SEBORRHEIC DERMATITIS

More than meets the eye

Martijn Gerard Hendrik Sanders

Financial support for the printing of this thesis was kindly provided by:

La Roche-Posay	UCB
Chipsoft	LEO Pharma
Louis Widmer	L'Oréal
Lilly	Merz Pharma
Van der Bend B.V.	Olmed

ISBN: 978-94-6361-558-7
Cover illustration by Sara van der Linde
Cover design, lay-out and printing by Optima Grafische Communicatie

Seborrheic Dermatitis
More than meets the eye

Seborroïsch eczeem
Niet alles is wat het lijkt

Proefschrift

ter verkrijging van de graad van doctor aan de
Erasmus Universiteit Rotterdam
op gezag van de rector magnificus
Prof.dr. F.A. van der Duijn Schouten
en volgens besluit van het College voor Promoties.

De openbare verdediging zal plaatvinden op
donderdag 24 juni 2012 om 10:30 uur

Door

Martijn Gerard Hendrik Sanders
geboren te Almelo

Erasmus University Rotterdam

PROMOTIECOMMISSIE

Promotor:	prof. dr. T.E.C. Nijsten
Overige leden:	prof. dr. E.P. Prens prof. dr. A.G. Uitterlinden prof. dr. J.L.W. Lambert
Copromotor:	dr. L.M. Pardo Cortes

CONTENTS

1 General introduction

INTRODUCTION

Seborrheic dermatitis is a chronic relapsing inflammatory skin disease characterized by erythema with greasy scaling and occurring in areas rich in sebaceous glands (i.e. the scalp, eyebrows, nasolabial folds and the chest). In most cases, seborrheic dermatitis presents as a mild skin disease in which diagnosis and treatment is straightforward. However, severe and recalcitrant forms do occur and seborrheic dermatitis can negatively influence quality of life, with 4.2 million people who sought medical attention in one year in America.[1,2] The etiology of seborrheic dermatitis is not fully understood. *Malassezia* yeasts are often pointed out as the cause of the disease, but the bacterial microbiome, genetic susceptibility, environmental factors, barrier function and the immune system may all influence disease risk.

EPIDEMIOLOGY

Studies using medical records have shown that the prevalence of seborrheic dermatitis is roughly 4%, and that males are more likely to be affected than females.[3-5] The first peak of seborrheic dermatitis occurs during infancy. However, infantile seborrheic dermatitis is a term that is used for a wide variety of eczematous and psoriasiform eruptions seen in infants and the discussion remains if infantile seborrheic dermatitis is an entity on its own. There is no convincing evidence that infantile seborrheic dermatitis is linked to adult seborrheic dermatitis.[6,7] If infantile seborrheic dermatitis is not considered, the first presentation of seborrheic dermatitis may start during puberty. After puberty, the incidence increases with age, with a peak between 30-50 years.[3,5,8] In several prospective studies, in which participants were screened for skin disorders, considerably higher prevalence estimates were found, with estimates around 12%,[8,9] suggesting that medical care is not always sought for seborrheic dermatitis.

CLINICAL SPECTRUM AND DIAGNOSIS

Seborrheic dermatitis is a chronic relapsing heterogeneous disease and ranges from very mild to very severe forms. To date, there are no clear diagnostic criteria for seborrheic dermatitis. Characteristic lesions are erythematous, squamous, greasy and itchy and have a symmetric distribution in areas rich of sebaceous glands. Scalp, eyebrows and nasolabial folds are most often affected, but seborrheic dermatitis can also be found in ear canals, intertriginous areas and on the chest.[10] Dandruff can be seen as a very mild form of seborrheic dermatitis confined to the scalp, which is covered with fine white scales, but without erythema. At the other end of the clinical spectrum is erytrodermic seborrheic dermatitis, which is very rare.

Table 1 gives an overview of a possible differential diagnosis including differentiating characteristics. The differential diagnosis of seborrheic dermatitis includes rosacea, atopic dermatitis, allergic contact dermatitis or lupus erythematosus if the face is affected.[11] When intertriginous areas are affected, psoriasis, erythrasma or Hailey-Hailey might be considered and tinea capitis or psoriasis might be part of the differential diagnosis if only the scalp is affected.[12,13]

Table 1. Differential diagnosis of seborrheic dermatitis.

Diagnosis	Typical sites	Morphology	Other characteristics
Psoriasis	Extensor surfaces of the knees and elbows and on the scalp	Sharply demarcated erythematous plaques with thick scales	Candle wax sign, Auspitz sign, nail abnormalities, arthritis
Tinea capitis	Scalp	Erythematous patches with squamae, central healing and active border. May be accompanied by a kerion, diffuse pustules and alopecia	KOH preparation +, possible fluorescence by Wood's light
Atopic dermatitis	Starts with cheeks, scalp and extensor sites in infant, but shifts to flexural sites with age	Diffuse lichenificated erythematosquamous plaques, secondary crusts and impetigo	History of hay-fever or asthma, xerosis cutis, extreme itch
Contact dermatitis	Distribution of allergen or irritant, when airborne, periocular area is most affected	Chronic lesions may resemble atopic dermatitis. Acute lesions may be accompanied by blistering and/or oedema	
Rosacea	Nose, cheek, forehead and eyes	Erythematous papules and pustules and/or erythema and telangiectasia	Facial flushing, rhinophyma
Cutaneous lupus erythematosus	Photodistribution	Indurated erythematous squamous plaques with adnexa destruction and potential scarring	Malar erythema and butterfly rash in acute lupus erythematosus
Erythrasma	Intertriginous regions	Erythematous moist plaques with squamae	KOH preparation +
Hailey-Hailey	Intertriginous regions, scalp might be affected	Erythematous, eroded, moist plaques	Fetid odour
Pemphigus foliaceus	Often starts at the trunk but may also occur on scalp and face	Very fragile superficial vesicles. Patients usually present with crusted erosions	Nikolsky sign

The differentiation between seborrheic dermatitis and psoriasis can be especially difficult. It has even been argued that seborrheic dermatitis is variant of psoriasis, "sebopsoriasis". However, the distinction between the two is relevant. Misdiagnosis could lead to unnecessary long-term use of corticosteroids and the accompanying side effects, where (concomitant) treatment with anti-fungals might have been sufficient in seborrheic dermatitis.[14] The differential diagnosis between seborrheic dermatitis and psoriasis is usually based on the clinical presentation and body distribution. Psoriatic plaques are more sharply demarcated and are most often present on the extensor surfaces of the knees and elbows and on the scalp. In

addition, psoriasis is more likely if there is a positive candle wax sign or Auspitz sign and there might be a Woronoff ring around a healing psoriasis plaque. Furthermore, up to 79% of the psoriasis patients have nail abnormalities.[10,15]

Skin cultures or biopsies are rarely necessary for the diagnosis and might be performed if there is no response to topical treatment. Acute seborrheic dermatitis shows features of spongiotic dermatitis. Longer lasting seborrheic dermatitis can look quite similar to the histology of psoriasis and might show focal parakeratosis, hyperkeratosis and psoriasiform epidermal hyperplasia. However, other characteristics of psoriasis like Munro micro abscesses, exocytosis of neutrophils and confluent parakeratosis are absent.[10,16]

CLINICAL MANAGEMENT

Due to the chronic nature of seborrheic dermatitis, long-term treatment is often necessary. A Cochrane meta-analysis showed that the total clearance after four weeks is comparable for topical antifungals, corticosteroids and calcineurin inhibitors.[17] In mild seborrheic dermatitis, topical antifungals, such as ketoconazole crème, should be the first line of therapy as these were found as affective, but with less adverse effects in long-term use. When topical antifungals are not effective enough in reducing the symptoms, calcineurin inhibitors or topical corticosteroids should be considered. For short-term treatment, adverse effects were more commonly reported for calcineurin inhibitors and predominately included mild burning and irritation in the induction phase. Long-term data is not available for calcineurin inhibitors. Extended use of topical steroids has been associated with skin atrophy and acneiform dermatitis.[18] For scalp seborrheic dermatitis, shampoos containing coal tar or salicylic acid may be of additional use.[19,20]

Treatment with systemic anti-fungals, corticosteroids or retinoids and therapy with ultraviolet light may be considered in therapy resistant cases. Also, a recent case series showed a possible beneficial effect of apremilast. However, evidence for these treatments is limited.[21-24] Based on the considerations above; we suggest a treatment ladder for seborrheic dermatitis in Table 2.

PATHOPHYSIOLOGY

Malassezia yeasts are often suggested to be the cause of seborrheic dermatitis. This yeast is more abundant on the skin of individuals affected with seborrheic dermatitis, and the most common treatment for seborrheic dermatitis, ketoconazole, is aimed at eliminating

Table 2. Treatment ladder for seborrheic dermatitis

Step 1*	Ketoconazole shampoo and/ or crème	In case of total clearance, consider to continue ketoconazole once a week to prevent relapse.[25]
Step 2	(Add) topical corticosteroid or calcineurin inhibitor	Face: calcineurin inhibitor (pimecrolimus or tacrolimus crème) or low to mild potent corticosteroids. Scalp: corticosteroid shampoo (e.g. clobetasol shampoo) or a corticosteroid lotion (e.g. triamcinolone lotion, desoximetason lotion), coal tar shampoos might be of additional use.
Step 3	Systemic anti-fungal	e.g. Itraconazole, 1 week 100 mg twice a day, followed by 200 mg/day for first 2 days of the following 3 months.[26] Terbinafine, fluconazole, ketoconazole or pramiconazole might also be considered.
Step 4	UVB-therapy[22]	High relapse rate, might be considered during severe inflammation.
Step 5	Consider low-dose oral isotretinoin, systemic corticosteroids or apremilast.	Based on small studies or case reports. [21,23,27]

*Start with step two in more severe cases.

this yeast. *Malassezia* yeasts are lipophilic, breaking down triglycerides into free fatty acids. DeAngelis et al. conducted a study in which they applied free fatty acids (represented by the oleic acid) to the skin and they found that this could induce seborrheic dermatitis, but only in people who suffered from seborrheic dermatitis before.[25] However, there are several uncertainties to the role of *Malassezia* in seborrheic dermatitis. This yeast can be considered as a commensal, which is present on the skin of both healthy and affected individuals. Corticosteroids with low potency and calcineurin inhibitors can be very effective in treating seborrheic dermatitis, although these ointments do not have anti-fungal properties. Also, it is questioned if ketoconazole decreases the abundance of *Malassezia* yeasts in vivo.[26] In other skin diseases associated with *Malassezia* yeasts, the hyphae of the yeast have been shown to be pathogenic by penetrating the epidermis, where in seborrheic dermatitis this is not the case. It seems that the yeast is associated with the disease, but probably only in patients with an intrinsic susceptibility and in specific circumstances. Some features have been investigated, others are largely unknown (Figure 1).

In contrast to the recognized role of the *Malassezia* in seborrheic dermatitis, the role of other microbiome organisms is less known. For other inflammatory skin diseases, such as atopic dermatitis, there are several associations with bacterial dysbiosis.[27] For seborrheic dermatitis, bacterial dysbiosis was found on the scalp and face in small case series, but these have not been replicated in well-powered studies.[28,29]

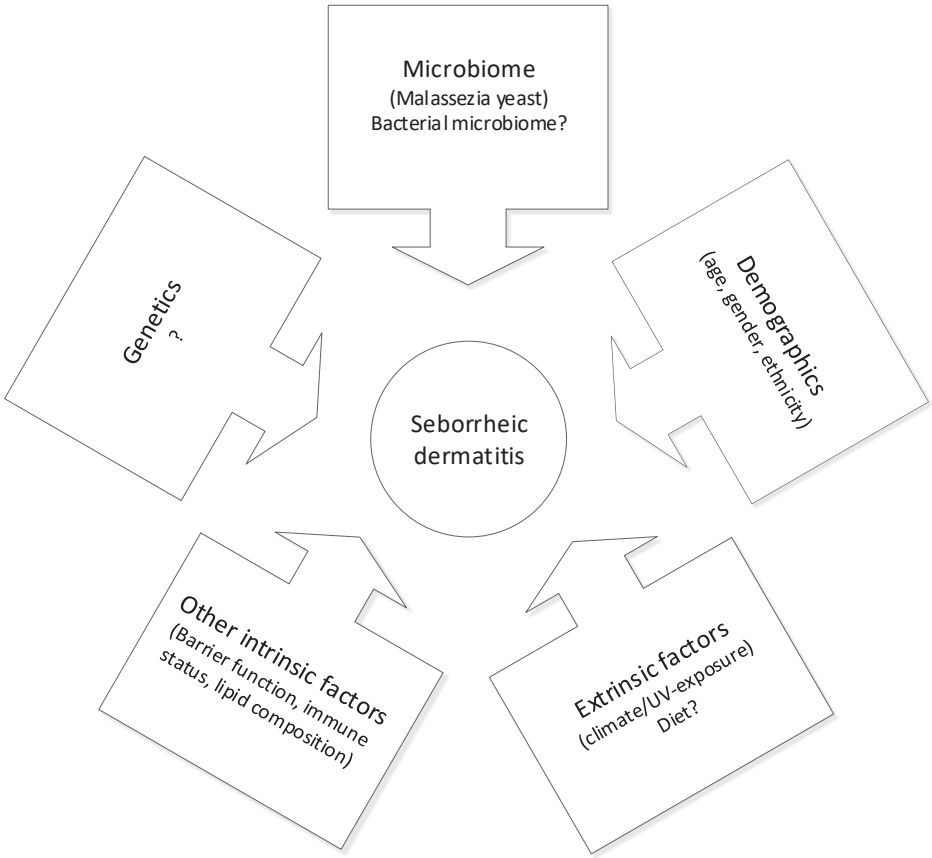


Figure 1. Possible factors that influence seborrheic dermatitis.

THE ROTTERDAM STUDY

Data collected in the Rotterdam study was used to answer a large proportion of the research questions in this thesis. The Rotterdam Study is an ongoing prospective population based cohort study of chronic diseases in a mainly white-skinned elderly population in the Ommoord district in Rotterdam, The Netherlands.[30] The study started in 1990 and now comprises over 15,000 individuals aged 45 years or older, and from 2010 forward participants underwent a full body skin examination (FBSE). The FBSE was conducted by a dermatology-trained physician with a focus on common skin diseases. Seborrheic dermatitis is one of those skin diseases. Examinations take place every 3 to 6 years. When conducting the studies in this thesis, data were available for one FBSE for most participants.

AIMS OF THIS THESIS

As mentioned above, there are still many uncertainties regarding the etiology of seborrheic dermatitis. Although seborrheic dermatitis can be treated well in most cases, it can affect quality of life, and there are diagnostic and therapeutic difficulties. In this thesis, we aim to give more insight in this common skin disease by investigating the occurrence and determinants of seborrheic dermatitis.

The global burden of disease project showed that the non-fatal skin disease burden is very high. However, as many skin disorders are often not identified, or neglected, prevalence rates are not well documented. In **Chapter 2**, we aimed to estimate the point prevalence of and age- and sex-adjusted standardized prevalence rates of seborrheic dermatitis and other common inflammatory and (pre)malignant skin diseases in the Rotterdam Study.

Many host and environmental determinants have been implicated in the pathogenesis of seborrheic dermatitis. However, most of these studies were small and/or conducted in a selected population. In **Chapter 3.1**, we aimed to validate which of these lifestyle and physiological determinants are associated with seborrheic dermatitis. The suggestion that nutrition may influence inflammatory skin diseases is investigated in **Chapter 3.2.**

In **Chapter 4**, we explore the possible role of genetic susceptibility for seborrheic dermatitis with two approaches. First, we investigated whether variants previously associated with atopic dermatitis or psoriasis are also associated with an increased risk of seborrheic dermatitis (hypothesis driven). Second, we conducted the first genome-wide association study (GWAS) to identify novel genetic variants associated with seborrheic dermatitis (hypothesis free).

Since seborrheic dermatitis was first described at the end of the 18th century, it is often assumed that *Malassezia* yeast cause and maintain the disease. However, as the yeasts are also part of the healthy skin microbiome, causal relation between de two has been at debate in the past years. Recently, it has been suggested that bacterial dysbiosis might influence the disease as well, which has been confirmed in small patient groups. In **Chapter 5**, we profiled the bacterial microbiome of the nasolabial fold of participants of the Rotterdam Study to characterize the bacterial microbiome of healthy and diseased skin.

REFERENCES

1 Szepietowski JC, Reich A, Wesolowska-Szepietowska E *et al.* Quality of life in patients suffering from seborrheic dermatitis: influence of age, gender and education level. *Mycoses* 2009; **52**: 357-63.

2 Lim HW, Collins SAB, Resneck JS, Jr. *et al.* The burden of skin disease in the United States. *J Am Acad Dermatol* 2017; **76**: 958-72 e2.

3 Palamaras I, Kyriakis KP, Stavrianeas NG. Seborrheic dermatitis: lifetime detection rates. *J Eur Acad Dermatol Venereol* 2012; **26**: 524-6.

4 Zander N, Sommer R, Schafer I *et al.* Epidemiology and dermatological comorbidity of seborrhoeic dermatitis: population-based study in 161 269 employees. *Br J Dermatol* 2019; **181**: 743-8.

5 Plunkett A, Merlin K, Gill D *et al.* The frequency of common nonmalignant skin conditions in adults in central Victoria, Australia. *Int J Dermatol* 1999; **38**: 901-8.

6 Alexopoulos A, Kakourou T, Orfanou I *et al.* Retrospective analysis of the relationship between infantile seborrheic dermatitis and atopic dermatitis. *Pediatric dermatology* 2014; **31**: 125-30.

