. It is of interest to note that CD and UC carry different microbial signatures, and that different changes in the microbiome may therefore be expected to play a role in these two disease entities. This makes it tempting to speculate that the microbiome is one of the distinguishing characteristics between these two IBD subgroups which may explain the association with skin diseases (as described above) in one (CD) but to a lesser extent the other (UC). While at first glance a connection between an intestinal microbiome and external skin phenotype might not seem logical, animal experiments have shown that a gut-skin axis does exist, and that without an intestinal flora, psoriasis-like skin conditions are less likely to develop[15].

We investigated therefore, in the second part of the thesis, the intestinal microbial signatures of patients with skin diseases. In **chapter 5** and **6**, we have described micro-

bial intestinal signatures of patients with psoriasis and HS in comparison with microbial signatures in IBD and healthy controls. In psoriasis, we demonstrated a microbial disturbance similar to IBD, i.e. a decrease of *F. prausnitzii* and an increase of *E. coli,* while in HS we did not[16]. Clinically, this observation is not easy to explain, since HS exhibits more clinical resemblance to IBD than psoriasis. However, psoriasis, in comparison with HS, is known to have more genetic overlap with IBD[17-19]. In addition, the sample size might have been too small to find (smaller) differences in HS. As it is becoming increasingly clear that genetic factors shape the immunological interaction with the environment and the microbiome composition[20-22], it is conceivable that diseases which share more genetic overlap also show more overlap in microbial dysbiosis.

Besides the bacterial microbiome, the fungal microbiome is of interest. For example, a depletion of *Saccharomyces cerevisiae* has been demonstrated in IBD[23]. In **Chapter 8**, we have demonstrated that psoriasis patients also carry a depletion of *S. cerevisiae,* a yeast with immunomodulatory properties classified as beneficial[23], suggesting that IBD and psoriasis may have a common etiology.

It is safe to conclude that the microbiome is important for health and chronic inflammatory diseases. But the question remains whether the current findings play a causal role or are a secondary consequence of alterations of physiological systems in the body, a question which will be addressed below.

Dysbiosis: cause or consequence of chronic inflammation in disease?

The mode of action of current therapies for chronic inflammatory immune-mediated diseases is the immunosuppression of symptoms. However, if we would consider dysbiosis as the disease and IBD as the symptom, restoration of bacterial homeostasis by bacteria-targeted treatments might restore the immunologic abnormalities, as a result of the immunomodulatory properties of the bacteria[24]. However, to date, it is uncertain whether the dysbiosis is the cause or the result of the inflammation. Most evidence is derived from animal studies, which support a causative role of the gut microbiota – colitis may be conferred by transplantation of inflammation-associated faeces and several studies have demonstrated that induction of colitis is reduced in germ-free mice[25]. However, while human studies on dysbiosis and disease have shown associations, direct causality is yet to be proven. Most likely, a disease trigger from microbial, immunological or even environmental origin might be the kickoff for a vicious cycle in which immune factors and microbes are aggravating each other with chronic inflammation as a result. This would imply that the immune system and microbes are interacting in a bidirectional manner, and while the gut microbiome is not fully restored in patients with medication-induced remission, it is conceivable that therapeutic modulation of the gut microbiota may dampen the immune response in these patients. Probably in some disease subsets,

dysbiosis is the cause, whereas in others, dysbiosis is the consequence. Black-and-white would be easy, but unlikely, particular in a disease which is already recognized to be multifactorial in origin.

Microbiome and the Environment: underestimated in microbiome research?

The description of clinical characteristics of the patients (age, gender, medication use, diet etc.) is of tremendous importance for microbiome research. But even to date, despite the fact that we know that environmental factors can influence the intestinal microbiome, some studies do not describe patient characteristics at all. Even when presented, correction for these factors is difficult due to relatively small sample sizes in many studies and deficient knowledge about the influence of these factors. One of the obvious established factors to influence the microbiome composition is oral antibiotic use, which is an exclusion criteria in almost all studies now. Further confounding could be prevented by a paired set-up since most environmental factors, such as diet, are considered relatively constant within a person, and it is becoming ever more clear that inter-personal variation in microbiota often exceeds the effects of treatments within one person.

The role of diet is slowly getting more and more focus in terms of its microbiome modulation and effect on disease. In **Chapter 4** we describe that bowel symptoms in IBD are worsened by certain foods such as spicy food and fatty food, which might interact with the gut microbiota[26]. Several studies have demonstrated that diet modulates the gut microbiome composition[27-29]. The link between the gut microbiome and obesity, a food-related disorder, is published in animal and human studies[30-33]. Interestingly, a microbiota transplant from overweight mice to lean mice, was sufficient to induce obesity in the lean mice[34]. Such effects of microbiome on health status are now recognized to be the effect of changes in metabolic products that accompany the microbial changes. Large sudden dietary changes have been demonstrated to change the microbiome composition very rapidly (1-2 days)[27]. While changes induced by short term diets are relatively short lasting, long term diets are linked with enterotype switches which may significantly impact our health[28,35,36]. It has even been suggested that the Paleolithic diet, reverting to the way our early ancestors ate, would be beneficial for diseases such as IBD[37]. One the possible mechanisms suggested is through dietary modulations on our microbiome, thereby reverting the dysbiosis commonly observed in patients with IBD.[38]

Looking at the total quantity of microbial studies performed in IBD, the focus on diet is still limited, although a believe in the involvement of diets in IBD is starting to grow[38]. Difficulties with adherence, motivation, influence of other confounders, and individual differences complicate this type of research. Spicy food for example might not aggravate all patients, but only certain subsets.