7 Mimouni K, Mukamel M, Zeharia A *et al.* Prognosis of infantile seborrheic dermatitis. *J Pediatr* 1995; **127**: 744-6.

8 Johnson MT, Roberts J. Skin conditions and related need for medical care among persons 1-74 years. United States, 1971-1974. *Vital Health Stat 11* 1978: i-v, 1-72.

9 Breunig Jde A, de Almeida HL, Jr., Duquia RP *et al.* Scalp seborrheic dermatitis: prevalence and associated factors in male adolescents. *Int J Dermatol* 2012; **51**: 46-9.

10 Bolognia JL, Schaffer JV, Cerroni L. Dermatology, 4th edition. *London: Elsevier.* 2017.

11 Dessinioti C, Antoniou C. The "red face": Not always rosacea. *Clin Dermatol* 2017; **35**: 201-6.

12 Metin A, Dilek N, Demirseven DD. Fungal infections of the folds (intertriginous areas). *Clin Dermatol* 2015; **33**: 437-47.

13 Auchus IC, Ward KM, Brodell RT *et al.* Tinea capitis in adults. *Dermatol Online J* 2016; **22**.

14 Gupta AK, Versteeg SG. Topical Treatment of Facial Seborrheic Dermatitis: A Systematic Review. *Am J Clin Dermatol* 2017; **18**: 193-213.

15 de Jong EM, Seegers BA, Gulinck MK *et al.* Psoriasis of the nails associated with disability in a large number of patients: results of a recent interview with 1,728 patients. *Dermatology* 1996; **193**: 300-3.

16 Barnhill RL, Crowson AN, Magro CM *et al. Dermatopathology*: McGraw-Hill Medical New York. 2010.

17 Kastarinen H, Oksanen T, Okokon EO *et al.* Topical anti-inflammatory agents for seborrhoeic dermatitis of the face or scalp. *Cochrane Database Syst Rev* 2014; **5**: CD009446.

18 Hengge UR, Ruzicka T, Schwartz RA *et al.* Adverse effects of topical glucocorticosteroids. *J Am Acad Dermatol* 2006; **54**: 1-15; quiz 6-8.

19 Paghdal KV, Schwartz RA. Topical tar: back to the future. *J Am Acad Dermatol* 2009; **61**: 294-302.

20 Borda LJ, Perper M, Keri JE. Treatment of seborrheic dermatitis: a comprehensive review. *The Journal of dermatological treatment* 2018: 1-12.

21 Gupta AK, Richardson M, Paquet M. Systematic review of oral treatments for seborrheic dermatitis. *J Eur Acad Dermatol Venereol* 2014; **28**: 16-26.

22 Pirkhammer D, Seeber A, Honigsmann H *et al.* Narrow-band ultraviolet B (ATL-01) phototherapy is an effective and safe treatment option for patients with severe seborrhoeic dermatitis. *Br J Dermatol* 2000; **143**: 964-8.

23 de Souza Leao Kamamoto C, Sanudo A, Hassun KM *et al.* Low-dose oral isotretinoin for moderate to severe seborrhea and seborrheic dermatitis: a randomized comparative trial. *Int J Dermatol* 2017; **56**: 80-5.
24 Kose O, Erbil H, Gur AR. Oral itraconazole for the treatment of seborrhoeic dermatitis: an open, noncomparative trial. *J Eur Acad Dermatol Venereol* 2005; **19**: 172-5.
25 DeAngelis YM, Gemmer CM, Kaczvinsky JR *et al.* Three etiologic facets of dandruff and seborrheic dermatitis: Malassezia fungi, sebaceous lipids, and individual sensitivity. *J Investig Dermatol Symp Proc* 2005; **10**: 295-7.
26 Zani MB, Soares RC, Arruda AC *et al.* Ketoconazole does not decrease fungal amount in patients with seborrhoeic dermatitis. *Br J Dermatol* 2016; **175**: 417-21.
27 Paller AS, Kong HH, Seed P *et al.* The microbiome in patients with atopic dermatitis. *J Allergy Clin Immunol* 2019; **143**: 26-35.
28 Park T, Kim HJ, Myeong NR *et al.* Collapse of human scalp microbiome network in dandruff and seborrhoeic dermatitis. *Exp Dermatol* 2017.
29 Tanaka A, Cho O, Saito C *et al.* Comprehensive pyrosequencing analysis of the bacterial microbiota of the skin of patients with seborrheic dermatitis. *Microbiol Immunol* 2016; **60**: 521-6.
30 Hofman A, Brusselle GG, Darwish Murad S *et al.* The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol* 2015; **30**: 661-708.

2 | Dermatological screening of a middle-aged and elderly population: the Rotterdam Study

M.G.H. Sanders
L.M. Pardo
J.A.C. Verkouteren
S.A.S. Hamann
M.A. Hamer
T. Nijsten

British Journal of Dermatology (2017) 177, ppe98–e100

To the Editor,

The prevalence of several skin diseases increases with age, partly due to age-related physiopathological alterations.[1] The global burden of disease project (GBD)[2] presented a comprehensive overview of the burden due to skin diseases across different age groups. However, the point prevalence's of many common skin diseases, especially in older people and noninstitutionalized settings, are still unknown.[3] In this study, we estimated the point prevalence and age- and sex-adjusted standardized prevalence rates of common inflammatory and (pre)malignant skin diseases in a cross-sectional study in a middle-aged to elderly population (Appendix S1; see Supporting Information).

In 2010, full body skin examinations (FBSEs) were introduced in the Rotterdam Study,[4] a prospective population-based cohort study of people aged ≥ 45 years. Since the start of study, in 1989, all citizens of Ommoord (a district of Rotterdam) were invited to participate, with an overall response of 72% during three invitation cycles. The study design and follow-up are detailed in Hofman *et al.*[4] At FBSE, all participants were aged ≥ 50 years. The FBSEs were carried out by dermatology-trained physicians who checked for common skin diseases, including (pre-)malignancies, eczema, psoriasis, seborrheic dermatitis and clinical signs of venous insufficiency (Appendix S2; see Supporting Information). Age- and sex-adjusted standardized prevalence rates (PR) were calculated per 100 000 persons aged ≥ 50 years and standardized to the Revised European Standard Population (Appendix S3; see Supporting Information).

In total, 5365 participants (median age 67.2 years; interquartile range 61.6–74.8) were examined. The age- and sex-adjusted prevalence of skin diseases are presented in Table 1. Xerosis cutis was present in 66% of participants. Pre-malignant and malignant skin diseases were very common, comprising 48% of all identified skin diseases (Figure 1). Actinic keratosis (AK) was found in 1399 (26.1%) participants; 234 (4.4%) showed one or more cutaneous malignancies. Basal cell carcinomas (BCCs) were most common, but melanomas, squamous cell carcinomas (SCC) and mycosis fungoides were also diagnosed (Table 1). Seborrheic dermatitis was the most common non-malignant disorder, with a standardized prevalence rate of 17 685 per 100 000 men and 9588 per 100 000 women.

Skin disorders are often not identified, or neglected. In this cohort, we found a high prevalence of (pre)malignant disorders. About a quarter of participants were diagnosed with AK, a potential precursor of SCC. The point prevalence of SCCs is lower than expected compared with melanomas. This could be because melanomas more often develop on less visible body sites and are often subclinical, contrasting with SCC. Therefore, melanomas are more likely to be detected during screening. About one in 25 participants was diagnosed with at least one cutaneous malignancy. This high prevalence suggests that primary care practitioners should be more alert to detect suspicious skin lesions in the middle-aged and elderly population.

Table 1. Characteristics of screened Rotterdam Study participants (*n* = 5,365) with standardized prevalence rates per 100,000.

	Participants *n* = 5,365	Crude prevalence % (CI)	Standardized prevalence rate per 100,000 males (CI)	Standardized prevalence rate per 100,000 females (CI)
Sex				
Male	2,346	43.7 (42.4-45.1)		
Female	3,019	56.3 (54.9-57.6)		
Age at visit (years) median (IQR)	67.2 (61.6-74.8)		67.2 (61.6-74.6) [3]	67.2 (61.6-75.2) [3]
Actinic keratosis				
1-3	747	13.9 (13.0-14.9)	13,333 (9,149-19,562)	11,342 (8,281-15,860)
4-9	351	6.5 (5.9 -7.2)	8,170 (5,352-13,154)	3,704 (2,163-6,758)
≥10	301	5.6 (5.0-6.3)	7,978 (5,354-12,763)	2,386 (1,275-5,036)
Skin cancer type				
Confirmed BCC[1]	153	2.8 (2.4-3.3)	3,562 (1,645-7,640)	2,153 (818-4,587)
Potential BCC[2]	62	1.2 (0.9-1.5)	942 (219-3,928)	1,222 (367-3,613)
SCC	10	0.2 (0.1-0.4)	206 (36-2,700)	139 (21-1,879)
Melanoma	8	0.1 (0.0- 0.3)	153 (16-2,620)	81 (2-1,788)
Mycosis fungoides	1	0.02 (0.0-0.1)	38 (1-2,417)	-
Psoriasis	175	3.3 (2.8-3.8)	3,407 (1,593-7,383)	2,905 (1,439-5,865)
Eczema	411	7.7 (7.0-8.4)	8,787 (5,472-14,190)	7,746 (4,900-12,014)
Seborrheic dermatitis	713	13.3 (12.4-14.2)	17,685 (12,719-24,696)	9,588 (6,458-14,142)
Rosacea	93	1.7 (1.4-2.1)	2,019 (718-5,518)	1,743 (670-4,333)
Rhinophyma	89	1.7 (1.3-2.1)	2,728 (1,285-6,379)	611 (79-2,718)
Xerosis cutis	3,541	66.0 (64.7-67.3)	60,772 (51,027-72,492)	66,681 (57,794-76,918)
Venous insufficiency				
C0 No visible or palpable signs of venous disease	1,802	33.6 (32.3-34.9)		
C1 Telangiectasia's or reticular veins	2,085	38.9 (37.6-40.2)	29,405 (22,639-38,158)	45,810 (38,678-54,326)
C2 Varicose veins ≥ 3 mm	1,147	21.4 (20.3-22.5)	18,546 (13,655-25,497)	20,755 (16,073-26,830)
C3 Oedema	250	4.7 (4.1-5.3)	5,273 (2,751-9,906)	5,875 (3,492-9,695)
C4 Changes in skin and subcutaneous tissue	60	1.1 (0.9-1.5)	1,114 (285-4,192)	962 (242-3,238)
C5 Healed venous ulcer	5	0.1 (0.0-0.2)	230 (6-2,780)	119 (3-1,859)
C6 Active venous ulcer	4	0.1 (0.0-0.2)	180 (5-2,686)	20 (1-1,673)

CI, confidence interval; IQR, interquartile range; SCC, squamous cell carcinoma. [1] Seventeen participants had ≥ 2 basal cell carcinomas (BCCs). [2] Participants with one or more suspected BCCs referred to a general practitioner or another hospital (not Erasmus MC) and of whom we had no further medical information or pathological confirmation. [3] Age at visit (years), median (IQR).

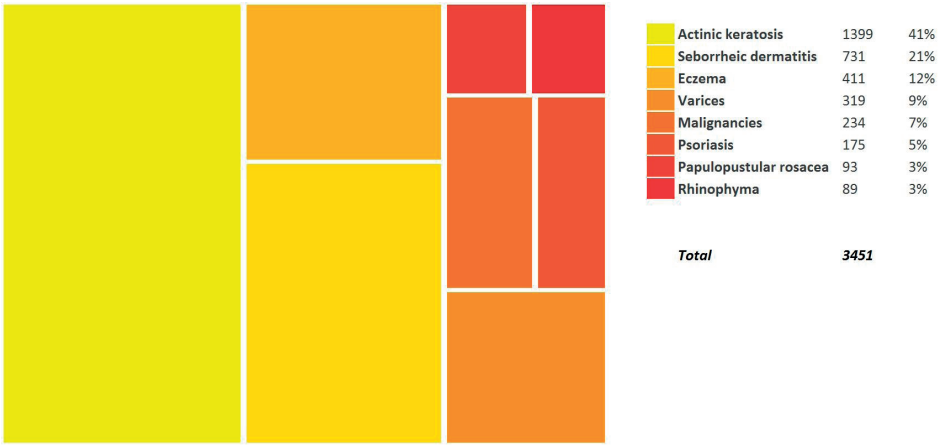


Figure 1. Total identified skin diseases.
*Varices: Large varicose veins with venous edema and/or skin changes.

The U.S. Preventive Services Task Force recently concluded that there is insufficient evidence for skin cancer screening in asymptomatic adults.[5] However, screening via self-examination, case finding by physicians or screening of high-risk subgroups were considered. The German skin screening programme showed that screening of all adults had a limited impact on melanoma-related mortality. However, the lower number needed to treat in elderly patients, and the higher skin cancer prevalence in men, suggest that elderly men are potentially a target group for skin screening.[5] Also, skin cancer awareness campaigns should not be restricted to melanomas but should include keratinocytic cancers given their prevalence and associated disease burden.[6]

Although most prevalent diseases identified in this study (e.g. seborrheic dermatitis, psoriasis, venous disease and eczema) have low mortality rates, they can severely affect quality of life.[7] Simple measures such as education on general skin care (e.g. using mild cleansers instead of water and soap in xerosis cutis) might be a fast and easy way to manage some of these conditions.[8] Also, treatment of varicose veins in patients with oedema (C3) or skin changes (C4) might reduce symptoms and prevent the development of venous leg ulcers.[9]

Most skin diseases may only cause a relatively small burden on the individual patient level, but due to the high prevalence, the societal burden is substantial. As stated in a recent report from the World Health Organization on ageing and health initiative,[10] changes in health care will be needed to adapt to the continuing increase in life expectancy and the subsequent higher proportion of the elderly population living with comorbidity, including skin diseases. The high prevalence of skin malignancies found in this population-based screening indicates that the total burden of skin diseases is beyond the recent estimates presented by the GBD

project. Therefore, the diagnosis, prevention and treatment of skin disease should get sufficient priority in general health care education and policies.

SUPPORTING INFORMATION

Appendix 1: Selection

The selection of common skin conditions was based on experience in clinical dermatology in Dutch middle-aged and elderly people (author T.N).

Appendix 2: Case definitions

Trained physicians conducted a full body skin examination and documented common skin diseases, which are described below.[11] All examinations occurred at the Rotterdam Study research facility in Ommoord, Rotterdam, the Netherlands.

Actinic keratosis: Defined as rough (keratotic) lesions with adherent scaling and erythema, not fitting another diagnosis.[12] The number of lesions were counted and classified in three groups, namely; 1–3, 4–9 and ≥ 10 lesions.

Skin cancer type: Lesions resembling a cutaneous malignancy were preferably referred to the Erasmus Medical Center for histological confirmation and follow-up and classified as basal cell carcinoma (BCC), squamous cell carcinoma, melanoma and mycosis fungoides. Lesions clinically suspect for BCCs that were referred to a general practitioner or another hospital (not Erasmus Medical Centre) were labelled as potential BCCs.

Psoriasis: Characterized by red, scaly, indurated and sharply demarcated plaques, with candle wax sign.

Eczema: Defined as dry and/or pruritic erythematous lesions, which may include scaling, excoriation, fissuring, hyperkeratosis and lichenification.

Seborrheic dermatitis: Defined as greasy scaling with erythema and a characteristic distribution in areas rich in sebaceous glands.

Rosacea: Defined as permanent erythema and telangiectasia of the face, with papules and/or pustules.

Rhinophyma: Defined as fibrous thickening of the nose with prominent pores.

Xerosis cutis: Defined as dry skin of the extensor sides of the extremities or generalized dry skin if more extensive.

Varicose veins: Classified in six groups based on the clinical parameter of the CEAP-classification.[13] C0: No visible or palpable signs of venous disease; C1: Telangiectasias or reticular veins; C2: Varicose veins ≥ 3 mm; C3: Venous oedema; C4: Changes in skin and subcutaneous tissue; C5: Healed venous ulcer; C6: Active venous ulcer.

Appendix 3: Statistical analysis

Direct standardization, with 5-year age-strata based on the revised European Standard Population,[14] was used to calculate the age- and sex-adjusted standardized prevalence rates.[15] Only strata above the age of 50 years were considered and calculations were done separately for men and women. Firstly, (crude) prevalence estimates per 100 000 persons were calculated for each age stratum. Then, these strata were multiplied by the number of persons of the corresponding stratum of the standard population. Finally, in order to get the standardized rates, we added the outcomes of the strata and divided by the total number of persons in the standard population (> 50 years of age). The confidence intervals of the standardized prevalence rates were calculated with the methods described by Tiwari *et al*.[16] Confidence intervals of the crude prevalence estimates were calculated with the Wilson procedure.[17]

REFERENCES

1 Farage MA, Miller KW, Berardesca E *et al.* Clinical implications of aging skin: cutaneous disorders in the elderly. *Am J Clin Dermatol* 2009; **10**: 73-86.

2 Karimkhani C, Boyers LN, Prescott L *et al.* Global burden of skin disease as reflected in Cochrane Database of Systematic Reviews. *JAMA Dermatol* 2014; **150**: 945-51.

3 Hahnel E, Lichterfeld A, Blume-Peytavi U *et al.* The epidemiology of skin conditions in the aged: A systematic review. *J Tissue Viability* 2016.

4 Hofman A, Brusselle GG, Darwish Murad S *et al.* The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol* 2015; **30**: 661-708.

5 Wernli KJ, Henrikson NB, Morrison CC *et al.* Screening for Skin Cancer in Adults: Updated Evidence Report and Systematic Review for the US Preventive Services Task Force. *JAMA* 2016; **316**: 436-47.