In addition to diet, other factors may impact the intestinal microbiome. In **Chapter 8** we have demonstrated, *in vitro* and *in vivo*, that medication use, in this case dimethylfumarate therapy (DMF), can increase the abundance of *S. cerevisiae* and may restore the depletion of this yeast that is present in psoriasis patients. Furthermore, we found a correlation with a higher abundance of *S. cerevisiae* and the presence of gastrointestinal side-effects seen with DMF use. This study shows that we need to take the host-microbiome into consideration in the treatment effects of prescribed medications, something that is until now rarely taken into account. In an important recently published population-based analysis it was demonstrated that several factors could explain 16.4% of the variability of the gut microbiome, including medication use, diet, lifestyle, blood parameters, anthropometrics, and bowel habits and health status[39,40]. One factor that hasn't been described previously, although seasonal variation of the microbiome is known[41], is the influence of UV light on our microbiome. In **Chapter 7**, we investigated the effects of UVB light on the skin and gut microbiome in dermatological patients undergoing UVB therapy. We found that UVB light can alter the skin and gut microbiome composition, increasing *Actinobacteria* on the skin, while a decrease in the faecal microbiota of this phylum was demonstrated, among other findings. The meaning of these microbial changes needs to be elucidated, but the findings do demonstrate that medical interventions and daily environmental exposures have enormous consequences for our external, but also internal microbiome.

Taking all these factors together, modern lifestyle and the environment are undeniably related to the microbiome composition and therefore related to the increased presence of chronic inflammatory immune-mediated diseases in our society.

A microbial perspective: Personalized medicine

From the knowledge of disease-association studies, microbiome research has started investigating the functional end of the bacteria, metabolomics, which will give more insight into the meaning of our results. Since the interindividual variability of the microbiome composition seems to be larger than the intraindividual variability, future studies should have a paired-set up to be able to find the relevant alterations. And in addition to the use of microbes as therapeutics, they might also be useful for biomarker purposes. Biomarkers are of tremendous importance in diagnostics and prognostics in clinical practice in healthcare and especially in IBD where subset recognition remains an important issue. The optimal biomarker should detect early aberrations even before the disease has manifested. As functional changes (or dysbiosis) might precede disease manifestation[42], detection of a "functional dysbiotic state" might provide a critical window of opportunity for prevention, e.g. through minimal invasive treatments such as microbial supplements or dietary approaches. In a visionary outlook, faecal fingerprint-

ing might be proposed guiding health status assessment and potential intervention. Moreover, now the first stool banks have been created, autologous-transplant with previously stored faeces of healthier days is imaginable. But even more important is prevention, and early-life might be the window of opportunity to prevent disease by human influences such as breast-feeding, exposure to the right microbes, good diet, lifestyle and no antibiotics.

In contrast to the genome, the microbiome is changeable and therefore an interesting target for novel therapies. In **Chapter 9** we presented a model for future therapeutic intervention in IBD-subsets including microbial interventions. More non-invasive therapies such as dietary adjustments, lifestyle adjustments and pre-probiotics can be added to the first line of therapies. We envision a model where gut microbiota consortia (cocktails of bacteria) and systemic immunosuppressants will be introduced in a later phase or only in IBD-subsets which have a more severe phenotype. A prospective personalized treatment approach should involve the patients' own preference discussing pros and cons of treatment modalities. An advantage of immunosuppressive therapy is a lower demand of discipline and efforts, while (long-term) adverse effects are the drawback. The microbiological "lifestyle" approach, on the contrary, will require a permanent diet switch and will therefore not be suitable for all patients. With patients' preference prominent for the choice of therapy, this will already contribute to the expected effect of the therapy and lead to an improvement in their quality of life (also called the "placebo-effect"). Therapies will be based on patients' IBD subset type, which is related to expected disease severity. A mild-onset IBD phenotype might be more suitable for the "lifestyle" and/or "supplementary" approach, whereas a severe IBD phenotype will be more suitable for a medicament from the top of the pyramid, possibly combined with microbiota modulation. For example steroids could be given for induction, whereas when remission is obtained, gut microbiota consortia and/or diet modification could be used for maintenance. In addition, a highly-restricted diet might be used for induction, and a more flexible diet for maintenance. This personalized medicine approach can also be applied to other chronic inflammatory diseases. Hypothetically, future treatment of chronic inflammatory immune-mediated diseases will not be mainly based on disease diagnosis (such as IBD or psoriasis diagnosis) but on individual signatures, such as microbial and genetic signatures. As stated before, the role of microbial factors in these other chronic inflammatory diseases are not yet as fully established as in IBD, but a gut-skin axis has already been established, and the role of the intestinal microbiome clearly extends beyond the gut.

Although personalized medicine might be called specialized medicine, this does not mean further sub specialization of the specialists. On the contrary, for patients with more than one chronic inflammatory disease, personalized medicine should involve an improved collaboration between specialists, for IBD and psoriasis/HS, dermatologists

and gastroenterologists. When a patient is diagnosed with both a bowel and a skin disease, personalized treatment should offer a specific combined treatment targeting both diseases. This provides better monitoring of treatment of the patient, might prevent overtreatment and provides answers to the patients.

In conclusion, although we need to be careful not to overestimate the gut microbiome involvement in chronic inflammatory immune-mediated diseases, there is ever more support for a future therapy shift from solely immunosuppressive approaches to targeted and personalized approaches including microbiota modulation, ranging from dietary adjustments to gut microbiota consortia. Although we are becoming more aware of the importance of prevention and impacts of lifestyle on health, a sudden dewesternalized life-style change is not soon to be expected. A comparison might be made with smoking: although we already know for decades it can cause lung cancer, a decreasing incidence of smokers is only slowly observed. It is important to educate patients on the influence of the environmental factors on their disease and their options for self-management in disease.

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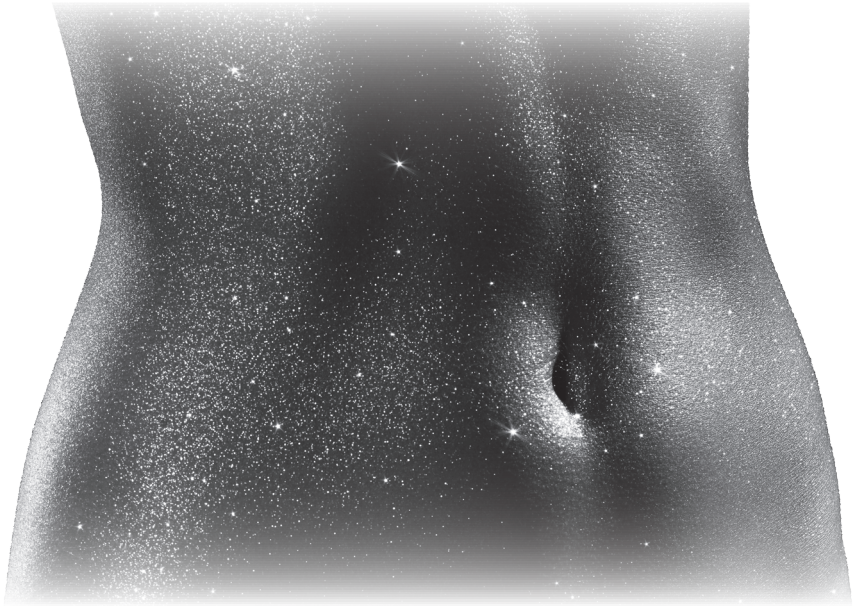