6 Hollestein LM, de Vries E, Aarts MJ *et al.* Burden of disease caused by keratinocyte cancer has increased in The Netherlands since 1989. *J Am Acad Dermatol* 2014; **71**: 896-903.

7 Parna E, Aluoja A, Kingo K. Quality of life and emotional state in chronic skin disease. *Acta Derm Venereol* 2015; **95**: 312-6.

8 Humbert P, Dreno B, Krutmann J *et al.* Recommendations for managing cutaneous disorders associated with advancing age. *Clin Interv Aging* 2016; **11**: 141-8.

9 National Institute for Health and Care Excellence. Varicose Veins in the Legs: The Diagnosis and Management of Varicose Veins. (Clinical guideline 168). 2013. http://guidance.nice.org.uk/CG168.

10 World Health O. World report on ageing and health. 2015. *Luxembourg, Luxembourg* 2015: 1-260.

11 Burns T, Breathnach S, Cox N *et al.* Rook's textbook of dermatology. In: Blachwell. 2010.

12 Flohil SC, van der Leest RJ, Dowlatshahi EA *et al.* Prevalence of actinic keratosis and its risk factors in the general population: the Rotterdam Study. *J Invest Dermatol* 2013; **133**: 1971-8.

13 Eklof B, Rutherford RB, Bergan JJ *et al.* Revision of the CEAP classification for chronic venous disorders: consensus statement. *J Vasc Surg* 2004; **40**: 1248-52.

14 Pace M, Lanzieri G, Glickman M *et al. Revision of the European Standard Population: report of Eurostat's task force*: Publications Office of the European Union. 2013.

15 Ahmad OB, Boschi-Pinto C, Lopez AD *et al.* Age standardization of rates: a new WHO standard. *Geneva: World Health Organization* 2001; **9**.

16 Tiwari RC, Clegg LX, Zou ZH. Efficient interval estimation for age-adjusted cancer rates. *Stat Methods Med Res* 2006; **15**: 547-69.

17 Wilson EB. Probable inference, the law of succession, and statistical inference. *Journal of the American Statistical Association* 1927; **22**: 209-12.

3.1 Prevalence and determinants of seborrheic dermatitis in a middle aged and elderly population: the Rotterdam Study.

M.G.H. Sanders
L.M. Pardo
O.H. Franco
R.S. Ginger
T. Nijsten

Br J Dermatol 2018; 178:22–23.

ABSTRACT

Background Seborrheic dermatitis is a chronic relapsing inflammatory skin disease with unclear pathophysiological mechanisms.

Objective To establish which lifestyle and physiological determinants are associated with seborrheic dermatitis.

Methods Seborrheic dermatitis was diagnosed by a trained physician during a full body skin examination within the Rotterdam Study, a prospective population-based cohort study in middle aged and elderly participants. The current design is a comparative cross-sectional study embedded in the Rotterdam Study. Potential factors were identified from the literature and analyzed in a multivariable logistic regression, including: age, sex, obesity, skin colour, stress, depression, education level, hypertension, climate, xerosis cutis, alcohol and tobacco use.

Results Of the 5,498 participants, 788 participants were diagnosed with seborrheic dermatitis (14.3%). We found associations between seborrheic dermatitis and male sex (adjusted OR 2.09; 95% CI: 1.77-2.47), darker skin (adjusted OR 0.39; 95% CI: 0.22-0.69), season (summer vs winter: adjusted OR 0.63; 95% CI: 0.48-0.82) and generalized xerosis cutis (adjusted OR 1.41; 95% CI 1.12-1.80).

Conclusion Seborrheic dermatitis is one of the most common inflammatory dermatoses in middle and elderly aged individuals, especially during winter. Men, and people with a light and dry skin were most likely to have seborrheic dermatitis.

INTRODUCTION

Seborrheic dermatitis is a common relapsing inflammatory skin disease, characterized by poorly demarcated erythematous patches with greasy scaling.[1] The diagnosis is mainly clinical and the disease spectrum is heterogeneous, ranging from a mild form of scaling limited to the scalp (dandruff),[2] to a severe form of erythema and scaling on scalp, face and chest. Cross-sectional and retrospective studies using medical records showed a prevalence between 2% and 8%,[3] but the prevalence in prospective studies was found to be as high as 23% in selected populations.[4,5] Despite the high frequency of seborrheic dermatitis in the population and the impaired quality of life associated with seborrheic dermatitis,[6] studies investigating the etiology of seborrheic dermatitis are scarce.

Both host and environmental determinants have been implicated in the pathogenesis of seborrheic dermatitis. It has been postulated that overgrowth of commensal *Malassezia* species, in a predisposed host (e.g. those with a decreased barrier function), are causal in the development of seborrheic dermatitis.[7] Several other determinants of seborrheic dermatitis have been studied as well, including elderly age,[3,8,9] male sex,[3,8-11] obesity,[4,12] skin color,[4] stress,[13-15] depression,[13,16] winter climate,[17,18] alcohol consumption,[12,19] tobacco exposure, hypertension,[12] HIV [20] and Parkinson's disease.[21,22] However, several of these studies were small (less than 100 participants), or conducted in a selected population (e.g. dermatology outpatients). Knowledge about determinants could provide new insights in the pathophysiology of seborrheic dermatitis, which might lead to preventative strategies and/or new treatment regimes.

The aim of this study is to investigate the reported determinants of seborrheic dermatitis in a middle aged and elderly population based study in the Netherlands (Rotterdam Study), in which people were diagnosed with seborrheic dermatitis by a physician during skin examination.

METHODS

Study population

The current design is a comparative cross-sectional study embedded in the Rotterdam Study, an ongoing prospective population based cohort study of chronic diseases in a middle aged and elderly population in the Ommoord district of Rotterdam, The Netherlands.[23] The study started in 1990 and now comprises 14,926 subjects aged 45 years or older and since 2010, 5,498 participants had a skin examination.

Case definition

A full body skin examination (FBSE) was conducted by dermatology-trained physicians. The diagnosis of seborrheic dermatitis was-based on greasy scaling, erythema and a characteristic distribution in areas rich in sebaceous glands. All three criteria had to be present for the diagnosis.

Factors associated

A literature search for potential determinants of seborrheic dermatitis was carried out using PubMed, Embase, Web-of-science and Google Scholar and was limited to observational studies on humans, published in English and updated until January 2016. The following determinants of seborrheic dermatitis were identified: elderly age, male sex, obesity, white skin colour, stress, depression, hypertension, HIV infection, Parkinson's disease, winter climate, high educational level, alcohol abuse and smoking. Xerosis cutis was chosen as a proxy for barrier dysfunction.

Age, sex, weight, height, blood pressure, presence of xerosis cutis (extensor side extremities or generalized) and skin colour [24] were registered at the FBSE. Body Mass Index (BMI) <25 was defined as normal weight, 25-30 as overweight and >30 as obesity.[25] Diastolic blood pressure of >90 mmHg or systolic blood pressure of >140 mmHg was defined as hypertension.[26] The date of FBSE was used to determine the season. Other determinants were assessed at a home interview, including: education level (low: primary education, medium: lower-intermediate vocational education, high: general secondary education and higher), alcohol (glasses/week), tobacco consumption (never and former vs current), depression and anxiety. The home interviews were conducted before the FBSE. The time between the home interview and the FBSE was less than 6 months for >95% of the participants. To assess depression, the Centre for Epidemiologic Studies Depression Scale was used and a score of ≥ 22 was the cut-off point for depression.[27] Anxiety disorders were used as a proxy of stress and were assessed by a slightly adapted version of the Munich Composite International Diagnostic Interview.[28] Participants without any anxiety disorder were compared to participants with one or more anxiety disorders.

Statistical analysis

Patient characteristics were compared between those with or without the disease using a chi-square test for categorical variables and a t-test for continuous variables. Univariable logistic regression analysis was used to calculate the crude odds ratio (OR) with 95% confidence interval (CI) to assess the strength of the association between the disease and the factors selected: age, sex, BMI, skin colour, season, smoking, alcohol, education level, hypertension, depression, stress and xerosis cutis. Subsequently, these variables were all included in a multivariable logistic regression to adjust for possible confounders. The fully conditional specification imputation method with 20 imputations was used to replace missing values

on the determinants mentioned above.[29] The outcome measurement (seborrheic dermatitis) was not imputed. A sensitivity analysis was carried out using only complete cases to verify the imputation method in the multivariable model. Statistical analyses were performed in IBM SPSS Statistics for Windows version 21.0 (Armonk, NY).

Subgroup analysis

Subgroup analysis was done to test whether previously reported sex differences in seborrheic dermatitis were due to hormonal differences. Hormone levels were available for a previous RS visit. On average, the hormones were measured 7.5 years before the FBSE. We tested the association between seborrheic dermatitis and total testosterone in men, using a logistic regression adjusting for age, skin colour, xerosis cutis and season. We used the same model in women, but replaced total testosterone by the Free Androgen Index (FAI). Also, we determined the p-value of the interaction terms between sex and the other determinants in the full model.

RESULTS

A total of 5,498 participants from the Rotterdam Study underwent a FBSE (Table 1). The median age of participants at date of FBSE was 67.9 (IQR: 61.9-76.4) and the proportion of woman was 57%. Of the 5,498 participants, 788 participants were diagnosed with seborrheic dermatitis (point prevalence of 14.3%). The percentage of missing data per variable was low (<5%), except for alcohol use (17.3%; Table 1).

In the univariable logistic regression we found significant associations between an increased prevalence of seborrheic dermatitis and age, male sex, a high education level, generalized xerosis cutis and alcohol consumption, while darker skin colour and summer season were associated with a decreased prevalence (Table 2). In the multivariable model, male sex (adjusted OR 2.09; 95% CI: 1.77-2.47), a darker skin colour (white-olive and brown skin vs white skin: adjusted OR 0.39; 95% CI: 0.22-0.69), season (summer vs winter: adjusted OR 0.63; 95% CI: 0.48-0.82) and generalized xerosis cutis (adjusted OR 1.41; 95% CI: 1.11-1.80) remained significant predictors for seborrheic dermatitis (Table 2). All significant variables remained significant in the model without imputed data and the effect sizes were comparable (data not shown).

In the logistic regression analyses between seborrheic dermatitis and total testosterone in men (adjusted OR 1.00, 95% CI: 0.98-1.02) and seborrheic dermatitis and the FAI in woman (adjusted OR 1.01, 95% CI: 0.92-1.10) no significant associations were found. Also, none of the interaction terms between sex and the other determinants were found significant (data not shown).

Table 1. Characteristics of participants by having or not having seborrheic dermatitis

	Coding	SD (%)	No SD (%)	P-value[1]
Participants		788 (14.3)	4710 (85.7)	
Sex	Male	466 (59.1)	1922 (40.8)	<0.001
Age (years)	Median (IQR)	70.1 (63.1-77.4)	67.7 (61.7-76.3)	0.027
BMI	< 25	215 (27.3)	1418 (30.1)	0.241
	25-30	383 (48.6)	2165 (46.0)	
	> 30	189 (24.0)	1115 (23.7)	
	Missing	1 (0.1)	12 (0.3)	
Skin colour	Very white - White	694 (88.1)	3858 (81.9)	<0.001
	White - Olive	81 (10.3)	650 (13.8)	
	Light brown - Black	13 (1.6)	202 (4.3)	
Education	Low	74 (9.4)	477 (10.1)	0.038
	Average	454 (57.6)	2884 (61.2)	
	High	250 (31.7)	1286 (27.3)	
	Missing	10 (1.3)	63 (1.3)	
Depression	(CESD ≥ 22)	31 (3.9)	199(4.2)	0.687
	Missing	7 (0.9)	52 (1.1)	
Anxiety disorder('s)	Yes	43 (5.5)	234 (5.0)	0.623
	Missing	27 (3.4)	227 (4.8)	
Xerosis cutis	No	271 (34.4)	1815 (38.5)	0.017
	Extensor-side extremities	390 (49.5)	2293 (48.7)	
	Generalized	121 (15.4)	557 (11.8)	
	Other	6 (0.8)	43 (0.9)	
	Missing	0 (0.0)	2 (0.04)	
Hypertension	Yes	418 (53.6)	2413 (51.7)	0.329
	Missing	8 (1.0)	43(0.9)	
Season	Winter	224 (28.4)	1091 (23.2)	0.001
	Spring	172 (21.8)	1056 (22.4)	
	Summer	92 (11.7)	756 (16.1)	
	Autumn	300 (38.1)	1807 (38.4)	
Smoking	Current	115 (14.6)	682 (14.5)	0.937
	Missing	1 (0.1)	8 (0.2)	
Alcohol (Glass/week)	Median (IQR)	5.8 (0.8-14.6)	4.4 (0.6-11.8)	0.004
	Missing	119 (15.1)	831 (17.6)	

[1]Chi-square test for categorical and t-test for continuous variables. BMI: body mass index. SD: Seborrheic dermatitis. CESD: Centre for Epidemiologic Studies Depression Scale. IQR: Interquartile range.

Table 2. Associations between seborrheic dermatitis and reported determinants.

Variable	Coding	Crude Odds Ratio	Adjusted Odds ratio
Sex	Male	2.10 (1.8-2.45) **	2.09 (1.77-2.47) **
Age at FBSE (years)	Continuous	1.01 (1.00-1.02) *	1.01 (0.99-1.02)
BMI	< 25	Reference	Reference
	25-30	1.17 (0.97-1.40)	1.05 (0.87-1.27)
	> 30	1.12 (0.91-1.38)	1.12 (0.90-1.39)
Skin colour	Very white - White	Reference	Reference
	White - Olive	0.69 (0.54-0.88) *	0.70 (0.53-0.86) *
	Light brown - Black	0.36 (0.20-0.63) **	0.39 (0.22-0.69) *
Education	Low	0.98 (0.76-1.28)	0.99 (0.75-1.29)
	Average	Reference	Reference
	High	1.23 (1.04-1.45) *	1.14 (0.85-1.53)
Depression	(CESD ≥ 22)	1.03 (0.64-1.65)	1.06 (0.70-1.59)
Anxiety disorder('s)	Yes	1.24 (0.83-1.84)	1.33 (0.93-1.89)
Xerosis cutis	No	Reference	Reference
	Extensor-side extremities	1.14 (0.97-1.34)	1.14 (0.96-1.35)
	Generalized	1.46 (1.17-1.81) *	1.41 (1.11-1.80) *
	Other	0.94 (0.60-1.46)	0.79 (0.33-1.89)
Hypertension	Yes	0.97 (0.82-1.16)	0.99 (0.85-1.16)
Season	Winter	Reference	Reference
	Spring	0.79 (0.64-0.98) *	0.84 (0.67-1.05)
	Summer	0.59 (0.46-0.77) **	0.63 (0.48-0.82) **
	Autumn	0.81 (0.67-0.98) *	0.81 (0.67-0.98) *
Smoker	Yes (current)	1.01 (0.81-1.25)	0.98 (0.78-1.22)
Alcohol (Glass/week)	Continuous	1.01 (1.01-1.02) **	1.01 (0.99-1.01)

FBSE: full body skin examination. BMI: BMI: body mass index. Confidence intervals are presented in brackets. * P-value <0.05, ** P-value < 0.001. CESD: Centre for Epidemiologic Studies Depression Scale

Table 3 presents an overview of the determinants for seborrheic dermatitis from previous published papers in comparison with the data of the current study. We replicated previous associations between seborrheic dermatitis and sex, skin colour and season, while we did not replicate several other associations such as obesity, depression, hypertension, alcohol consumption and tobacco use.

Table 3. A summary of reported and tested risk factors for seborrheic dermatitis.

		Previous studies		Rotterdam Study
Determinants		**Summary**	**Measurements**	**Summary (table 2)**
Male sex	[3,8-11]	Higher prevalence of SD for males in adult population.	Ratio: Male : female ≈ 1.4:1	SD has a strong associating with male sex.
Age	[3,8,9]	Peak between 30-50, which mostly persist in elderly age.	Prevalence graphs	No association. Relatively high prevalence in this elderly cohort.
Obesity	[4,12]	Increased triceps skin fold in male adolescent with SD. Higher percentage of obesity in adult population with SD.	Adjusted PR = 1.56, 95% CI; 1.12-2.18 16.3% vs 15.3%, p = 0.014	No association.
Skin colour	[4]	Higher prevalence of SD in white skinned male adolescent	Adjusted PR (white skin) = 1.42, 95% CI: 1.06-1.92	Decreased risk in white-olive and brown skin vs white skin.
Education	[4,12]	No significant effect in social economic status in two studies. Higher prevalence of SD in high social economic state in one study.	- 25.4% vs 19.1%, p = 0.001	No association
Hypertension	[12]	One study found an association between hypertension and SD	Adjusted OR=1.23, 95% CI: 1.12-1.35	No association
Depression	[13,16]	Increased prevalence of SD in depressive patients, not adjusted.	36% vs 9%, p < 0.01	No association
Stress	[13-15]	Self-reported triggering factor. More stressful life events in SD patients. Higher perceived stress scale.	- 5.47 (± 3.92) vs 3.27 (± 2.96), p<0.05 Adjusted OR = 1.065 (1.021 to 1.110), p = 0.003	No association
Season	[17,18]	Self-reported seasonal influences: higher disease severity in winter and lowest in summer. Visits for the diagnosis SD to the outpatient clinic.	52 % reported seasonal influences: more skin problems in winter (50%), less in summer (77%). Spring 42%, Summer 7%, Fall 25%, Winter 25%	Lowest risk for SD in summer.
Smoking	[4,12]	One study showed no effect of smoking Another study showed a slightly lower incidence of smokers in the SD group, not adjusted.	Adjusted PR (smoking)= 1.06, 95% CI: 0.75–1.50, p = 0.7 16.2% vs 19.6%, P = 0.001	No association.
Alcohol	[12,19]	No study with control group shows an association.	Alcohol abuse 0.3% (SD) vs 0.5% (control), p = 0.13	No association.