CHAPTER 11

Summary / Samenvatting

SUMMARY

Chronic inflammatory diseases, such as inflammatory bowel disease (IBD) and the skin diseases psoriasis and hidradenitis suppurativa (HS), take a prominent place in modern healthcare. And while our knowledge about the separate diseases is growing, the link between these diseases of the gut and skin, is still underexplored. In this thesis, our aim was to explore the gut-skin link, focusing on clinical and microbial factors in these diseases.

In **Chapter 1** we introduced the content of this thesis. IBD, as well as psoriasis and HS are chronic inflammatory immune-mediated diseases of respectively the gut and skin, which show considerable overlap in both etiology and epidemiology. Psoriasis, characterized by erythematosquamous plaques on the skin, has a prevalence of around 2-3% in the general population and is often co-prevalent with the inflammatory joint disorder psoriatic arthritis. The prevalence of HS, characterized by painful inflammations and fistulas/abscesses in mainly axillary, inguinal and perianal area, is estimated to be around 4%. IBD, which includes Crohn's disease and ulcerative colitis, is seen in about 0.4% of the general population, and is therefore less prevalent than these skin disorders. In several previous studies, an association has been confirmed between these bowel and skin diseases, although it is still unclear if co-prevalence of the diseases affects the disease courses of either of the individual complaints. The etiology of the diseases is highly complex, with several factors, including genetic, immunologic and environmental factors, combined inducing the chronic inflammatory process seen in these patients. In IBD, intestinal microbial factors are known to be a fourth factor contributing to its etiology, but whether this is also the case in IBD-related skin disorders remains to be investigated.

The microbiome is the collection of all the microbes in and on our body. The largest collection of microbes is located in our intestines, but other highly colonized areas include skin, mouth, lungs and vagina. From birth, or possibly already in utero, we "humans" become colonized by maternal and environmental microbes. From this moment, in a complex interaction with the developing immune system en the host genetic make-up, the composition of the microbiome is shaped and stabilizes in early adulthood. The microbiome influences different physiologic processes, including metabolism, hormone production, immunologic reactions, and digestion. The human body can be seen as an ecosystem in symbiotic balance with its microbial inhabitants. Previously, only the role of microbes as intruders and cause of diseases was given credence, but now a shift has taken place, as we have discovered their protective function in health and disease.

The vast majority of our microbiome indeed consists of beneficial, commensal bacteria which can protect us from invasion of pathogens, harmful bacteria. This is one of the reasons that caution is warranted when prescribing antibiotics, which erase not only the harmful but also beneficial bacteria. In particular when given at a young age, such antibiotic use can result in a permanent shift in the composition of our microbiome.

PART I. CLINICAL SIGNATURES

In the first part of the thesis we have described the clinical characteristics of patients diagnosed with both a chronic inflammatory skin disease and a chronic inflammatory bowel disease. In **Chapter 2** we showed that the prevalence of IBD in a psoriasis cohort was four times higher than in the general population. The risk was the most prominent for psoriasis patients with concomitant psoriatic arthritis. Crohn's disease was just as often diagnosed as ulcerative colitis in these patients. IBD and psoriasis were diagnosed at a younger age in patients suffering from both complaints, as compared to patients suffering from IBD or psoriasis alone. Most often these patients were first diagnosed with IBD, and consequently with psoriasis. In addition, the IBD phenotype, in particular Crohn's disease, was more severe (abscesses, fistulas and perianal disease) than in patients with solely IBD. In contrast, the psoriasis phenotype was mild, similar to patients with solely psoriasis. Other auto-immune diseases were also more prevalent in patients with both IBD and psoriasis and more aggressive medication was used in these patients. This study indicates that the disease course of patients suffering from both IBD and psoriasis differs from that of patients with solely IBD or psoriasis. Since treatments overlap between the diseases, increased collaboration between specialists is required for the correct and most effective treatment of both diseases.

In **Chapter 3** we examined the clinical characteristics of patients with both IBD and HS. It was apparent that most patients were female and were overweight. Besides obesity, smoking is also a risk factor for the development of HS. Patients with both IBD and HS smoked less often than patients with solely HS. In the majority, IBD was the first diagnosis to be made, and HS the second. Most patients had mild-moderate HS, and data showed that the IBD disease course was more severe (more ileocolonic and penetrating disease in Crohn's disease) in patients with both IBD and HS. Notably, the patients were more often diagnosed with Crohn's disease than with ulcerative colitis (86%).

In **Chapter 4** we investigated whether bowel and/or skin symptoms in patients with IBD, with and without skin disorders, were aggravated by certain foods. The study confirmed that certain foods, in particular fatty foods and spicy foods worsened bowel complaints

in respectively 42% en 32% of the IBD patients. Skin symptoms were not reported to be affected by the use of certain foods. Dietary behavior was not reported to be different between IBD patients with and IBD patients without skin disorders. More research into the role of diet in IBD, preferably in interaction with the microbiome, is justified.

PART II. MICROBIAL SIGNATURES

In the second part of the thesis we focused on the microbial characteristics. Because it is known that microbial factors are prominent in IBD etiology, we investigated whether patients with IBD-associated skin disorders show similar disturbances of the microbiome composition.

One of the most specific and consistent findings in the microbial signatures in IBD is a decrease of the protective gut bacterium *Faecalibacterium prausnitzii*, which possesses several anti-inflammatory properties such as butyrate production. In **Chapter 5** we analyzed whether the intestinal abundance of *F. prausnitzii* was similarly decreased in psoriasis and HS patients, in comparison with IBD patients and healthy controls, using quantitative PCR. As expected based on earlier studies, faecal samples from IBD patients showed a significant reduction in *F. prausnitzii* abundance, as compared to healthy controls. In psoriasis, but not HS, a similar depletion of *F. prausnitzii* was found, mimicking the findings in IBD patients. Furthermore, we found an increase in *Escherichia coli* abundance in both psoriasis and IBD patients, as compared to healthy controls, however this was again not found in HS patients. Two (smaller) cohorts of patients with both IBD and psoriasis, and both IBD and HS, were also analyzed for these bacteria. It was apparent that the dysbiosis of both bacteria was further increased in patients with both diseases, compared to the patients with one disease. This study supports the presence of a gut-skin link in IBD and psoriasis and demonstrates that patients with both diseases might have a distinct disease pattern.

The microbiome consists of more bacterial species than the ones previously investigated, and we considered the fact that a gut-skin link in HS might be found by studying the remaining bacteria. Therefore, we investigated the microbiome composition in HS patients on a deeper level in **Chapter 6**. In an exploratory study we determined the composition of the gut and skin microbiome of HS patients in comparison with healthy controls, using 16SrRNA sequencing. Again, we did not find evidence for microbiome involvement in HS patients. Only in the one patient with severe HS, a severe disturbance of the skin microbiota was demonstrated, probably secondary to the inflammation. Interestingly, this was accompanied by a disturbed gut microbiome in this patient.

Thus, while the missing pathogenic link between HS and CD was not found in this study, future studies with a larger sample size might find a link within a subpopulation of the HS patients.

In **Chapter 7** we have examined whether UV light has effect on the skin and gut microbiome composition. It is known that inflammatory skin disorders such as psoriasis improve in summer, and this is why UV therapy has been standard care for several skin disorders. In this study, we investigated the effects of UVB light on the skin and gut microbiome in dermatological patients undergoing UVB therapy in a paired setting, using 16SrRNA sequencing. The results indicate that UVB light alters the composition of both the skin and the gut microbiome in dermatological patients. An increase, but also a decrease of certain species was observed on the skin. However, in the faeces only a decrease of several taxa was observed. On the skin, among other findings, an increase of the prevalent phylum *Actinobacteria* was observed, whereas in the gut a decrease of this phylum was shown. Whether the observed alterations are favorable or harmful to our microbiome, will need further investigation.

Besides bacteria, the microbiome also consists of fungi/yeasts, on which we focused our attention in **Chapter 8**. In IBD, one of the prominent findings of the fungal microbiome is a depletion of *Saccharomyces cerevisiae*, a beneficial yeast possessing several immunomodulatory properties. In this chapter, we demonstrated that in psoriasis patients also a faecal *S. cerevisiae* depletion was present, which appeared to be restored with the use of dimethylfumarate therapy (DMF), a commonly used therapy for psoriasis. Besides these *in vivo* findings, we confirmed *in vitro* that DMF stimulates *S. cerevisiae* growth. Interestingly, patients who had gastrointestinal side-effects of DMF had a higher *S. cerevisiae* abundance than patients without these side-effects. This study demonstrates that not only are intestinal bacterial alterations, mimicking those in IBD, present in psoriasis patients, fungal alterations can also be found. Furthermore, it shows that medication use can affect the microbiome composition, and that *S. cerevisiae* might have beneficial, but also harmful (gastrointestinal) side-effects.

In **Chapter 9**, the newest gut microbiota developments, with emphasis on IBD, were discussed**.** Even though the microbiome research field is relatively young compared to for instance the field of genetic research, resulting in studies with smaller sample sizes, it is a very promising area of research. The microbiome may present as a possible therapeutic target, as it is changeable, whereas the genome is not. Increasing evidence shows that the microbiome is involved in the etiology of IBD, although there is still no consensus about whether the microbial alterations are primary or secondary effects of the inflammation. The definition of IBD-subsets will be required in the future in order to provide

individualized and optimal treatments. The development of disease in each individual is dependent on the convergence of different factors, none of which are the same in two patients, and resulting in disease courses which are just as variable. Following our previous studies, IBD patients with for example concurrent psoriasis might be classified as a distinct subset. Hopefully, future developments will be able to combine the discovery of certain microbial patterns with the genetic patterns, to identify individuals belonging to these different disease subsets. But even more important is prevention of disease, for which the window of opportunity may be early-life, at which time the major changes in microbiome composition occur. Now that many associations between chronic inflammatory diseases and microbial alterations have been uncovered, further microbiome research will have to elucidate the functional consequences of these bacterial changes. New insights offer opportunities for new non-invasive treatments such as lifestyle and diet modifications. Besides this, gut microbiota consortia (cocktails of bacteria) are being developed, to restore the dysbiosis of the microbiota. It is now thought that a pill containing such bacteria is more likely to be the future than faecal microbiota transplantation. Therapeutic interventions targeting the microbiome are still in a developmental phase, but do contain promise for the future. The optimum treatment will be made upon individual characteristics combined with patients' preference.

NEDERLANDSE SAMENVATTING

Chronische inflammatoire (ontstekings) ziekten, zoals inflammatoire darmziekten (IBD) en de huidziekten psoriasis en hidradenitis suppurativa (HS), nemen een steeds prominentere plaats in de zorg in. En terwijl onze kennis toeneemt over de afzonderlijke ziekten, blijft het verband tussen de aandoeningen onderbelicht. In dit proefschrift hebben wij getracht de link tussen de darm en huid te exploreren, waarbij wij ons gericht hebben op klinische en microbiële factoren die betrokken zijn bij deze ziekten.