SD: seborrheic dermatitis. PR: prevalence rate. CI: confidence interval

DISCUSSION

In this middle aged and elderly population, the point prevalence of seborrheic dermatitis was 14.3%, which is higher than the 2% to 8% in previous studies.[1,3,30] This difference may be due to the fact that most of these studies were based on patient records, and therefore most likely included patients with moderate to severe seborrheic dermatitis. In contrast, the Rotterdam Study is a population-based study in which all grades of seborrheic dermatitis were considered. Furthermore, the Rotterdam Study focusses on elderly and seborrheic dermatitis prevalence increases with age.[5,31] Indeed, several physio-pathological alterations occur in the skin of elderly (i.e., a decreased amount of lipids in the in stratum corneum and thinning of epidermis and dermis) resulting to a higher vulnerability to external stimuli in this age group.[31,32] In contrast to other studies, age was not significant in the multivariable analysis, most likely due to the advanced age of the entire study population.

Of the reported determinants of seborrheic dermatitis, sex, skin colour and season were also seen in the Rotterdam Study, while obesity, depression, hypertension, alcohol consumption and tobacco use were not (Table 3). We found that men were twice as likely to develop seborrheic dermatitis compared to women. Hormonal differences between men and women may explain this association, since seborrheic dermatitis has an incidence peak during puberty, where the levels of androgens in men are high.[1,30] However, logistic regression analyses on seborrheic dermatitis and total testosterone in men and seborrheic dermatitis and the FAI in woman suggested no direct association between previously measured hormone levels and presence of seborrheic dermatitis among the participants of the Rotterdam Study.[33] However, as the hormone measurements were taken several years before the FBSE, replication of our findings with more recent data is recommended. Sex differences could also be explained in part by cutaneous differences in pH and/or use of skin products, which might influence microbial colonization and barrier integrity.[34,35] Unfortunately, these variables were not available in the Rotterdam Study and therefore were not investigated. Other studies will be needed to assess the behavioural or biological differences underlying the statistical associations between gender and seborrheic dermatitis.

In line with previous studies, the prevalence of seborrheic dermatitis was lowest during summer, which could be explained by increased ultraviolet light (UV) exposure. UV induced cutaneous immunosuppressive effects might reduce the inflammatory response to environmental stimuli and UV has a direct effect on the microbiome, including on *Malassezia* yeast of the skin.[36,37] The decreased risk of seborrheic dermatitis in people with coloured skin is again consistent with a previous finding.[4] Although this may be due to a diagnostic bias of seborrheic dermatitis in dark-skinned individuals, because erythema is less striking, it might also be due to superior barrier function in more pigmented skin.[38]

Several studies underline the importance of skin barrier function in the pathogenesis of seborrheic dermatitis.[2,7,32] In this study, xerosis cutis was taken as a proxy for skin barrier dysfunction and an association between generalized xerosis cutis and seborrheic dermatitis was observed. Although we could not rule out overdiagnosis of seborrheic dermatitis due to excessive squamae that may accompany xerosis cutis of the scalp and face, the association implies that participants with seborrheic dermatitis more often have a skin barrier dysfunction and that this is not limited to the areas affected by seborrheic dermatitis. Therefore, interventions aiming to improve skin barrier function may become a target in the treatment of patients with seborrheic dermatitis.

In contrast to previous studies,[12,19] we did not find associations for other determinants, including smoking, alcohol and education (Table 3). None of these previous studies adjusted for the important seborrheic dermatitis predictors such as sex, age, or skin colour, leading to residual confounding and spurious associations, especially since differences in smoking and alcohol consumption between men and women are well known.[39] In addition, the definition of determinants between studies differed and might explain the lack of replication of other determinants such as obesity, stress and depression. A large study with 9255 seborrheic dermatitis cases and 9246 controls suggested that seborrheic dermatitis and hypertension were associated (Adjusted OR 1.23, 95% CI: 1.12-1.35), but we did not replicate this finding.[12]

We did not investigate the association between seborrheic dermatitis and Parkinson's disease and HIV, because the data were incomplete and/or not available. Another limitation lies in the cross-sectional design of the analysis that does not allow to make causal inferences between seborrheic dermatitis and determinants. Furthermore, this study covers a middle aged and elderly population, which limits the generalizability to younger patients. The strength of our study is the population based setting, the large sample size, the physician based diagnosis and the availability of different epidemiological determinants that allowed us to control for potential confounders.

In conclusion, one out of seven people had seborrheic dermatitis and men and those with generalized xerosis cutis were even more likely to have seborrheic dermatitis. Participants with a darker complexion and those assessed during the summer period were less likely to have seborrheic dermatitis. The association between generalized xerosis cutis and seborrheic dermatitis suggests that patients might have an underlying barrier dysfunction.

REFERENCES

1 Gupta AK, Bluhm R. Seborrheic dermatitis. *J Eur Acad Dermatol* 2004; **18**: 13-26.
2 Turner GA, Hoptroff M, Harding CR. Stratum corneum dysfunction in dandruff. *Int J Cosmet Sci* 2012; **34**: 298-306.
3 Palamaras I, Kyriakis KP, Stavrianeas NG. Seborrheic dermatitis: lifetime detection rates. *J Eur Acad Dermatol Venereol* 2012; **26**: 524-6.
4 Breunig Jde A, de Almeida HL, Jr., Duquia RP *et al.* Scalp seborrheic dermatitis: prevalence and associated factors in male adolescents. *Int J Dermatol* 2012; **51**: 46-9.
5 Mastrolonardo M, Diaferio A, Vendemiale G *et al.* Seborrhoeic dermatitis in the elderly: inferences on the possible role of disability and loss of self-sufficiency. *Acta Derm Venereol* 2004; **84**: 285-7.
6 Szepietowski JC, Reich A, Wesolowska-Szepietowska E *et al.* Quality of life in patients suffering from seborrheic dermatitis: influence of age, gender and education level. *Mycoses* 2009; **52**: 357-63.
7 DeAngelis YM, Gemmer CM, Kaczvinsky JR *et al.* Three etiologic facets of dandruff and seborrheic dermatitis: Malassezia fungi, sebaceous lipids, and individual sensitivity. *J Investig Dermatol Symp Proc* 2005; **10**: 295-7.
8 Johnson MT, Roberts J. Skin conditions and related need for medical care among persons 1-74 years. United States, 1971-1974. *Vital Health Stat 11* 1978: i-v, 1-72.
9 Plunkett A, Merlin K, Gill D *et al.* The frequency of common nonmalignant skin conditions in adults in central Victoria, Australia. *Int J Dermatol* 1999; **38**: 901-8.
10 Ratzer MA. The incidence of skin diseases in the West of Scotland. *Br J Dermatol* 1969; **81**: 456-61.
11 Baghestani S, Zare S, Mahboobi AA. Skin disease patterns in Hormozgan, Iran. *Int J Dermatol* 2005; **44**: 641-5.
12 Linder D, Dreiher J, Zampetti A *et al.* Seborrheic dermatitis and hypertension in adults: a cross-sectional study. *J Eur Acad Dermatol Venereol* 2014; **28**: 1450-5.
13 Oztas P, Calikoglu E, Cetin I. Psychiatric tests in seborrhoeic dermatitis. *Acta Derm Venereol* 2005; **85**: 68-9.
14 Misery L, Touboul S, Vincot C *et al.* [Stress and seborrheic dermatitis]*Ann Dermatol Venereol* 2007; **134**: 833-7.
15 Bae JM, Ha B, Lee H *et al.* Prevalence of common skin diseases and their associated factors among military personnel in Korea: a cross-sectional study. *J Korean Med Sci* 2012; **27**: 1248-54.
16 Maietta G, Fornaro P, Rongioletti F *et al.* Patients with mood depression have a high prevalence of seborrhoeic dermatitis. *Acta Derm Venereol* 1990; **70**: 432-4.
17 Bergbrant IM. Seborrhoeic dermatitis: A clinical survey. *J EUR ACAD DERMATOL VENEREOL* 1992; **1**: 285-90.
18 Hancox JG, Sheridan SC, Feldman SR *et al.* Seasonal variation of dermatologic disease in the USA: a study of office visits from 1990 to 1998. *Int J Dermatol* 2004; **43**: 6-11.
19 Dinis-Oliveira RJ, Magalhaes T, Moreira R *et al.* Clinical and forensic signs related to ethanol abuse: a mechanistic approach. *Toxicol Mech Methods* 2014; **24**: 81-110.
20 Marino CT, McDonald E, Romano JF. Seborrheic dermatitis in acquired immunodeficiency syndrome. *Cutis* 1991; **48**: 217-8.
21 Binder RL, Jonelis FJ. Seborrheic dermatitis in neuroleptic-induced parkinsonism. *Arch Dermatol* 1983; **119**: 473-5.

22 Mastrolonardo M, Diaferio A, Logroscino G. Seborrheic dermatitis, increased sebum excretion, and Parkinson's disease: a survey of (im)possible links. *Med Hypotheses* 2003; **60**: 907-11.
23 Hofman A, Brusselle GG, Darwish Murad S *et al.* The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol* 2015; **30**: 661-708.
24 Jacobs LC, Hamer MA, Verkouteren JA *et al.* Perceived skin colour seems a swift, valid and reliable measurement. *Br J Dermatol* 2015; **173**: 1084-6.
25 Flegal KM, Carroll MD, Kit BK *et al.* Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 2012; **307**: 491-7.
26 Whitworth JA, Chalmers J. World health organisation-international society of hypertension (WHO/ISH) hypertension guidelines. *Clin Exp Hypertens* 2004; **26**: 747-52.
27 LS R. The CES-D Scale: A self-report depression scale for research in general population. *Appl Psychol Measurement* 1977; **1:385**.
28 Wittchen HU, Lachner G, Wunderlich U *et al.* Test-retest reliability of the computerized DSM-IV version of the Munich-Composite International Diagnostic Interview (M-CIDI). *Soc Psychiatry Psychiatr Epidemiol* 1998; **33**: 568-78.
29 Sterne JA, White IR, Carlin JB *et al.* Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 2009; **338**: b2393.
30 Dessinioti C, Katsambas A. Seborrheic dermatitis: Etiology, risk factors, and treatments: Facts and controversies. *Clin Dermatol* 2013; **31**: 343-51.
31 Farage MA, Miller KW, Berardesca E *et al.* Clinical implications of aging skin: cutaneous disorders in the elderly. *Am J Clin Dermatol* 2009; **10**: 73-86.
32 Schwartz JR, Messenger AG, Tosti A *et al.* A comprehensive pathophysiology of dandruff and seborrheic dermatitis - towards a more precise definition of scalp health. *Acta Derm Venereol* 2013; **93**: 131-7.
33 Rosner W, Auchus RJ, Azziz R *et al.* Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. *J Clin Endocrinol Metab* 2007; **92**: 405-13.
34 Chen W, Mempel M, Traidl-Hofmann C *et al.* Gender aspects in skin diseases. *J Eur Acad Dermatol Venereol* 2010; **24**: 1378-85.
35 Kim MK, Patel RA, Shinn AH *et al.* Evaluation of gender difference in skin type and pH. *J Dermatol Sci* 2006; **41**: 153-6.
36 Lee CH, Wu SB, Hong CH *et al.* Molecular Mechanisms of UV-Induced Apoptosis and Its Effects on Skin Residential Cells: The Implication in UV-Based Phototherapy. *Int J Mol Sci* 2013; **14**: 6414-35.
37 Wikler JR, Janssen N, Bruynzeel DP *et al.* The effect of UV-light on pityrosporum yeasts: ultrastructural changes and inhibition of growth. *Acta Derm Venereol* 1990; **70**: 69-71.
38 Man MQ, Lin TK, Santiago JL *et al.* Basis for enhanced barrier function of pigmented skin. *J Invest Dermatol* 2014; **134**: 2399-407.
39 Brady KT, Randall CL. Gender differences in substance use disorders. *Psychiat Clin N Am* 1999; **22**: 241-+.

3.2 | Association between diet and seborrheic dermatitis: a cross-sectional study

Martijn G H Sanders
Luba M Pardo
Rebecca S Ginger
Jessica C. Kiefte-de Jong
Tamar Nijsten

Journal of Investigative Dermatology (2019) 139, 108e114

ABSTRACT

Current treatments for seborrheic dermatitis only provide a temporary relief. Therefore, identifying modifiable lifestyle factors may help to reduce disease burden. The objective of this study was to determine whether specific dietary patterns or total antioxidant capacity are associated with seborrheic dermatitis. Participants of the Rotterdam Study with a skin examination and a food-frequency questionnaire were included. Total antioxidant capacity was assessed by the ferric reducing antioxidant potential of each food item. Dietary patterns were identified with principal component analysis (PCA). Multivariable logistic regression analyses were used to assess the association between total antioxidant capacity, dietary pattern-derived PCA factors and seborrheic dermatitis adjusted for confounders. In total, 4,379 participants were included, of whom 636 (14.5%) had seborrheic dermatitis. The PCA identified a "Vegetable", "Western", "Fat-rich" and "Fruit" dietary pattern. The "Fruit" pattern was associated with a 25% lower (Q1vsQ4: adjusted OR 0.76; 95%CI: 0.58-0.97, p-trend 0.03) risk and the "Western" pattern with a 47% increased risk (Q1vsQ4: adjusted OR 1.47; 95%CI: 0.98-2.20, p-trend 0.03), but only for females. Other factors were not associated with seborrheic dermatitis. In conclusion, a high fruit intake was associated with less seborrheic dermatitis, whereas high adherence to a "Western" dietary pattern in females was associated with more seborrheic dermatitis.

INTRODUCTION

Seborrheic dermatitis is a common chronic relapsing skin disease.[1] While there is no known cause for seborrheic dermatitis, evidence does suggest that certain risk factors may predispose people to seborrheic dermatitis. These factors include male sex, genetic predisposition, light skin color, winter season and high abundance of *Malassezia* yeasts on the skin.[2,3] Since treatment options for seborrheic dermatitis such as topical or oral antifungals and topical corticosteroids provide at most temporary relief, it is essential to identify modifiable lifestyle factors that may reduce the burden of this condition.

Several studies suggested that nutrition may influence inflammatory skin diseases such as acne vulgaris and to a lesser extent psoriasis.[4,5] However, there are no studies investigating the effect of diet on seborrheic dermatitis. Numerous components in our diet may affect skin health in vitro. For instance antioxidants, which are commonly found in fruits and vegetables, may be beneficial for inflammatory skin diseases.[6,7] Other dietary components that might affect skin diseases include vitamin A (i.e. involved in keratinization, immunomodulation and the regulation of sebaceous gland activity), omega-3 fatty acids (i.e. anti-inflammatory properties) and psoralen in citrus fruits (i.e., photo-carcinogenic properties).[4,8,9] The effect of a single dietary component on a disease is often too small to detect in individuals and the single components are part of an overall dietary pattern. Therefore, assessing dietary patterns and the overall antioxidant capacity may be more suitable to identify potential nutritional risk factors for seborrheic dermatitis.[10]

In a recent cross-sectional study in the Rotterdam Study, we demonstrated that seborrheic dermatitis occurrence was associated with male gender, light skin color, dry skin and winter season.[2] In this cross-sectional study of a middle aged and elderly population, we aim to determine whether the total dietary antioxidant intake or a specific *a posteriori* defined dietary pattern is associated with seborrheic dermatitis.

RESULTS

Study population

In total, 5,498 participants underwent a FBSE of which 4,379 (RS-1: 801, RS-II: 1441, RS-III: 2137) had complete nutrition data. Of the 4,379 eligible participants, 636 had seborrheic dermatitis lesions during the FBSE (point prevalence 14.5%). The median age of all participants was 68.9 (IQR: 62.6-77.4) and the proportion of women was 57.6%. The distribution of the demographic characteristics and possible confounding factors between people with and without seborrheic dermatitis at time of FBSE are presented in table 1.

Table 1. Characteristics of participants by having or not having seborrheic dermatitis

		SD	No SD	P-value
Participants		636 (14.5%)	3743 (85.5%)	
Sex	Male	378 (59.4%)	1477 (39.5%)	<0.01
Age (years)	Median (IQR)	69.9 (63.9-77.5)	68.8 (62.4-77.4)	0.14
BMI	< 25	179 (28.1%)	1176 (31.4%)	0.23
	25-30	310 (48.7%)	1714 (45.8%)	
	> 30	147 (23.1%)	845 (22.6%)	
	Missing	0 (0.0%)	8 (0.2%)	
Skin color	Very white - White	567 (89.2%)	3103 (82.9%)	<0.01
	White - Olive	61 (9.6%)	524 (14.0%)	
	Light brown - Black	8 (1.3%)	116 (3.3%)	
Education	Low	54 (8.5%)	357 (9.5%)	0.45
	Average	378 (59.4%)	2267 (60.6%)	
	High	195 (30.7%)	1068 (28.5%)	
	Missing	9 (1.4%)	51 (1.4%)	
Xerosis cutis	No	214 (33.6%)	1421 (38.0%)	0.07
	Extensor-side extremities	325 (51.1%)	1850 (49.4%)	
	Generalized	93 (14.6%)	440 (11.8%)	
	Other	4 (0.6%)	31 (0.8%)	
	Missing	0 (0.0%)	1 (0.03%)	
Physical activity (METhours/week)	Median (IRQ)	40.1 (15.0-75.8)	43.0 (17.5-81.6)	0.04
	Missing	24 (3.8%)	191 (5.1%)	
Season	Winter	187 (29.4%)	861 (23.0%)	<0.01
	Spring	137 (21.5%)	800 (21.4%)	
	Summer	77 (12.1%)	605 (16.2%)	
	Autumn	235 (36.9%)	1477 (39.5%)	
Smoking	Current	546 (85.8%)	3262 (87.1%)	0.37
Alcohol (Glass/week)	Median (IQR)	8.3 (1.4-21.0)	6.8 (0.9 - 17.3)	0.04
Energy intake (kcal/day)	Median (IQR)	2118.1 (1721.3-2626.2)	2077.2 (1670.9-2542.3)	0.07
Weekly use of supplements	Yes	296 (46.5%)	1686 (45.0%)	0.48

BMI: body mass index. MET: metabolic equivalent of task. SD: Seborrheic dermatitis, IQR: Interquartile range, The χ2-test was used for categorical variables and the t-test for continuous variables.