In **hoofdstuk 1** hebben wij de verschillende hoofdstukken van dit proefschrift ingeleid. IBD, psoriasis, en HS zijn allen chronische inflammatoire immuun-gemedieerde ziekten. Hoewel bij deze ziekten verschillende organen zijn aangedaan, respectievelijk de darmen en de huid, bestaat het vermoeden van een aanzienlijke overlap tussen deze ziektebeelden, zowel qua presentatie als qua oorzaak. Psoriasis, gekarakteriseerd door rode, schilferende plaques op de huid, komt voor in ongeveer 2-3% van de algehele populatie en wordt vaak samen gezien met artritis psoriatica, een chronische inflammatoire gewrichtsziekte. Het voorkomen van de ziekte HS, gekarakteriseerd door pijnlijke ontstekingen en abcessen in de oksels, liezen en het perianale gebied, wordt op ongeveer 4% van de algehele populatie geschat. IBD, een verzamelnaam voor de ziekte van Crohn en colitis ulcerosa, komt in ongeveer 0.4% van de algehele populatie voor, en wordt daarmee minder vaak gediagnosticeerd dan de huidziekten psoriasis en HS. In eerdere studies is een associatie aangetoond tussen het voorkomen van deze huidziekten en IBD, al is het nog onduidelijk wat het effect hiervan is op het klinische beeld van de ziekten. De oorzaak van chronische inflammatoire ziekten is complex, en is opgebouwd uit een combinatie van genetische, immunologische en omgevingsfactoren. In IBD spelen daarnaast ook microbiële factoren in de darm een prominente rol. In hoeverre dat tevens bij IBD - geassocieerde huidziekten zo is, is nog niet eerder onderzocht.

Het microbioom is de verzameling van alle micro-organismen in en op ons lichaam. Het grootste aantal microben bevindt zich in de darm, maar ook in de mond, huid, longen en vagina bevinden zich grote hoeveelheden micro-organismen. Vanaf de geboorte, of mogelijk zelfs in de baarmoeder, worden wij als mens gekoloniseerd met micro-organismen afkomstig van de moeder en de omgeving. Vanaf dit moment wordt in interactie met het zich ontwikkelende immuunsysteem en afhankelijk van de genetische aanleg, de samenstelling van het individuele microbioom gevormd, welke op volwassenen leeftijd stabiliteit bereikt. Het microbioom heeft invloed op diverse fysiologische processen in het lichaam zoals het metabolisme, de hormoonproductie, afweerreacties, en de spijsvertering. De mens kan gezien worden als een ecosysteem waarin micro-

organismen ons in symbiose bewonen. Werd er eerder gedacht dat bacteriën voornamelijk ziekten veroorzaken, de laatste tientallen jaren is juist de beschermende rol van deze organismen naar voren gebracht. De grootste meerderheid van het microbioom bestaat uit commensale, goede bacteriën die een beschermende functie hebben en ons beschermen tegen invasie van pathogene, schadelijke bacteriën. Dit is ook een van de redenen dat voorzichtigheid is geboden met antibioticagebruik, daar deze niet alleen de schadelijke, maar ook goede bacteriën uitroeien en, met name indien gegeven op jonge leeftijd, voor een permanente verandering van het microbioom kunnen zorgen.

DEEL I. KLINISCHE KARAKTERISTIEKEN

In het eerste deel van dit proefschrift hebben wij de klinische karakteristieken van patienten die zowel een huidziekte als een darmziekte hebben beschreven.

In **hoofdstuk 2** stelden we vast dat de ziekte IBD vier keer vaker voorkomt in patiënten met psoriasis dan in de algehele populatie in Nederland. Het risico was met name hoog voor psoriasis patiënten die ook artritis psoriatica hadden. De ziekte van Crohn werd even vaak gezien als Colitis ulcerosa in patiënten met psoriasis. Bovendien bleek dat bij aanwezigheid van twee ziektebeelden, de IBD, vooral de ziekte van Crohn, ernstiger was (meer abcessen, fistels en perianale ziekte) dan bij patiënten die alleen IBD hadden. De psoriasis was echter juist mild in deze patiënten, en niet ernstiger dan in patiënten met alleen psoriasis. Indien een patiënt zowel IBD als psoriasis had, ontwikkelden deze ziekten zich op jongere leeftijd dan wanneer een patiënt slechts één van deze ziekten had. Hierbij werd IBD vaak eerder gediagnosticeerd dan psoriasis. Ook werden andere auto-immuunziekten vaker gezien en werd er agressievere medicatie gebruikt. Al met al, lijkt het erop dat het ziekteverloop in patiënten met zowel IBD als psoriasis anders is dan in patiënten met een van beide ziekten. Omdat IBD en psoriasis reageren op dezelfde behandelingen, is een goede samenwerking tussen specialisten essentieel om tot de beste en meest efficiënte behandeling te komen voor patiënten die meer dan één chronische inflammatoire ziekte hebben.

In **hoofdstuk 3** hebben we de klinische karakteristieken van patiënten met zowel IBD als HS besproken. Het viel op dat de meeste patiënten vrouw waren, en vaak overgewicht hadden. Naast overgewicht, is ook roken een bekende risicofactor voor het ontwikkelen van HS. Patiënten met zowel IBD en HS bleken echter minder te roken dan patiënten met alleen HS. In de meerderheid van de patiënten werd eerst IBD gediagnosticeerd, en daarna pas HS. De meeste patiënten hadden mild-matig ernstige HS, en de data gaven aan dat het ziekteverloop van IBD ernstiger is (meer ziekte in zowel ileum en colon,

penetrerende ziekte in de ziekte van Crohn) in patiënten met zowel IBD en HS. Ook het systemische medicatiegebruik in deze patiënten was fors. Verder was het opvallend dat de HS patiënten vaker de ziekte van Crohn hadden dan colitis ulcerosa (86%), wat suggereert dat de ziekte van Crohn meer overeenkomsten heeft met de ziekteoorzaak van HS dan colitis ulcerosa.

In **hoofdstuk 4** werd onderzocht of patiënten met IBD, zonder en met huidaandoeningen, ook verergering van hun huid- en darmklachten ervaarden door het gebruik van bepaalde voedingsmiddelen in hun dieet. Dit bleek inderdaad het geval, met name vet en pittig eten veroorzaakten in respectievelijk 42% en 32% van de patiënten een verergering van de darmklachten. Bij de huidklachten werd geen noemenswaardige verergering door bepaalde voedingsmiddelen opgemerkt. De samenstelling van het dieet leek niet te verschillen tussen IBD patiënten met- en zonder een huidaandoening. Meer onderzoek naar de invloed van dieet in IBD is aangewezen.

DEEL II. MICROBIËLE KARAKTERISTIEKEN

In het tweede deel van het proefschrift hebben wij ons gericht op het karakteristieken van het microbioom. Omdat wij weten dat microbiële factoren een belangrijke plaats innemen in de etiologie van IBD, en we in het eerste deel van dit proefschrift een duidelijke epidemiologische link tussen IBD en huidziekten hebben laten zien, hebben wij onderzocht of het microbioom van patiënten met huidziekten overeenkomsten vertoont met de veranderingen die bij IBD worden gezien.

Een van de meest specifieke en consistente bevindingen in het IBD microbioom onderzoek is een verminderde aanwezigheid van de anti-inflammatoire darmbacterie *Faecalibacterium prausnitzii*. In **hoofdstuk 5** hebben wij onderzocht in faeces monsters of de commensaal *F. prausnitzii* ook verminderd aanwezig is in de darmen van psoriasis patiënten, in vergelijking met IBD patiënten en gezonde controles, gebruik makend van kwantitatieve PCR. Ditzelfde hebben wij onderzocht in HS patiënten. Zoals verwacht naar aanleiding van eerdere studies, vonden wij in IBD patiënten een verlaagde aanwezigheid van *F. prausnitzii* ten opzichte van de gezonde controles. In psoriasis, maar niet in HS patiënten, vonden wij een zelfde verlaging van *F. prausnitzii* in de darmen. Voor *Escherichia coli* vonden wij een verhoogde aanwezigheid in psoriasis patiënten, die vergelijkbaar was met de verhoging in IBD patiënten. Ook deze verandering zagen wij niet terug in de faeces van HS patiënten. Dezelfde veranderingen in de hoeveelheden van deze bacteriën werden ook gevonden in twee (kleinere) cohorten met patiënten die ofwel psoriasis samen met IBD, ofwel HS samen IBD hadden. Deze patiënten leken

een nog sterkere verandering te tonen van deze bacteriën dan patiënten met slechts één van de genoemde ziekten. Deze studie ondersteunt het bestaan van een gut-skin link in psoriasis en IBD, en pleit tevens voor het bestaan van een ander ziektepatroon in patiënten die meer dan één chronische inflammatoire ziekte hebben.

Daar het microbioom bestaat uit meer bacteriën dan enkel de bovengenoemde, en we bij HS geen aanwijzingen voor de gut-skin link konden aantonen op basis van de geteste bacteriën, wilden wij dit dieper onderzoeken in **hoofdstuk 6**. In een exploratieve studie hebben wij van de HS patiënten in vergelijking met gezonde controles, huid- en faecesmonsters geanalyseerd door middel van 16SrRNA sequencing. Ook hier vonden wij geen verschillen in de samenstelling van het microbioom tussen HS patiënten en gezonde controles. Slechts bij één patiënt, de enige patiënt met een ernstige HS, leek het huid microbioom ernstig verstoord te zijn, waarschijnlijk secundair aan de inflammatie. In deze patiënt werd ook een verstoord darm microbioom aangetroffen. Hoewel er in deze exploratieve studie opnieuw geen gut-skin link werd aangetoond tussen HS en de ziekte van Crohn, is het mogelijk dat een subpopulatie van HS patiënten deze link wel vertoont, en dat inclusie van meer patiënten deze link aan het licht zou kunnen brengen.

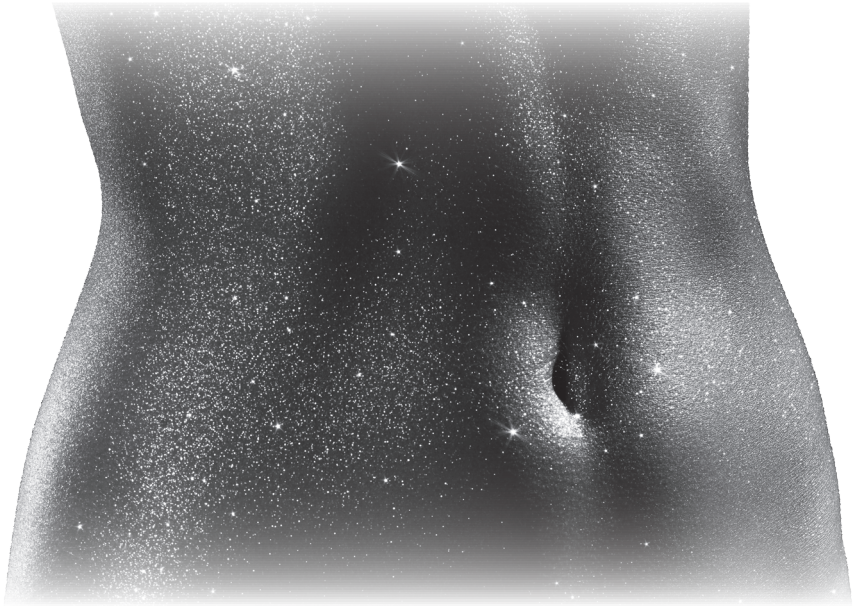
In **hoofdstuk 7** onderzochten we of UV straling de samenstelling van het huid- en darmmicrobioom kan beïnvloeden. Van chronische inflammatoire huidziekten zoals psoriasis is het bekend dat de symptomen verbeteren in de zomer, en daarom is UV therapie een standaard therapie voor deze aandoening, maar ook voor andere huidaandoeningen zoals atopisch eczeem. In deze studie onderzochten wij het effect van UVB straling op het huid- en darmmicrobioom bij patiënten die UVB therapie ondergingen. Wij vergeleken de samenstelling van het huid- en darmmicrobioom vóór en na UVB therapie, gebruik makend van 16SrRNA sequencing. De resultaten gaven een sterke indicatie dat UVB licht de compositie van zowel het huidmicrobioom als het darmmicrobioom kan beïnvloeden. Zowel een toename, als een afname van bepaalde bacteriesoorten werden gevonden. Op de huid werd er bijvoorbeeld een toename van de veelvoorkomende *Actinobacteria* gezien, terwijl in de darm er juist een afname van dit phylum werd gezien. Of deze resultaten juist gunstig of niet gunstig zijn voor het microbioom en de ziekten, moet nog verder onderzocht worden.