Total antioxidant capacity

The median FRAP-score at an intake of 2000 kcal was 24.3 (IQR: 17.3-30.5). The crude and multivariable logistic regression between the FRAP-score of the diet and seborrheic dermatitis is shown in table 2. We did not find an association between antioxidant intake and seborrheic dermatitis (FRAP-score, quartile 1 vs quartile 4: adjusted OR 0.94; 95% CI: 0.73-1.19, p for trend 0.88).

Table 2. Multivariable logistic regression between the total antioxidant capacity of the diet and the risk of having seborrheic dermatitis.

FRAP	Crude OR[1]	95% CI Lower	Upper	P-value	Adjusted OR[2]	95% CI Lower	Upper	P-value
Q1 (ref)								
Q2	0.81	0.63	1.03	0.09	0.80	0.63	1.03	0.08
Q3	1.08	0.86	1.37	0.51	1.04	0.82	1.32	0.75
Q4	0.96	0.75	1.22	0.74	0.93	0.73	1.19	0.57
P for trend				0.68				0.91

1: Crude OR: odds ratio adjusted for age and sex

2: Adjusted OR: odds ration adjusted for age, sex, total energy intake, skin color, smoking, alcohol, BMI, season, physical activity education and supplement use.

Dietary patterns

The PCA yielded four independent components of interest, explaining 26.2% of the total variation of the diet patterns. The first component was characterized as a "Vegetables" dietary pattern, the second as a "Western" pattern, characterized by meat, potato and alcohol consumption, the third as a "Fruit" pattern and the fourth as a "Fat" pattern, which correlated most with consuming olive oil and with other healthy and unhealthy fats (Table 3).

Table 4 shows the effect of adherence to the diet patterns on the risk of having seborrheic dermatitis. In the crude model, there seems to be a negative effect of the Western pattern, a positive effect of the Fruit pattern (borderline significant) and no effect of Vegetable or Fat pattern consumption. In the adjusted model, adherence to the Western pattern seems to be associated with a higher risk for seborrheic dermatitis, but only significant for the highest quartile (quartile 1 vs quartile 4: adjusted OR 1.34; 95% CI: 1.03-1.75, p value for trend 0.07). Adherence to the Fruit pattern was associated with a lower risk for seborrheic dermatitis (quartile 1 vs quartile 4: adjusted OR 0.75; 95% CI: 0.58-0.97, p value for trend 0.03) and adherence to the other patterns did not influence seborrheic dermatitis risk.

In the additional analysis, we tested for interaction between the dietary outcomes and all other variables. A significant interaction was found between the Western dietary pattern and sex (p value 0.013). Therefore, we decided to stratify this dietary pattern. For males, there was no significant association between the Western pattern and seborrheic dermatitis. However, for females, a higher adherence to this pattern was associated with an increased risk of seborrheic dermatitis (Table 5).

Table 3. Principal component analysis of 34 food groups.

	Vegetables	Western	Fruit	Fat
Greenleaf vegetables	**.846**	.039	.040	.047
Vegetables other	**.825**	.110	.013	.030
Yellow leaf vegetables	**.760**	-.086	.152	.000
Meat unprocessed	.048	**.727**	.006	.021
Meat processed	-.045	**.670**	-.042	.070
Potatoes	.164	**.478**	.012	.096
Alcoholic drinks (not wine)	-.062	**.366**	-.067	.040
Citrus fruits	.050	-.013	**.903**	.002
Fruits (non-citrus)	.134	-.021	**.883**	-.011
Olive oil	.111	-.099	.075	**.758**
Unhealthy fats	-.061	.086	-.079	**.692**
Healthy fats	-.013	.144	-.009	**.596**
Soups and sauces	.135	.273	.028	.330
Sweets	-.065	.056	.123	.112
Savory snacks	.018	.152	-.117	.027
Refined grains	.143	.113	-.163	.105
Nuts and seeds	.110	-.061	.063	.013
Fatty fish	.145	-.005	.039	.010
Lean fish	.113	-.008	.007	.029
Shellfish	-.012	.142	.029	-.010
Whole grains	.075	.032	-.084	.222
Yoghurt	.110	-.044	.175	-.192
Mineral water	.088	-.006	.060	-.006
Poultry	.197	.185	.025	-.029
Black tea	.021	.123	.079	-.017
Coffee	.087	.148	-.007	-.045
Soy	.127	-.113	.033	-.004
Pulses	.028	.178	-.037	-.054
Herb tea	.140	-.161	.110	.042
Wine	.073	-.022	-.027	-.026
Cheese	.051	.160	.070	.023
Soft drinks	-.030	.130	-.031	-.081
Milk	.063	-.080	.088	.010
Eggs	-.066	.295	-.018	.076

Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. Factor loadings with a low predictive value ($-0.2 \leq x \leq 0.2$) are presented in gray. Factors loadings with a high predictive value (>0.35) are presented in bold.

Table 4. Multivariable logistic regression between adherence to the dietary patterns and the risk of having seborrheic dermatitis.

Pattern	Crude OR[1]	95% C.I Lower	Upper	P-value	Adjusted OR[2]	95% C.I Lower	Upper	P-value
Vegetables								
Q1 (ref)								
Q2	1.04	0.82	1.33	0.75	1.028	.81	1.31	0.82
Q3	1.13	0.89	1.44	0.31	1.134	.89	1.45	0.31
Q4	1.12	0.87	1.43	0.37	1.122	.87	1.45	0.38
P for trend				0.28				0.28
Western								
Q1 (ref)								
Q2	1.29	1.00	1.66	**0.05**	1.27	1.11	1.44	0.07
Q3	1.19	0.92	1.54	0.18	1.16	0.89	1.50	0.27
Q4	1.35	1.05	1.73	**0.02**	1.34	1.03	1.75	**0.03**
P for trend				0.05				0.07
Fruit								
Q1 (ref)								
Q2	0.87	0.69	1.09	0.22	0.83	0.74	0.93	0.12
Q3	0.80	0.63	1.02	0.07	0.77	0.60	0.99	**0.04**
Q4	0.80	0.63	1.03	0.08	0.75	0.58	0.97	**0.03**
P for trend				0.06				**0.03**
Fat								
Q1 (ref)								
Q2	1.26	0.99	1.61	0.06	1.28	1.00	1.63	0.05
Q3	0.96	0.75	1.24	0.76	0.98	0.77	1.24	0.85
Q4	1.20	0.94	1.53	0.14	1.22	.945	1.58	0.14
P for trend				0.47				0.47

1: Crude OR: odds ratio adjusted for age and sex
2: Adjusted OR: odds ration adjusted for age, sex, skin color, smoking, BMI, season, total energy intake, physical activity, education and supplement use.
Boldface: p-values < 0.05

Table 5. Multivariable logistic regression between the "Western" dietary pattern and seborrheic dermatitis, stratified for sex.

	Male				Female			
	OR	95% C.I Lower	Upper	P-value	OR	95% C.I Lower	Upper	P-value
Q1 (ref)								
Q2	1.19	0,99	1.44	0.35	1.26	0.88	1.81	0.21
Q3	0.87	0,60	1.27	0.47	1.50	1.04	2.18	**0.03**
Q4	1.18	0,83	1.67	0.37	1.47	0.98	2.20	0.06
P for trend				0.67				**0.03**

This stratified multivariable logistic regression was conduction because of the significant interaction between the "Western" dietary pattern and sex (p-value 0.013).OR: odds ratio adjusted for odds ration adjusted for age, sex, skin color, smoking, BMI, season, total energy intake, physical activity, education and supplement use. Boldface: p-values < 0.05

DISCUSSION

In the study, we found that participants with a dietary pattern characterized by high fruit intake had lower odds of having seborrheic dermatitis after adjustment for confounders. Furthermore, we found that a "Western" dietary pattern was associated with higher odds of having seborrheic dermatitis, but only for females. We did not find an association between the presence of seborrheic dermatitis and dietary patterns characterized by vegetables or fat; nor did we find an association between seborrheic dermatitis and total dietary antioxidant capacity.

The consumption of fruits might reduce the likelihood of having seborrheic dermatitis via consumption of a wide variety of vitamins and other compounds (e.g. flavonoids, antioxidant) that have been demonstrated to reduce inflammation in several diseases.[11,12] Also, fruits contain several nutrients that can serve as methyl donors, which can prevent the expression of inflammatory genes.[13] Another possible hypothesis by which fruits affect skin health involves psoralen. Psoralen is highly present in citrus fruits and increases the sensitivity of the skin to UV radiation.[14] This increased sensitivity to UV could have a positive effect on seborrheic dermatitis since it is less frequently present in summer months.[2,15]

Western diets and diets high in meat and processed food consumption have often been associated with markers of inflammation.[16,17] Omega-6 fatty acids is one of the markers that can significantly change due to a diet and have been suggested to induce chronic inflammation. However, intervention studies with omega-6 supplementation did not substantiate this.[18] The stratified analysis of our data showed that females with a high adherence to the "Western" pattern seem to have higher odds of having seborrheic dermatitis. However, higher adherence to the "Western" pattern was not associated with seborrheic dermatitis in males. Previous dietary intervention studies showed that there are differences in response to diet between males and females. For example, a healthy diet improved insulin homeostasis in males, but not in females. Sex differences in body fat distribution might be one explanation for this.[19] Also, it is known that immune response in females differs from males and that females are more susceptible to autoimmune and inflammatory diseases.[20] This study underlines the importance of possible interactions between sex and nutrition, in which adding sex as a confounder in the final model is not sufficient.

Because seborrheic dermatitis is a chronic inflammatory disease, and reactive oxygen species may promote chronic inflammation or aggravate inflammatory skin diseases,[21] we expected individuals with a high total antioxidant intake to have a lower prevalence of skin disease. Two previous studies on the total antioxidant level in serum and the antioxidant levels of scalp scrapes suggested that oxidative stress might be higher in seborrheic dermatitis pa-

tients.[22,23] In contrast to these studies and the hypothesis, participants with a higher overall dietary antioxidant capacity did not have a decreased odds of having seborrheic dermatitis. This observation suggests that the effect of oral antioxidants in the treatment of seborrheic dermatitis might be limited. However, it has been questioned if methods assessing the overall antioxidant capacity should be used to make claims concerning the antioxidant defense system.[24] In addition, we did not find a consistent association between dietary antioxidant capacity and inflammatory markers in The Rotterdam Study.[25] Therefore, other biomarkers of antioxidant capacity or oxidative stress may provide additional insights in the role of antioxidants in seborrheic dermatitis.

In this study we investigated the role of diet in seborrheic dermatitis. The strengths of our study are the large sample size, the population-based setting, the physician-based diagnosis and the availability of different epidemiological factors that allowed us to control for potential confounders. There are several limitations to the study. The cross-sectional design does not allow to make causal inferences and since this study covers a middle aged and elderly population, the generalizability to younger patients might be limited. Unfortunately, the disease severity and distribution of seborrheic dermatitis was not specifically documented during the full body skin examination. Therefore, we cannot elaborate further on the relation between location or severity of the disease and the associations with the diet components. Also, because we did not investigate individual dietary components, this study cannot be used to select possible supplements that might reduce seborrheic dermatitis risk. Furthermore, the use of an FFQ to assess dietary intake is prone to measurement error. To account for systematic measurement errors and to limit the influence of outliers, we adjusted our analyses for total energy intake and categorized the dietary data into quartiles. Although self-reported dietary intake is subject to measurements error when it concerns absolute intake, it has been shown that the FFQ is able to adequately rank the intake of individuals according to their food group.[26] To account for potential confounding by food supplementation, we adjusted the analyses for any dietary supplement use. For RS-I and RS-II, the FFQ was conducted in the same period as the FBSE. For RS-III, the FFQ data was registered five years earlier than the FBSE. However, we previously showed that dietary patterns are relatively stable in this population (in the same quartile of intake), in particular for components as vegetables (73%), fruits (93%), dietary fiber (91%), saturated fat (92%) and alcohol (79%).[27] There might always be residual confounding of variables that were not documented in this cohort. Self-reported stress, for example, could be such a variable that might have influenced both dietary choices and disease risk.[28] However, we previously showed that measurements of anxiety and depression (proxies of stress) were not associated with seborrheic dermatitis in this cohort.[29] Also, including these variables in the multivariable logistic regression of this study did not influence the associations between the dietary patterns and seborrheic dermatitis (data not shown).

In conclusion, a high intake of fruit was associated with lower odds of seborrheic dermatitis and a high adherence to the "Western" dietary pattern seems to be associated with a higher risk of seborrheic dermatitis in females. These findings were not driven by the overall dietary antioxidant capacity. While the results of this study cannot be used to provide exact recommendations, it seems legitimate to advise seborrheic dermatitis patients to follow national diet guidelines regarding fruit, which recommends at least 200g/day of fruit in the Netherlands.[30] Furthermore, although a high adherence to the western dietary pattern was only associated with an increased disease risk for females, it might be beneficial for both sexes to reduce meat consumption considering recent literature linking meat intake with an increased risk of mortality.[31] Dietary pattern studies seem appropriate as initial dietary association study and might be meaningful in other skin diseases as well. Replication of our findings in an independent cohort, or conducting an interventional study, would be necessary to substantiate these claims. A prospective study measuring relapse rate in seborrheic dermatitis patients with a high or low fruit intake would be of high value.

METHODS

Study design

The Rotterdam Study (RS) is an ongoing prospective population based cohort study of chronic diseases in a middle aged and elderly population in the Ommoord district of Rotterdam, The Netherlands.[32] The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC (registration number MEC 02.1015) and by the Dutch Ministry of Health, Welfare and Sport (Population Screening Act WBO, license number 1071272-159521-PG). The Rotterdam Study has been entered into the Netherlands National Trial Register (NTR; www.trialregister.nl) and into the WHO International Clinical Trials Registry Platform (ICTRP; www.who.int/ictrp/network/primary/en/) under shared catalogue number NTR6831. All participants provided written informed consent to participate in the study and to have their information obtained from treating physicians. The study started in 1990 and to date comprises three cohorts (RS-1, RS-II, and RS-III) with a total of 14,926 subjects aged 45 years or older. Dermatological examinations were introduced in 2010, and since then, 5,498 participants had a skin examination. The current study is a cross-sectional study containing all participants with a skin examination and available nutrition data.

Case definition

Seborrheic dermatitis was diagnosed by dermatology-trained physicians during a scheduled full body skin examination (FBSE). The diagnoses were based on a greasy scaling, erythema and a characteristic distribution in areas rich in sebaceous glands. Participants without seborrheic dermatitis were considered as controls.

Nutritional data

Dietary intake was assessed by means of a food-frequency questionnaire (FFQ), which included 389 questions regarding the consumption of food over the last month. The Dutch Food Composition Table of 2006 and 2011[33] was then used to transform the data into daily macronutrient intake and total energy intake (kcal/day). This FFQ was based on a validated FFQ for Dutch adults.[26,34] This FFQ was validated against 3 day food records, 4-5 months apart and showed an energy- and sex-adjusted correlation for macronutrients between 0.47 (fat) to 0.79 (polysaccharides).

Total antioxidant capacity

The total antioxidant capacity was calculated as described earlier.[35] In short, an Antioxidant Food Table[6] was used to assess the antioxidant capacity of each dietary item. This Antioxidant Food Table contains the antioxidant capacity of food items assessed on the basis of an existing table that evaluated the ferric reducing ability of plasma (FRAP) for more than 3000 food items.[6] For every participant, the consumption frequency of each dietary item was multiplied by the FRAP-value in this table. The total antioxidant capacity was then adjusted for the total energy intake using the residual method and categorized into quartiles.[36]

Dietary patterns

In order to define dietary patterns, we used the posteriori dietary pattern analysis described in Hu et al (1999). [37] The list of 389 food items from the FFQ was categorized into 34 main food groups. The groups were categorized based on of the Nevo table, while accounting for effects of specific subgroups (e.g. vegetables were split up because of the high vitamin A content in green leafy vegetables, fruits were split up because of the high vitamin C content in citrus fruits). These food groups were then analyzed with a PCA to identify dietary patterns that explained the maximum variation of food intake. To minimize correlation between the dietary patterns, a Varimax rotation was used. In determining the number of dietary patterns (factors) that should remain; we interpreted the Scree Test for factors with an Eigenvalue of ≥1.0. The factors were then used to rank the participants in low to high adherence for each of the dietary patterns. These ranks were used to create quartiles, which explain how well a participant fits in a specific pattern.