Naast bacteriën, bestaat het microbioom ook uit diverse schimmels/gisten, waar we **hoofdstuk 8** aan hebben gewijd. Binnen het humane schimmelrijk is een verlaagde aanwezigheid van *Saccharomyces cerevisiae,* welke geclassificeerd is als een "goede" gist met afweer modulerende eigenschappen, een van de meest prominente bevindingen in IBD. In hoofdstuk 8 toonden we aan met behulp van kwantitatieve PCR dat *S. cerevisiae* ook verlaagd is in psoriasis patiënten, maar dat deze depletie kan worden hersteld door

het gebruik van dimethylfumuraat (DMF), een standaard therapie voor psoriasis. Naast de *in vivo* bevindingen, hebben we tevens *in vitro* bevestigd dat DMF een groei van *S. cerevisiae* kan bewerkstelligen. Patiënten die gastro-intestinale bijwerkingen van DMF ondervonden, bleken een hogere *S. cerevisiae* hoeveelheid in hun darmen te hebben dan patiënten die geen bijwerkingen van de medicatie hadden. Deze studie toont aan dat niet alleen de hoeveelheid aanwezige bacteriën verlaagd kan zijn in psoriasis, maar dat ook de aanwezigheid van schimmels, zoals *S. cerevisiae*, aangedaan kan zijn. Daarnaast toonde de studie aan dat medicatie gebruik invloed kan hebben op het microbioom, en dat deze gist mogelijk zowel goede als minder goede gevolgen (bijwerkingen) kan hebben.

De nieuwste ontwikkelingen op het gebied van het darmmicrobioom werden besproken in **hoofdstuk 9**, met de nadruk gelegd op IBD. Ondanks dat microbioom onderzoek een relatief nieuw veld is, zeker in de vergelijking met genetica studies, wat zich onder andere toont in kleinere patiënten aantallen, is het wel een veelbelovend veld. Het microbioom is te manipuleren, en te gebruiken als therapeutisch target, terwijl het genoom onveranderlijk is. Bewijs stapelt zich op dat het microbioom een grote rol speelt in IBD, al weten we nog niet of een microbioom verstoring in IBD de kip of het ei is. Definitie van IBD-subsets zal in de toekomst nodig zijn om de beste en individuele behandelingen aan patiënten te kunnen bieden. Het ontstaan van de ziekte in elk individu is een samenloop van verschillende omstandigheden en samenkomen van verschillende factoren, die bij geen ieder gelijk is, net zoals het ziekteverloop verschilt tussen patiënten. Patiënten met IBD die bijvoorbeeld ook psoriasis hebben worden daarbij mogelijk geclassificeerd als aparte subset. Hopelijk is het in de toekomst mogelijk om een microbioom patroon, mogelijk in combinatie met een genetisch patroon, vast te stellen om een individu en een ziekte subset goed te kunnen karakteriseren. Maar belangrijker nog is preventie, waarbij we ons met name moeten richten op de kindertijd omdat dat de periode is waarin de microbioom compositie en de aanleg voor bepaalde ziekten gevormd wordt.

Microbioom onderzoek zal de komende jaren steeds meer informatie prijsgeven over de daadwerkelijke functies van de bacteriën, nu diverse ziekte-associaties zijn aangetoond. Nieuwe microbioom inzichten bieden meer therapeutische mogelijkheden voor IBD patiënten, zoals dieetaanpassingen en lifestyle-interventies, met relatief weinig bijwerkingen. Hiernaast worden er cocktails van bacteriën ontwikkeld, waarmee getracht wordt om de disbalans van het microbioom te herstellen. Het is meer waarschijnlijk dat dit in een vorm van een pil zal zijn, dan dat poeptransplantatie de toekomst zal hebben. Therapeutische interventies met als target het microbioom staan nog in de kinderschoenen, maar zullen ongetwijfeld deel gaan uit maken van het IBD-repertoire in de toekomst. Een optimale behandeling zal dan worden ingesteld aan de hand van individuele karakteristieken in aansluiting op de wensen van de patiënt.



CHAPTER 12

Appendices

List of co-authors
List of publications
PhD portfolio
Dankwoord
Curriculum Vitae

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LIST OF PUBLICATIONS

Hester Eppinga, Sergey R. Konstantinov, Maikel P. Peppelenbosch, H. Bing Thio. The microbiome and psoriatic arthritis. *Current rheumatology reports* 2014 Mar;16(3):407

Hester Eppinga, H. Bing Thio, Maikel P. Peppelenbosch, Sergey R. Konstantinov. The gut microbiome dysbiosis and its potential role in psoriatic arthritis. *International Journal of Clinical Rheumatology* 2014 Dec; 9(6):559-565

Hester Eppinga, H. Bing Thio, C. Janneke van der Woude. Characteristics of patients with hidradenitis suppurativa and inflammatory bowel disease. *Clinical Gastroenterology and Hepatology* 2016 Mar;14(3):482-3

Hester Eppinga, Maikel P. Peppelenbosch. Worsening of bowel symptoms through diet in patients with inflammatory bowel disease. *Inflammatory Bowel Diseases* 2016 Feb;22(2):E6-7

Hester Eppinga, Christa J. Sperna Weiland, H. Bing Thio, C. Janneke van der Woude, Tamar E.C. Nijsten, Maikel P. Peppelenbosch, Sergey R. Konstantinov. Similar depletion of protective *Faecalibacterium prausnitzii* in psoriasis and inflammatory bowel disease, but not in hidradenitis suppurativa. *Journal of Crohn's and Colitis*, 2016 Sep;10(9):1067-75

Hester Eppinga, Gwenny M. Fuhler, Maikel P. Peppelenbosch, Gail A. Hecht. Gut microbiota developments with emphasis on inflammatory bowel disease: report from the Gut Microbiota for Health World Summit 2016. *Gastroenterology* 2016 Aug;151(2):e1-4

PHD PORTFOLIO

Name PhD student:	Hester Eppinga
PhD period:	2014-2016
Erasmus MC Department:	Gastroenterology and Hepatology
	Dermatology
Promotor:	Prof. Dr. M.P. Peppelenbosch
Copromotoren:	Dr. H.B. Thio
	Dr. G.M. Fuhler