Covariates

Age, sex, skin colour,[38] length, weight and season were documented during the visit to the research center. During the home interview participants were asked about their education level (low: primary education, medium: lower-intermediate vocational education, high: general secondary education and higher) and tobacco use (never and former vs current). Physical activity was assessed using the LASA Physical Activity Questionnaire (LAPAQ) and

expressed in METhours/week.[39] Participants were categorized as supplement users if they used supplements at least once a week.

Statistical analyses

Missing data on the covariates were imputed using the fully conditional specification imputation method with 20 imputations.[40] Multivariable logistic regression was used to calculate the strength of the association between total antioxidant capacity and seborrheic dermatitis and the PCA-extracted dietary patterns and seborrheic dermatitis, both adjusting for age, sex, skin color, smoking, total energy intake, BMI, season, physical activity, education and supplement use. In addition, we tested for interaction between the dietary outcomes and all other variables.

The threshold for significance was set at a p-value of 0.05. All analyses were conducted in IBM SPSS Statistics for Windows version 21.0 (Armonk, NY).

REFERENCE

1 Gupta AK, Bluhm R. Seborrheic dermatitis. *J Eur Acad Dermatol* 2004; **18**: 13-26.

2 Sanders MGH, Pardo LM, Franco OH *et al.* Prevalence and determinants of seborrhoeic dermatitis in a middle-aged and elderly population: the Rotterdam Study. *Br J Dermatol* 2018; **178**: 148-53.

3 Sanders MGH, Pardo LM, Uitterlinden AG *et al.* The Genetics of Seborrheic Dermatitis: A Candidate Gene Approach and Pilot Genome-Wide Association Study. *J Invest Dermatol* 2018; **138**: 991-3.

4 Millsop JW, Bhatia BK, Debbaneh M *et al.* Diet and psoriasis, part III: role of nutritional supplements. *J Am Acad Dermatol* 2014; **71**: 561-9.

5 Kwon HH, Yoon JY, Hong JS *et al.* Clinical and Histological Effect of a Low Glycaemic Load Diet in Treatment of Acne Vulgaris in Korean Patients: A Randomized, Controlled Trial. *Acta Derm-Venereol* 2012; **92**: 241-6.

6 Carlsen MH, Halvorsen BL, Holte K *et al.* The total antioxidant content of more than 3100 foods, beverages, spices, herbs and supplements used worldwide. *Nutr J* 2010; **9**: 3.

7 Bickers DR, Athar M. Oxidative stress in the pathogenesis of skin disease. *J Invest Dermatol* 2006; **126**: 2565-75.

8 Beckenbach L, Baron JM, Merk HF *et al.* Retinoid treatment of skin diseases. *Eur J Dermatol* 2015; **25**: 384-91.

9 Dugrand A, Olry A, Duval T *et al.* Coumarin and furanocoumarin quantitation in citrus peel via ultraperformance liquid chromatography coupled with mass spectrometry (UPLC-MS). *J Agric Food Chem* 2013; **61**: 10677-84.

10 Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. *Curr Opin Lipidol* 2002; **13**: 3-9.

11 Cepeda AM, Del Giacco SR, Villalba S *et al.* A Traditional Diet Is Associated with a Reduced Risk of Eczema and Wheeze in Colombian Children. *Nutrients* 2015; **7**: 5098-110.

12 He FJ, Nowson CA, MacGregor GA. Fruit and vegetable consumption and stroke: meta-analysis of cohort studies. *Lancet* 2006; **367**: 320-6.

13 Perdigoto CN, Valdes VJ, Bardot ES *et al.* Epigenetic regulation of epidermal differentiation. *Cold Spring Harb Perspect Med* 2014; **4**.

14 Wu S, Han J, Feskanich D *et al.* Citrus Consumption and Risk of Cutaneous Malignant Melanoma. *J Clin Oncol* 2015; **33**: 2500-8.

15 Pirkhammer D, Seeber A, Honigsmann H *et al.* Narrow-band ultraviolet B (ATL-01) phototherapy is an effective and safe treatment option for patients with severe seborrhoeic dermatitis. *Br J Dermatol* 2000; **143**: 964-8.

16 Ozawa M, Shipley M, Kivimaki M *et al.* Dietary pattern, inflammation and cognitive decline: The Whitehall II prospective cohort study. *Clin Nutr* 2017; **36**: 506-12.

17 Giugliano D, Ceriello A, Esposito K. The effects of diet on inflammation: emphasis on the metabolic syndrome. *J Am Coll Cardiol* 2006; **48**: 677-85.

18 Innes JK, Calder PC. Omega-6 fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* 2018.

19 Bedard A, Riverin M, Dodin S *et al.* Sex differences in the impact of the Mediterranean diet on cardiovascular risk profile. *Br J Nutr* 2012; **108**: 1428-34.

20 Klein SL, Flanagan KL. Sex differences in immune responses. *Nat Rev Immunol* 2016; **16**: 626-38.

21 Trouba KJ, Hamadeh HK, Amin RP *et al.* Oxidative stress and its role in skin disease. *Antioxid Redox Signal* 2002; **4**: 665-73.

22 Emre S, Metin A, Demirseren DD *et al.* The association of oxidative stress and disease activity in seborrheic dermatitis. *Arch Dermatol Res* 2012; **304**: 683-7.

23 Ozturk P, Arican O, Belge Kurutas E *et al.* Oxidative stress in patients with scalp seborrheic dermatitis. *Acta Dermatovenerol Croat* 2013; **21**: 80-5.

24 Turck D, Bresson JL, Burlingame B *et al.* Guidance for the scientific requirements for health claims related to antioxidants, oxidative damage and cardiovascular health. *EFSA Journal* 2018; **16**.

25 Stringa N, Brahimaj A, Zaciragic A *et al.* Relation of antioxidant capacity of diet and markers of oxidative status with C-reactive protein and adipocytokines: a prospective study. *Metabolism* 2017; **71**: 171-81.

26 Goldbohm RA, van den Brandt PA, Brants HA *et al.* Validation of a dietary questionnaire used in a large-scale prospective cohort study on diet and cancer. *Eur J Clin Nutr* 1994; **48**: 253-65.

27 Schoufour JD dJE, Kiefte-de Jong JC, van Lenthe FJ, Hofman A, Nunn SPT, Franco OH. Socio-economic indicators and dietary quality in an elderly population. *Submitted* 2017.

28 Misery L, Touboul S, Vincot C *et al.* [Stress and seborrheic dermatitis] Stress et dermatite seborrheique. *Ann Dermatol Venereol* 2007; **134**: 833-7.

29 Sanders MGH, Pardo LM, Franco OH *et al.* Prevalence and determinants of seborrheic dermatitis in a middle aged and elderly population: the Rotterdam Study. *Br J Dermatol* 2017.

30 Brink. L, Smeets. AP-, Stafleu. A *et al.* The Netherlands Nutrition Centre. Richtlijnen Schijf van Vijf. http://www.voedingscentrum.nl/professionals/schijf-van-vijf/naslag-richtlijnen-schijf-van-vijf.aspx. 2016.

31 Etemadi A, Sinha R, Ward MH *et al.* Mortality from different causes associated with meat, heme iron, nitrates, and nitrites in the NIH-AARP Diet and Health Study: population based cohort study. *BMJ* 2017; **357**: j1957.

32 Ikram MA, Brusselle GGO, Murad SD *et al.* The Rotterdam Study: 2018 update on objectives, design and main results. *Eur J Epidemiol* 2017; **32**: 807-50.

33 Dutch Food Composition Table. Nevo tabel 2006/2011. Voorlichtingsbureau voor de Voeding. The Hague, The Netherlands, 2011.

34 Feunekes GI, Van Staveren WA, De Vries JH *et al.* Relative and biomarker-based validity of a food-frequency questionnaire estimating intake of fats and cholesterol. *Am J Clin Nutr* 1993; **58**: 489-96.

35 Pantavos A, Ruiter R, Feskens EF *et al.* Total dietary antioxidant capacity, individual antioxidant intake and breast cancer risk: the Rotterdam Study. *Int J Cancer* 2015; **136**: 2178-86.

36 Willett WC, Howe GR, Kushi LH. Adjustment for total energy intake in epidemiologic studies. *Am J Clin Nutr* 1997; **65**: 1220S-8S; discussion 9S-31S.

37 Hu FB, Rimm E, Smith-Warner SA *et al.* Reproducibility and validity of dietary patterns assessed with a food-frequency questionnaire. *Am J Clin Nutr* 1999; **69**: 243-9.

38 Jacobs LC, Hamer MA, Verkouteren JA *et al.* Perceived skin colour seems a swift, valid and reliable measurement. *Br J Dermatol* 2015; **173**: 1084-6.

39 Stel VS, Smit JH, Pluijm SM *et al.* Comparison of the LASA Physical Activity Questionnaire with a 7-day diary and pedometer. *J Clin Epidemiol* 2004; **57**: 252-8.

40 Sterne JA, White IR, Carlin JB *et al.* Multiple imputation for missing data in epidemiological and clinical research: potential and pitfalls. *BMJ* 2009; **338**: b2393.

4 | The genetics of seborrheic dermatitis: a candidate gene approach and pilot genome-wide association study

Martijn G H Sanders
Luba M Pardo
André G Uitterlinden
Adrian M Smith
Rebecca S Ginger
Tamar Nijsten

Journal of Investigative Dermatology (2018) 138, 991e993;

To the editor,

Seborrheic dermatitis is a chronic inflammatory skin disease with a complex etiology. The genetic predisposition for seborrheic dermatitis has not been studied, resulting in the absence of candidate genes involved in the pathogenesis of this condition. However, seborrheic dermatitis shares several clinical features with other chronic inflammatory skin diseases, in particular with psoriasis (PSO) (sometimes coined 'sebopsoriasis') and atopic dermatitis (AD), which are much better characterized genetically.[1,2]

In this cross-sectional study, we used a candidate gene approach (CGA) to investigate whether genetic variants previously associated with AD and PSO are also associated with an increased risk of seborrheic dermatitis. In addition, we conducted a genome-wide association study (GWAS) to identify novel genetic variants associated with seborrheic dermatitis.

A detailed description of the methods can be found in the Supplementary Methods. In brief, we included participants of the Rotterdam Study (RS), an ongoing prospective cohort study of chronic diseases that consists a major cohort (RS-I) and two extensions (RS-II and RS-III). The Rotterdam Study has been approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands. All participants provided written informed consent to participate in the study and to obtain information from their treating physicians.[3] Seborrheic dermatitis was diagnosed during a full body skin examination by a physician. DNA extraction from whole blood, genotyping, imputation and quality control were carried out following standard protocols. After quality control, 9,008,729 markers were included.

The CGAs were performed in two steps. First, a SNP-based CGA of previously associated PSO and AD SNPs was performed using a logistic regression with an additive model, which was adjusted for age, sex, and four principal components of genetic variance (PCs). The latter covariates were used to account for possible population stratification and hidden relatedness. Second, a gene-based CGA was done to screen for additional variants within the regions of interest using the approach of Purcell et al. (2007) (details presented in Supplementary Methods). To discover new loci for seborrheic dermatitis, we conducted a GWAS for each cohort using the same model as for the SNP-based approach. The GWASs of the three separate cohorts were implemented in the ProbABEL package[4] and the meta-analysis was carried-out using metal (Supplementary Methods).[5]

In total, 4,050 participants were available for analysis, of which 609 (15%) had seborrheic dermatitis (Supplementary Table 1). The SNP-based and gene-based CGA did not yield any significant locus for seborrheic dermatitis after correcting for multiple testing (Supplementary Table 2-5), although some of the findings suggested an overlap between seborrheic dermati-

tis and PSO and AD. For example, the *LCE3* gene cluster, known to play a role in PSO and AD, showed suggestive associations with seborrheic dermatitis in both the data of the SNP-based CGA and the gene-based CGA (p-values, 0.0157 and 0.00869, respectively). *MICB* showed suggestive evidence in the gene-based CGA (p-value=0.0063) and *IL12B* showed suggestive evidence in the SNP-based CGA (p-value=0.02011) (Table 1).

Table 1. Top SNPs and loci detected in the candidate SNP-based and gene-based association analysis (p<0.05).

SNP	Chr	A1	A2	Disease	Locus	Freq	Direction of SNP	SNP P-value*	Gene p-value*
rs61813875	1	c	g	AD	*LCE3E*	0.9771	**- - -**	**0.008693**	**0.0192**
rs145809981	6	t	c	AD	*MICB*	0.1232	**-** + +	0.9425	**0.006299**
rs4809219	20	a	c	AD	*RTEL1*	0.7396	**+** - **+**	0.9414	**0.0162**
rs7512552	1	t	c	AD	*C1orf51/MRPS21*	0.4995	+ + +	**0.04366**	**0.024**
rs112111458	2	a	g	AD	intergenic	0.8739	- - -	0.2496	**0.0225**
rs4112788	1	a	g	PSO	*LCE3E-LCE3D del*	0.3582	**+ + +**	0.571	**0.0157**
rs3213094[2]	5	t	c	PSO	*IL12B*	0.1802	**- - -**	**0.02011**	0.1397
rs280519[2]	19	a	g	PSO	*TYK2*	0.5079	**+ + +**	**0.03388**	0.3912

Chr: Chromosome, A1: reference allele, A2: other allele, Disease: SNP associated with atopic dermatitis (AD) or psoriasis (PSO), Freq: frequency of reference allele, Direction: Effect direction of the reported SNP for each cohort (RS-I, RS-II and RS-III), bold if the direction was similar as previously reported *: No significant results when applying Bonferroni-correction. The full tables can be found in the supplement information.

In the GWAS, two genome-wide significant SNPs (p-value ≤ 5 x10-8) were identified. The first SNP was rs58331610, which mapped to an intronic region of the *MAST4* gene at chromosome 5 (p-value: 1.75 x 10^{-8}). The second SNP was rs16944244, which was part of a linkage disequilibrium (LD) block of 18 SNPs (r2=0.6) and mapped to an intergenic region at chromosome 17p12, between the genes *PIRT* and *SHISA6* (p-value: 2.10 x 10^{-8}) (Table 2, Supplementary Figure 3). In addition, we found that 68 SNPs in seven different loci were associated with seborrheic dermatitis with suggestive genome-wide associations (p-value< 5x 10-6) (Supplementary Table 6).

MAST4 belongs to a group of protein kinases that play a critical role in intracellular signal transmission cascades.[6] The precise function of *MAST4* is unknown, but the gene is expressed in hair follicle keratinocytes.[7] Further bioinformatics analysis showed two transcription factor binding site overlaying the chromosomal position of r58331610 (Supplementary Result). Another interesting locus was *PIRT*. This gene is 285 mega base pairs downstream of the second genome wide significant SNP (rs16944244) and it is known to be a modulator of *TRPV1* and *TRPM8*. *TRPV1* plays a role in itch and *TRPM8* has recently been linked as a regulator of epidermal homeostasis.[8-10]

Table 2. Top SNPs identified in the genome-wide association study ($p < 5 \times 10^{-7}$)

SNP	Genes of interest	Chr	Location	A1	A2	Freq	P-value
rs58331610	***MAST4***	**5**	**66020457**	**a**	**g**	**0.8615**	**1.75×10^{-8}**
rs16944244	***PIRT/SHISA6*** +	**17**	**11026693**	**c**	**g**	**0.9796**	**2.10×10^{-8}**
rs78160483	*PIRT/SHISA6* +	17	11026297	t	g	0.9818	8.38×10^{-8}
rs16944241	*PIRT/SHISA6* +	17	11026414	a	g	0.9818	8.38×10^{-8}
rs6546997	*TACR1/ GAPDHP57*+	2	75658780	t	c	0.1894	9.53×10^{-8}
rs8075550	*PIRT/SHISA6* +	17	11027059	a	g	0.9594	1.04×10^{-7}
rs11870758	*PIRT/SHISA6* +	17	11015902	a	t	0.9722	1.21×10^{-7}
rs12188593	*MAST4*	5	66030069	t	c	0.1359	1.27×10^{-7}
rs182131544	*PIRT/SHISA6* +	17	11023964	a	g	0.9788	1.32×10^{-7}
rs111448323	*PIRT/SHISA6* +	17	11022815	a	g	0.9786	1.50×10^{-7}
rs4791451	*PIRT/SHISA6* +	17	11027570	c	g	0.9808	1.54×10^{-7}
rs77699632	*PIRT/SHISA6* +	17	11020937	a	t	0.0214	1.73×10^{-7}
rs111341744	*PIRT/SHISA6* +	17	11021220	a	g	0.9784	1.79×10^{-7}
rs75205996	*PIRT/SHISA6* +	17	11021311	t	c	0.9784	1.79×10^{-7}
rs58692658	*PIRT/SHISA6* +	17	11019423	t	c	0.022	2.32×10^{-7}
rs77592872	*PIRT/SHISA6* +	17	11018841	a	t	0.9781	2.34×10^{-7}
rs138424360	*PIRT/SHISA6* +	17	11018089	a	g	0.0219	2.37×10^{-7}
rs80298958	*PIRT/SHISA6* +	17	11016351	c	g	0.9784	3.39×10^{-7}
rs76730906	*PIRT/SHISA6* +	17	11019589	t	g	0.9754	3.85×10^{-7}

Abbreviations: A1, reference allele; A2, other allele; Chr, chromosome; Freq, frequency of reference allele; SNP, single-nucleotide polymorphism. Boldface indicates genome-wide significance ($< 5 \times 10^{-8}$), +: Intergenic, A1: reference allele, A2: other allele, Freq: frequency of reference allele. The p-values were calculated by meta-analysis of the output per SNP for each RS cohort using p-values derived from a likelihood ratio test as summary statistics. The full table can be found in the Supplementary Results.

This study has several limitations. Seborrheic dermatitis is a clinical diagnosis without clear diagnostic criteria and it may wax and wane resulting in an underestimation of the seborrheic dermatitis prevalence. In our study, participants with seborrheic dermatitis were more likely to have PSO (Supplementary Table 1), suggesting that these diseases occur simultaneously. However, with a clinical presentation exclusively on the scalp for seborrheic dermatitis, it could also be due to misdiagnosis. Another limitation is that our study population was smaller than the previous GWAS in which SNPs associated with PSO and AD were discovered.[1,2] Therefore, we cannot exclude that the lack of associations we observed in our CGA analysis was due to a lack of power, since most of the PSO and AD SNPs had modest effects. Given the high proportion of PSO patients in our study it could be argued that these patients are also contributing to the suggestive associations in our CGA. However, we conducted a sensitivity analysis where PSO cases were excluded, and the associations in both the SNP-based and gene-based CGA were still significant, which showed that the associations were not only driven by the PSO patients (Supplementary Result). Despite the modest sample size, we

found genome-wide hits within or close to genes that can be linked to skin inflammation or immunity. To our knowledge, no other cohort of clinically assessed seborrheic dermatitis is available. A future study on the genetics of seborrheic dermatitis should be focused on replicating the findings in a larger independent sample, subsequently fine-mapping approaches will be required to identify the causal variant in the region of LD and might also identify new markers initially not identified.[11]

In conclusion, we did not find robust evidence for a shared genetic background between seborrheic dermatitis and AD or PSO. However, two genome-wide significant associations for seborrheic dermatitis were identified in a pilot GWAS, indicating that genetic susceptibility probably plays a role in seborrheic dermatitis. This study provides a basis for further genetic research on seborrheic dermatitis and could lead to a next step in the understanding of the seborrheic dermatitis pathogenesis.

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY METHODS

Study population.

The Rotterdam Study (RS) is an ongoing prospective cohort study of chronic diseases, including skin diseases, in the elderly. A detailed description of the study can be found elsewhere.[3] In brief, the RS consists of a major cohort (RS-I) and two extensions (RS-II and RS-III). RS-I started in 1990 and initially included 7,983 participants living in the Ommoord district in Rotterdam, The Netherlands. RS-II started in 2000 and includes 3,011 participants. RS-III is a further extension of the cohort, started in 2006, and includes 3,932 participants. In total, the RS consists of 14,926 subjects aged 45 years or over. However, dermatology was not introduced until 2010 and included 5,376 subjects at time of analysis. The cohort consists predominantly (90%) of participants of North-European ancestry, of which genotype data was collected.

Case definition

A total of 4,454 participants (RS-I: 823, RS-II: 1212, RS-III: 2419) from the RS with available genotype data underwent a full body skin examination by trained physicians. The clinical diagnosis of seborrheic dermatitis was based on the presence of greasy scaling, erythema and a characteristic distribution of the scalp, face and/or chest. Participants who did not have any sign of active disease and without a history of ketoconazole use ($\geq$3 prescriptions using pharmacy linkage data) were included as controls.

Genotyping and Imputation

The Illumina Infinium II HumanHap550 BeadChips were used to genotype the RS-I and RS-II cohorts, while Illumina Human610-Quad BeadChips were used to genotype the RS-III cohort. The imputation was done using the MACH-minimac v1.0.18 software and imputed to the 1,000 genomes data set, resulting in 29,079,142 genotyped and/or imputed markers.[12]

Quality control on the SNPs included removing SNPs with Hardy-Weinberg equilibrium deviations (p-value < 5 $x10^{-6}$), genotype call rate < 97%, gender mismatch and a high mean autosomal heterozygosity. First-degree relatives were removed and SNPs were filtered out if they had a minor allele frequency of less than 1% or an imputation quality (Rsq) of less than 0.3.

Selection of candidate loci

Pubmed was searched for well-powered GWAS in Caucasian populations that showed an association between SNPs and PSO or AD. We selected SNPs with a significance level of 5.0 $<10^{-8}$. The reported (lead) SNP was used for the SNP-based association analysis [1,13-19] and was used to select a locus region for a gene-based CGA.

For lead SNPs located within a gene, a 30kb region (plus 15kb downstream and 15kb upstream of the gene) was added to include regulatory elements close to the gene. If the lead SNP was intergenic and located more than 15kb away of a gene, we also added 15kb downstream and 15kb upstream from the base-pair position of the lead SNP. The NCBI Variation Viewer (http://www.ncbi.nlm.nih.gov/variation/view/) with build CRCh37 was used to retrieve the gene genomic coordinates. These coordinates were used to retrieve SNPs in the corresponding regions of the RS cohorts.

Candidate gene approach

The CGAs were performed in two steps. First, a SNP-based CGA of previously associated PSO and AD SNPs and second, a gene-based CGA to screen for additional variants within the regions of interest.

The SNP-based CGA of seborrheic dermatitis cases and controls was performed using the imputed dosage data of each RS cohort using a logistic regression with an additive model.[4] The output per SNP for each RS cohort was meta-analyzed using p-values derived from a likelihood ratio test as summary statistics.[5]

Best-guessed genotypes were used for the gene-based CGA. The genotypes of the three RS cohorts were estimated from the imputed dosage data using the GCTA software with default parameters.[20]

Gene-based logistic regression was conducted using the set-based test implemented in PLINK v1.90 (parameters: p-value = 0.05, maximum number of SNPs = 15, $r^2 = 0.5$, permutations = 10000).[21] Both SNP- and gene based associations were adjusted for age, sex, and four principal components (PCs). The adjustment for PCs is part of the statistical analysis in GWAS analysis to control for possible population stratification and hidden relatedness.[22] All SNP data were used to calculated the main axes of genomic variation (here PCs- eigenvectors) using the multi-dimensional scaling (MDS) method as implemented in the program Plink.[21] The first four axes of genetic variation calculated for all individuals are expected to account for most of the genetic variation in populations of similar ethnic background, such as the participants from the Rotterdam Study.[3]

Sensitivity analysis

The significant associations found in the SNP-based and gene-based CGA of the psoriasis genes might be driven by the proportion of psoriasis patients. Therefore, we conducted a sensitivity analysis in which we excluded all psoriasis patients and repeated the SNP- and gene based CGAs for associations with a p-value of < 0.05.

Genome-wide association study

To investigate the proportion of variance accounted for by genetic factors, we first calculated heritability of seborrheic dermatitis with the best guess genotype data using the approach of Yang *et al.*, 2010.[23] Furthermore, a GWAS of seborrheic dermatitis cases and controls was performed using the imputed dosage data of each RS cohort using a logistic regression with an additive model. The model was adjusted for age, sex, and four PCs. The GWAS analyses were implemented in the ProbABEL package.[4] The inflation factor λ was estimated between 1.00 and 1.02 for all three cohorts and was not further considered.

Next, the GWAS summary statistics per RS cohort were meta-analyzed. During the quality control of the GWAS results, a difference in p-values was noted between the Wald-test and the likelihood ratio test, which is likely due to the relatively small sample size. As a result, the beta coefficients of the logistic regression might be not reliable. When this happens, it is advised to use the p-value of the likelihood ratio test to test for statistical associations. Therefore, we decided to use the p-values derived from a likelihood ratio test for the meta-analysis of the GWAS per RS cohort.[24] The meta-analysis was performed assuming a fixed-effects model where the p-values of the associations for each allele were converted in a signed Z-score. The Z-scores across the cohorts were combined and weighted to the square root of the sample size for each cohort.[5] The threshold for the p-value of genome wide significance was set at a p-value $\leq 5 \times 10^{-8}$ and p-value $\leq 5 \times 10^{-6}$ was set to be suggestive evidence.

Bioinformatics Analysis

Two regulatory databases were queried to identify possible regulatory elements that overlap the chromosomal location of all SNPs passing genome wide significance. Crossover with the Open Regulatory Annotation database (ORegAnno, 22) was observed using the UCSC Genome Browser.[25] MAPPER 2 was queried directly; using the MAPPER search engine,[26] with subsequent hits visualized using the UCSC Genome Browser. The genomic locations around the SNPs associated with seborrheic dermatitis were further explored using the UCSC genome browser to identify any additional features of interest in their vicinity.

SUPPLEMENTARY RESULTS

Study population

After excluding first-degree relatives and controls who had history of ketoconazole use, we included 736 participants of RS-I, 1,095 participants of RS-II and 2,219 participants of RS-III for the CGA and GWAS of seborrheic dermatitis. Of these, 609 (15%) were diagnosed during a full body skin examination as having seborrheic dermatitis. Males were more likely to have seborrheic dermatitis than females (60.4% of cases vs 41.8% of controls were males; p-value < 0.001) and seborrheic dermatitis patients were older compared to those without seborrheic dermatitis (68.94 vs 67.97, p-value: 0.018). Seborrheic dermatitis cases were more likely to have the diagnosis of PSO than controls (4.8% vs 3.0%; p=0.035; Supplementary Table S1). The heritability of seborrheic dermatitis estimated with our data was 14% (confidence interval: 0-30%, p=0.045).

CGA – Sensitivity analysis

All suggestive associations of PSO SNPs and loci that were found in the CGA (Table 1) remained significant in the sensitivity analysis (p-value <0.05). The *LCE3E-LCE3D del* loci and the SNPs rs3213094 (*IL12B*) and rs280519 (*TYK2*) had p-values of 0.0174, 0.03197, and 0.04949, respectively.

Genome wide association study – Additional bioinformatics Analysis

The MAPPER search engine identified a transcription factor binding site that overlays the chromosomal position of r58331610, which may bind CCAAT/enhancer binding protein (C/EBP). Inspection of the Open Regulatory Annotation Database (ORegAnno)[27] using the UCSC Genome Browser[25] revealed that there is a literature-curated transcription factor binding site that also sits across the genomic location of rs58331610. This transcription factor is SMARCA4 (OReg ID 1274278), also known as BRG1. Further analysis of the genomic region surrounding rs58331610 places the SNP within both an interspersed DNA repeating element and a h3k27ac Histone H3 enhancer mark, and close to a DNase hypersensitivity region (Supplementary Figure S2).

The prediction of a C/EBP binding site overlaying the r58331610 suggests that this variant could modulate inflammatory response signals since C/EBP-β binding sites are particularly found in regulatory sequences of genes that are associated with the inflammatory response. The C/EBPβ-transcription factor regulates IL-17 responsive genes[28] and is expressed preferentially in differentiated keratinocytes.[29] The presence of a binding site for SMARCA4, a "chromatin remodeler" which promotes expression of genes involved in epidermal barrier repair,[30] as well as controlling hair follicle tissue remodeling,[31] suggests a potential role for MAST4 in the maintenance of epidermal integrity.

No transcription factor binding sites were found to overlay the chromosomal location of rs16944244 using the MAPPER and ORegAnno databases.[26,27] A review of the surrounding genomic region using the UCSC Genome Browser shows that rs16944244 is in the middle of two DNase hypersensitivity clusters and within a Long Interspaced Nuclear Element (LINE). This SNP is also within two reported mRNA sequences, JD106968 and KF274610. In addition, there is evidence of transcriptional activity in this region as rs16944244 sits in between two DNase Hypersensitivity regions and within a long interspersed nuclear element.

The two reported human mRNA sequences present at this location, suggesting there may be an unannotated gene close to the associated SNP which will require further investigation. Exploring the role of these loci in seborrheic dermatitis in future studies might bring important insights in the pathogenesis of seborrheic dermatitis.

Supplementary Table S1. Basic characteristics

Characteristics	Case (%)	Control (%)	P-value
n	609 (15.0)	3441(85.0)	
Male	368 (60.4)	1437 (41.8)	< 0.001
Age			
45-55	52 (8.5)	280 (8.1)	
>55-65	181 (29.7)	1221 (35.5)	
>65-75	220 (36.1)	1157 (33.6)	
>75-85	125 (20.5)	639 (18.6)	
>85	31 (5.1)	144 (4.2)	
Mean (SD)[1]	68.9 (9.3)[1]	67.97 (9.6)[1]	0.018
Psoriasis	29 (4.8)	104 (3.0)	0.035

This table includes the participants that remained after quality control.
1: Standard deviation (SD)

Supplementary Table S2. CGA of psoriasis SNPs

SNP	Genes of interest	Chr	Location	A1	A2	Freq	Direction	P value
rs3213094	*IL12B*	5	158750769	t	c	0.1802	---	0.02011
rs280519	*TYK2*	19	10472933	a	g	0.5079	+++	0.03388
rs20541	*IL13*	5	131995964	a	g	0.1938	---	0.07811
rs10789285	*intergenic*	1	69788482	t	g	0.779	---	0.1163
rs12580100	*IKZF4/RPS26*	12	56439209	a	g	0.8757	++-	0.1455
rs240993	*REV3L*	6	111673714	t	c	0.2733	--+	0.2069
rs33980500	*TRAF3IP2*	6	111913262	t	c	0.0717	--+	0.2414
rs7993214	*COG6*	13	40350912	t	c	0.3484	-++	0.2543
rs458017	*REV3L*	6	111696091	t	c	0.9313	+++	0.3084
rs2066808	*STAT2*	12	56737973	a	g	0.9286	+++	0.337
rs4795067	*NOS2*	17	26106675	a	g	0.6474	-++	0.4674
rs27524	*CAST/ERAP1*	5	96101944	a	g	0.3621	+-+	0.4876
rs842636	*REL*	2	61091950	a	g	0.4476	++-	0.4906
rs2546890	*IL21B*	5	158759900	a	g	0.5483	++-	0.4924
rs2675662	*CAMK2G*	10	75599127	a	g	0,5471	-++	0.4958
rs114934997	*intergenic*	5	40370724	a	c	0,0812	++-	0.5033
rs4649203	*IFNLR1*	1	24519920	a	g	0.7242	--+	0.5038
rs702873	*REL*	2	61081542	t	c	0.4419	++-	0.5116
rs12586317	*KIAA0391/MRP63P8/DRPXP3*	14	35682172	t	c	0.7544	--+	0.5272
rs4112788	*LCE3E-LCE3D*	1	152551276	a	g	0.3582	+++	0.571
rs17728338	*TNIP1/ANXA6*	5	150478318	a	g	0.0478	--+	0.6066
rs17716942	*KCNH7*	2	163260691	t	c	0.8597	++-	0.6177
rs4685408	*PLCL2*	3	16996035	a	g	0.536	+-+	0.6323
rs11209026	*IL23R*	1	67705958	a	g	0.068	-++	0.6691
rs7637230	*NFKBIZ*	3	101663555	a	g	0.806	-++	0.6759
rs2235617	*RNF114*	20	48554977	c	G	0.5656	-+-	0.746
rs1076160	*TSC1*	9	135776034	t	c	0.501	--+	0.75
rs6809854	*intergenic*	3	18784423	a	g	0.8043	++-	0.7513
rs1008953	*SDC4/SYS1*	20	43980726	t	c	0.2275	--+	0.7681
rs495337	*SPATA2*	20	48522330	a	g	0.4362	+-+	0.7851
rs8016947	*intergenic*	14	35832666	t	g	0.4398	++-	0.8202
rs12720356	*TYK2*	19	10469975	a	c	0.9341	--+	0.8281
rs2201841	*IL23R*	1	67694202	a	g	0.7139	-+-	0.8444
rs1975974	*intergenic*	17	21707060	a	g	0.7831	++-	0.8926
rs10782001	*FBXL19*	16	30942625	a	g	0.6498	-++	0.9359
rs610604	*TNFAIP3*	6	138199417	t	g	0.6776	-+-	0.9792
rs10484554	*intergenic*	6	31274555	-	-	-		-
rs12191877	*USP8P1/WASF5P*	6	31252925	-	-	-		-
rs2395029	*HLA-X*	6	31431780	-	-	-		-
rs3134792	*HLA-B*	6	31312326	-	-	-		-

*: No significant results when applying Bonferroni-correction, –: Not available in dataset. Chr: Chromosome. A1: reference allele. A2: other allele. Freq: frequency of reference allele. Direction: Effect direction for each cohort (RS-I, RS-II and RS-III).

Supplementary Table S3. CGA of atopic dermatitis SNPs

SNP	Genes of interest	Chr	Location	A1	A2	Freq	Direction	P value
rs61813875	*LCE3E*	1	152536650	c	g	0.9771	---	0.008693
rs7512552	*C1orf51/MRPS21*	1	150265704	t	c	0.4995	+++	0.04366
rs2212434	*C11orf30/LRRC32*	11	76281593	t	c	0.4367	+++	0.06397
rs7127307	*intergenic*	11	128187383	t	c	0.5223	+++	0.1308
rs10791824	*OVOL1*	11	65559266	a	g	0.4403	+++	0.1553
rs6419573	*IL18R1/IL18RAP*	2	103027103	t	c	0.2376	++-	0.1554
rs2944542	*ZNF365*	10	64369999	c	g	0.3862	-++	0.1935
rs2038255	*PPP2R3C*	14	35559126	t	c	0.1724	---	0.1999
rs10214237	*IL7R*	5	35883734	t	c	0.7152	+++	0.2237
rs112111458	*CD207/VAX2*	2	71100105	a	g	0.8739	---	0.2496
rs4312054	*NLRP10*	11	7977161	t	g	0.5985	-+-	0.2618
rs2592555	*PRR5L*	11	36371757	t	c	0.7092	+++	0.2657
rs6473227	*intergenic*	8	81285892	a	c	0.64	++-	0.2669
rs4713555	*HLA-DRB1*	6	32575524	t	g	0.271	-+-	0.3444
rs7625909	*SFMBT1*	3	53091164	t	c	0.3189	+++	0.407
rs2041733	*CLEC16A*	16	11229589	t	c	0.4489	-+-	0.4407
rs6602364	*IL2RA*	10	6038853	c	g	0.5816	++-	0.4671
rs4643526	*PUS10*	2	61184651	a	g	0.1731	---	0.4745
rs10199605	*LINC00299*	2	8495097	a	g	0.326	++-	0.646
rs2918307	*ACTL9*	19	8789722	a	g	0.8488	-+-	0.6531
rs2228145	*IL6R*	1	154426970	a	c	0.6084	+-+	0.7749
rs1249910	*CCDC80*	3	112391174	a	g	0.3219	+-+	0.8034
rs6827756	*KIAA1109*	4	123184411	t	c	0.383	+--	0.8744
rs12188917	*RAD50/IL13*	5	131991085	t	c	0.8045	--+	0.9412
rs4809219	*RTEL1*	20	62303115	a	c	0.7396	+-+	0.9414
rs145809981	*MICB*	6	31466217	t	c	0.1232	-++	0.9425
rs12951971	*STAT3*	17	40528131	t	g	0.8998	++-	0.9752

*: No significant results when applying Bonferroni-correction. Chr: Chromosome. A1: reference allele. A2: other allele. Freq: frequency of reference allele. Direction: Effect direction for each cohort (RS-I, RS-II and RS-III).