1. PHD TRAINING	**Year**	**Workload**
Courses		
Research management	2014	1 ECTS
Systematic literature search pubmed	2014	0.2 ECTS
Course Molecular Diagnostics	2014	0.2 ECTS
Basic course Excel	2014	0.3 ECTS
Basic course Access	2014	0.3 ECTS
Advanced course Access	2014	0.4 ECTS
Biomedical research techniques	2014	1.5 ECTS
Basic introduction in SPSS	2014	1 ECTS
Genetics for dummies	2014	0.5 ECTS
Biomedical English writing	2015	3 ECTS
Advanced Immunology	2015	1 ECTS
Course Endnote	2015	0.2 ECTS
Biostatistics for clinicians NIHES	2015	0.7 ECTS
BROK (Basic Course Clinical Research)	2015	1 ECTS
Graphpad Prism	2015	0.3 ECTS
Scientific integrity, Erasmus MC	2016	0.3 ECTS
Seminars, meetings and workshops		
PhD day Erasmus MC	2014	6 hours
PhD day Erasmus MC	2015	6 hours
PhD day Erasmus MC	2016	6 hours
IBD meetings, Erasmus MC	2014-2016	120 hours
Dermatology research meetings	2014-2016	80 hours
Journal club Dermatology	2015-2016	10 hours
Microbiome meetings	2015	4 hours
Reference meetings, "Skintermezzo", Erasmus MC	2014-2016	24 hours
MDL seminars	2014-2016	120 hours

Media contacts for researchers	2015	2.5 hours
Time management: "battling your brain"	2016	4 hours
Young-ECCO workshop	2016	2 hours
"Writing and reviewing clinical and scientific papers"		

Conferences

NVED, Dutch society for Experimental Dermatology, Lunteren	2014	1 ECTS
Gutday (Darmendag), Amsterdam	2014	1 ECTS
Pre- and probiotics, Wageningen	2014	1 ECTS
Wetenschappelijke vergadering Nederlandse Vereniging Dermatologie en Venerologie (NVDV), Amsterdam	2015	1 ECTS
Gutday (Darmendag), Rotterdam	2015	1 ECTS
Wetenschappelijke vergadering Nederlandse Vereniging Dermatologie en Venerologie (NVDV),	2015	1 ECTS
European Society for Dermatological Research (ESDR), Rotterdam	2015	1 ECTS
Wetenschappelijke vergadering Nederlandse Vereniging Gastroenterologie (NVGE), Veldhoven	2015	1 ECTS
European Crohn and Colitis Organization Congres (ECCO), Amsterdam	2016	1 ECTS
Gut microbiota for Health World Summit, Miami	2016	1 ECTS
Psychodermatology symposium, Amsterdam	2016	1 ECTS
Women's Microbes, Amsterdam	2016	1 ECTS
2nd PhD weekend Dermatology, Wassenaar	2016	1 ECTS
3rd PhD weekend Dermatology, Antwerpen	2016	1 ECTS
Wetenschappelijke vergadering Nederlandse Vereniging Gastroenterologie (NVGE), Veldhoven	2016	1 ECTS

Presentations

The intestinal microbiota in IBD and skin manifestations, Dutch society for Experimental Dermatology (NVED, oral)	2014	1 ECTS
The gut-skin axis in IBD and associated skin disorders, MDL seminar, Erasmus MC	2014	1 ECTS
Decreased abundance of *F. prausnitzii* in psoriasis, European Society for Dermatological Research (ESDR, poster)	2015	1 ECTS
Psoriatic arthritis, psoriasis and the microbiota, Department of Rheumatology, Erasmus MC (oral)	2015	1 ECTS
UVB therapy, skin disorders and the microbiome, Reinier de Graaf Gasthuis, Delft (oral)	2015	1 ECTS
Decrease of *F. prausnitzii* in IBD and psoriasis,	2015	1 ECTS

"Je bent wat je eet", PhD weekend Dermatology, Wassenaar (oral)	2015	1 ECTS
Inflammatory skin and gut diseases and the microbiome, Skintermezzo Dermatology Erasmus MC (oral)	2015	1 ECTS
F. prausnitzii MDL seminar, Erasmus MC (oral)	2015	1 ECTS
PhD overview, Gastroenterology, Erasmus MC (oral)	2016	1 ECTS
Similar depletion of *F. prausnitzii* in psoriasis and IBD, Gut Microbiota for Health World Summit, Miami (poster)	2016	1 ECTS
Similar depletion of *F. prausnitzii* in psoriasis and IBD, NVGE, (oral presented by C. Sperna Weiland)	2016	1 ECTS
Phenotype and prevalence of concurrent IBD and psoriasis, NVGE (oral)	2016	1 ECTS

2. TEACHING

Supervising microbiology students		
Sergio Chavez Chavez	2015	1 ECTS
Blerdi Blakaj	2015	1 ECTS
Supervising Master's thesis		
Ralph Setyo	2014	1 ECTS
Ruena Tahitu	2015	1 ECTS
Christa Sperna Weiland	2015	1 ECTS
Nicole Boden	2016	1 ECTS

Other	
Medical Research Advisory Committee grant (MRACE), Erasmus MC, for PhD project	2014
Interview psoriasis society "Darmflora onder de loep"	2015
Coach bachelor students medicine Erasmus MC	2016

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"It giet oan!"

CURRICULUM VITAE

De auteur van dit proefschrift, Hester Eppinga, is geboren op 11 september 1989 te Siegerswoude, gemeente Opsterland. Ze is opgegroeid op het Friese platteland in Siegerswoude, en behaalde in 2007 haar Gymnasium diploma aan de Christelijke Scholen Gemeenschap Liudger, locatie de Raai in Drachten. Zij werd aansluitend decentraal toegelaten tot de studie Geneeskunde aan de Erasmus Universiteit Rotterdam. Vanuit haar afstudeeronderzoek naar microbiële factoren bij Inflammatoire darmziekten en geassocieerde huidziekten bij de afdeling Dermatologie en Maag- Darm- Leverziekten van het Erasmus Medisch Centrum in Rotterdam, werd de basis gelegd voor dit proefschrift. Tijdens dit afstudeeronderzoek groeide haar interesse in dit onderwerp en verwierf zij in samenwerking met haar begeleiders een prestigieuze beurs (de MRACE beurs) voor een promotietraject in het verlengde van haar afstudeeronderzoek. Nadat zij haar artsenbul behaalde, startte zij in mei 2014 als arts-onderzoeker onder supervisie van Prof. Dr. M.P. Peppelenbosch, Dr. S.R. Konstantinov, Dr. G.M. Fuhler en Dr. H.B. Thio.

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