Supplementary Table S4. CGA of atopic dermatitis loci.

Reported SNP	Loci	Chr	Start	End	SNPs/set	Significant SNPs	Independent significant SNPs	P value
rs145809981	*MICB*	6	31447054	31493901	408	18	2	0.006299
rs4809219	*RTEL1*	20	62274163	62345051	252	7	3	0.0162
rs61813875	*LCE3E*	1	152523175	152554229	97	2	2	0.0192
rs112111458	*intergenic*	2	71085105	71115105	108	42	4	0.0225
rs7512552	*C1orf51/ MRPS21*	1	150250704	150280704	91	6	2	0.024
rs2228145	*IL6R*	1	154362669	154456926	221	7	3	0.1421
rs2212434	*intergenic*	11	76266593	76296593	126	8	2	0.1589
rs6419573	*IL18R1/ IL18RAP*	2	103012103	103042103	115	23	3	0.1788
rs10791824	*OVOL1*	11	65539505	65579690	128	26	8	0.2066
rs10199605	*intergenic*	2	8480097	8510097	95	3	2	0.2522
rs2944542	*ZNF365*	10	64118916	64446771	1117	71	15	0.2877
rs2918307	*intergenic*	19	8774722	8804722	113	1	1	0.2966
rs12951971	*STAT3*	17	40450342	40555586	233	1	1	0.3895
rs6602364	*IL2RA*	10	6037657	6119333	388	13	9	0.4047
rs2592555	*PRR5L*	11	36302725	36501754	848	59	12	0.5098
rs2041733	*CLEC16A*	16	11023345	11291046	816	29	11	0.6155
rs7127307	*intergenic*	11	128172383	128202383	89	8	3	0.6421
rs10214237	*IL7R*	5	35841977	35894705	205	1	1	0.7087
rs4643526	*PUS10*	2	61152548	61260365	199	2	1	0.9024
rs6827756	*KIAA1109*	4	123058488	123298914	510	2	1	0.9407
rs4713555	*intergenic*	6	32560524	32590524	65	0	0	1
rs12188917	*RAD50/IL13*	5	131976085	132006085	48	0	0	1
rs1249910	*intergenic*	3	112376174	112406174	65	0	0	1
rs2038255	*PPP2R3C*	14	35539678	35606868	266	0	0	1
rs6473227	*intergenic*	8	81270892	81300892	115	0	0	1
rs7625909	*SFMBT1*	3	52918221	53095089	436	0	0	1
rs4312054	*NLRP10*	11	7966156	8000059	104	0	0	1

Loci: Intergenic if the SNP was more than 15 MB away from a gene. Chr: Chromosome. Significant SNPs: Total number of SNPs with p-value < 0.05. Independent significant SNPs: Total number of significant SNPs (p-value < 0.05) also passing LD-criterion (r2 < 0.5). P value: No significant results when applying Bonferroni-correction.

Supplementary Table S5. CGA of psoriasis loci.

Reported SNP	Loci	Chr[2]	Start	End	SNPs /set	Significant SNPs[3]	Independent significant SNPs[4]	P value[5]
rs4112788	*LCE3E-LCE3D*	1	152536276	152566276	37	2	2	0.0157
rs12580100	*IKZF4,RPS26*	12	56424209	56454209	40	11	2	0.078
rs3134792	*HLA-B*	6	31306649	31339989	24	6	1	0.1383
rs2546890/ rs3213094	*IL12B*	5	158726790	158772481	101	25	5	0.1397
rs4649203	*IFNLR1*	1	24465646	24528765	249	43	11	0.1438
rs4685408	*PLCL2*	3	16959582	17147098	478	7	3	0.216
rs10789285	*Intergenic*[1]	1	69773482	69803482	74	4	2	0.2268
rs1076160	*TSC1*	9	135751735	135835094	184	1	1	0.2438
rs12586317	*KIAA0391/ MRP63P8/ DRPXP3*	14	35667172	35697172	100	4	1	0.2857
rs33980500	*TRAF3IP2*	6	111861581	111942477	248	9	1	0.3504
rs7993214	*COG6*	13	40214763	40380802	528	91	6	0.3849
rs280519/ rs12720356	*TYK2*	19	10446204	10506248	195	24	7	0.3912
rs1008953	*SDC4/SYS1*	20	43965726	43995726	80	4	1	0.3997
rs2675662	*CAMK2G*	10	75557259	75649349	128	1	1	0.4269
rs240993	*REV3L*	6	111605234	111819421	518	25	1	0.4543
rs458017	*REV3L*	6	111605234	111819421	518	25	1	0.4543
rs8016947	*Intergenic*[1]	14	35817666	35847666	183	7	3	0.4675
rs2235617	*RNF114*	20	48537914	48585422	174	6	1	0.5235
rs610604	*TNFAIP3*	6	138173325	138219451	81	3	1	0.5559
rs4795067	*NOS2*	17	26068791	26142555	241	25	5	0.576
rs11209026/ rs2201841	*IL23R*	1	67589590	67740662	450	51	7	0.5893
rs114934997	*Intergenic*[1]		40355724	40385724	105	1	1	0.6297
rs27524	*CAST/ERAP1*	5	96086944	96116944	137	1	1	0.673
rs17728338	*TNIP1/ANXA6*	5	150463318	150493318	139	3	2	0.7066
rs7637230	*NFKBIZ*	3	101644703	101731770	303	12	4	0.734
rs17716942	*KCNH7*	2	163212917	163710257	948	1	1	0.9801
rs495337	*SPATA2*	12	56720381	56769058	49	0	0	1
rs20541	*IL13*	5	131978865	132011801	56	0	0	1
rs6809854	*Intergenic*[1]	3	18769423	18799423	119	0	0	1
rs842636	*Intergenic*[1]	2	61076950	61106950	71	0	0	1
rs702873	*Intergenic*[1]	2	61066542	61096542	79	0	0	1
rs10782001	*FBXL19*	16	30919392	30975104	55	0	0	1
rs1975974	*Intergenic*[1]	17	21692060	21722060	31	0	0	1
rs2066808	*STAT2*	12	56720381	56769058	49	0	0	1
rs12191877	*USP8P1/WASF5P*	6	31237925	31267925	0	0	0	-

Supplementary Table S5. CGA of psoriasis loci. (continued)

Reported SNP	Loci	Chr[2]	Start	End	SNPs/set	Significant SNPs[3]	Independent significant SNPs[4]	P value[5]
rs10484554	*Intergenic*[1]	6	31259555	31289555	0	0	0	-
rs2395029	*HLA-X*	6	31414623	31445267	0	0	0	-

Intergenic if the SNP was more than 15 MB away from a gene. 2: Chromosome. 3: Total number of SNPs with p-value < 0.05. 4: Total number of significant SNPs (p-value < 0.05) also passing LD-criterion (r2 < 0.5). 5: just downstream of *REL*. 5: No significant results when applying Bonferroni-correction.

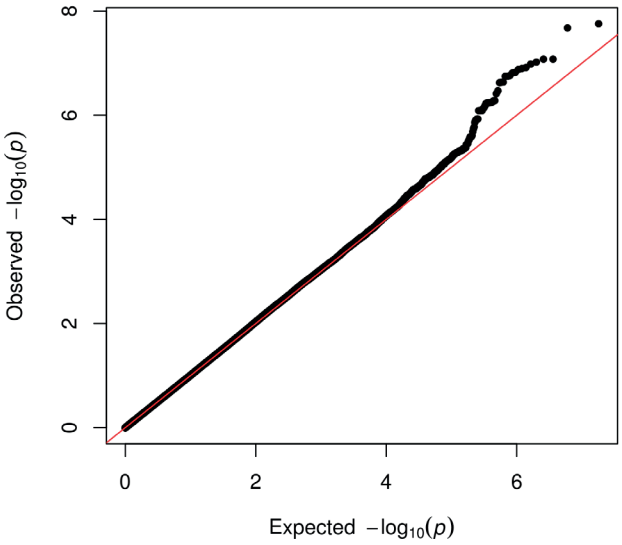
Supplementary Table S6. SNPs of interest ($p < 5 \times 10^{-6}$)

Genes of interest[1]	SNP	Chr[2]	Location	A1	A2	Freq	P value
MAST4	rs58331610	5	66020457	a	g	0.8615	1.75×10^{-8}*
Pirt/SHISA6 +	rs16944244	17	11026693	c	g	0.9796	2.10×10^{-8}*
Pirt/SHISA6 +	rs78160483	17	11026297	t	g	0.9818	8.38×10^{-8}
Pirt/SHISA6 +	rs16944241	17	11026414	a	g	0.9818	8.38×10^{-8}
TACR1/EVA1A +	rs6546997	2	75658780	t	c	0.1894	9.53×10^{-8}
Pirt/SHISA6 +	rs8075550	17	11027059	a	g	0.9594	1.04×10^{-7}
Pirt/SHISA6 +	rs11870758	17	11015902	a	t	0.9722	1.21×10^{-7}
MAST4	rs12188593	5	66030069	t	c	0.1359	1.27×10^{-7}
Pirt/SHISA6 +	rs182131544	17	11023964	a	g	0.9788	1.32×10^{-7}
Pirt/SHISA6 +	rs111448323	17	11022815	a	g	0.9786	1.50×10^{-7}
Pirt/SHISA6 +	rs4791451	17	11027570	c	g	0.9808	1.54×10^{-7}
Pirt/SHISA6 +	rs77699632	17	11020937	a	t	0.0214	1.73×10^{-7}
Pirt/SHISA6 +	rs111341744	17	11021220	a	g	0.9784	1.79×10^{-7}
Pirt/SHISA6 +	rs75205996	17	11021311	t	c	0.9784	1.79×10^{-7}
Pirt/SHISA6 +	rs58692658	17	11019423	t	c	0.022	2.32×10^{-7}
Pirt/SHISA6 +	rs77592872	17	11018841	a	t	0.9781	2.34×10^{-7}
Pirt/SHISA6 +	rs138424360	17	11018089	a	g	0.0219	2.37×10^{-7}
Pirt/SHISA6 +	rs80298958	17	11016351	c	g	0.9784	3.39×10^{-7}
Pirt/SHISA6 +	rs76730906	17	11019589	t	g	0.9754	3.85×10^{-7}
GRM3	rs6978155	7	86477894	a	t	0.0543	5.24×10^{-7}
GRM3	rs6957842	7	86477870	a	t	0.9457	5.28×10^{-7}
GRM3	7:86475369:I	7	86475369	d	i	0.9459	5.63×10^{-7}
GRM3	rs6960053	7	86473063	a	g	0.0541	5.68×10^{-7}
GRM3	rs6974507	7	86472032	t	g	0.9459	5.75×10^{-7}
GRM3	rs7801589	7	86470943	t	c	0.9459	5.76×10^{-7}
GRM3	rs6465087	7	86470488	t	c	0.9459	5.78×10^{-7}
GRM3	rs6954573	7	86472161	a	t	0.9455	5.86×10^{-7}
KIAA1324L	rs73382367	7	86525065	a	g	0.9436	6.51×10^{-7}
KIAA1324L	rs6465089	7	86564795	t	c	0.0563	7.17×10^{-7}
GRM3	7:86478749:I	7	86478749	d	i	0.9453	7.44×10^{-7}
GRM3	rs6947778	7	86476124	c	g	0.0545	8.11×10^{-7}
GRM3	rs6967992	7	86476311	t	c	0.9455	8.11×10^{-7}
GRM3	rs6955565	7	86472484	a	g	0.0545	8.12×10^{-7}
GRM3	rs6955452	7	86472584	c	g	0.9455	8.15×10^{-7}
GRM3	rs6955917	7	86472763	a	g	0.0545	8.15×10^{-7}
KIAA1324L	rs6958716	7	86594383	t	c	0.943	1.18×10^{-7}
KIAA1324L	rs6977048	7	86594543	t	g	0.057	1.18×10^{-6}
KIAA1324L	rs7801050	7	86634828	a	g	0.0553	1.21×10^{-6}
MAST4	5:66029161:D	5	66029161	d	i	0.1198	1.25×10^{-6}
GJB +	rs4308942	1	35100636	a	g	0.0559	1.37×10^{-6}

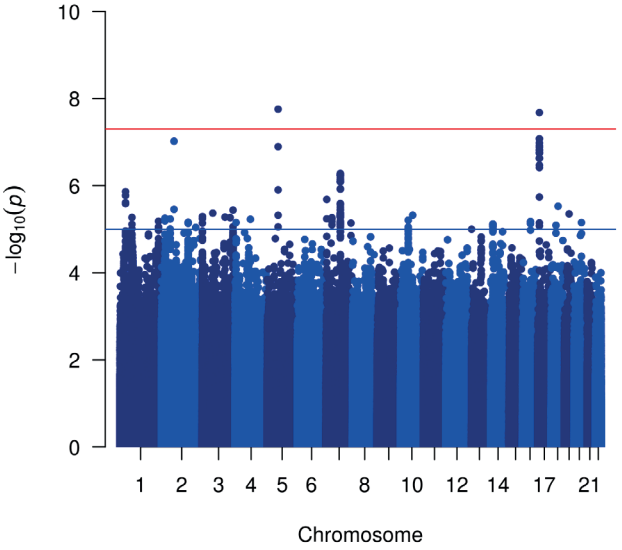
Supplementary Table S6. SNPs of interest ($p < 5 \times 10^{-6}$) (continued)

Genes of interest[1]	SNP	Chr[2]	Location	A1	A2	Freq	P value
GJB +	rs4390141	1	35100635	t	c	0.9448	1.68×10^{-6}
Pirt/SHISA6	17:11013544:I	17	11013544	d	i	0.9715	1.83×10^{-6}
GRM3	rs6463427	7	5403213	t	c	0.3735	2.07×10^{-6}
GJB +	1:35117047:D	1	35117047	d	i	0.0657	2.45×10^{-6}
KIAA1324L	rs7781922	7	86506679	t	c	0.054	2.58×10^{-6}
KIAA1324L	rs75714543	7	86506726	a	t	0.946	2.58×10^{-6}
GJB +	1:35117072:D	1	35117072	d	i	0.0651	2.59×10^{-6}
KIAA1324L	rs1999947	7	86593170	t	c	0.0698	2.77×10^{-6}
Intergenic	rs10502822	18	41494417	t	g	0.9569	2.97×10^{-6}
KIAA1324L	rs1999946	7	86593299	t	c	0.0701	3.13×10^{-6}
TACR1/EVA1A+	rs7563188	2	75658832	t	c	0.1497	3.49×10^{-6}
KIAA1324L	7:86593557:D	7	86593557	d	i	0.0738	3.50×10^{-6}
SENP2	rs13081203	3	185322643	a	g	0.3249	3.64×10^{-6}
GRM3	rs10263372	7	86486846	c	g	0.9174	3.67×10^{-6}
KIAA1324L	rs73384121	7	86606081	a	g	0.0734	4.19×10^{-6}
PRICKLE2	rs704417	3	64252424	t	c	0.4945	4.28×10^{-6}
KIAA1324L	rs73384108	7	86587659	t	c	0.0724	4.34×10^{-6}
KIAA1324L	rs73384111	7	86587766	t	c	0.0724	4.35×10^{-6}
KIAA1324L	rs1029367	7	86544248	t	c	0.0852	4.39×10^{-6}
KIAA1324L	rs73384112	7	86587902	t	c	0.0725	4.40×10^{-6}
Intergenic	rs7250483	19	29490697	a	g	0.0395	4.45×10^{-6}
KIAA1324L	rs61699848	7	86589789	a	g	0.0726	4.72×10^{-6}
Intergenic	rs11597510	10	72729492	t	c	0.7589	4.75×10^{-6}
MAST4	rs6860389	5	66022151	a	g	0.8707	4.77×10^{-6}
KIAA1324L	rs73384113	7	86588338	a	g	0.0726	4.78×10^{-6}
GRM3	rs28479466	7	86491021	a	t	0.9159	4.82×10^{-6}
GRM3	rs28755802	7	86491029	a	g	0.0841	4.82×10^{-6}
GRM3	rs7808623	7	86490154	t	g	0.9157	4.84×10^{-6}
GRM3	rs17161067	7	86490264	t	c	0.9159	4.86×10^{-6}
KIAA1324L	rs1999945	7	86593390	c	g	0.0727	4.92×10^{-6}

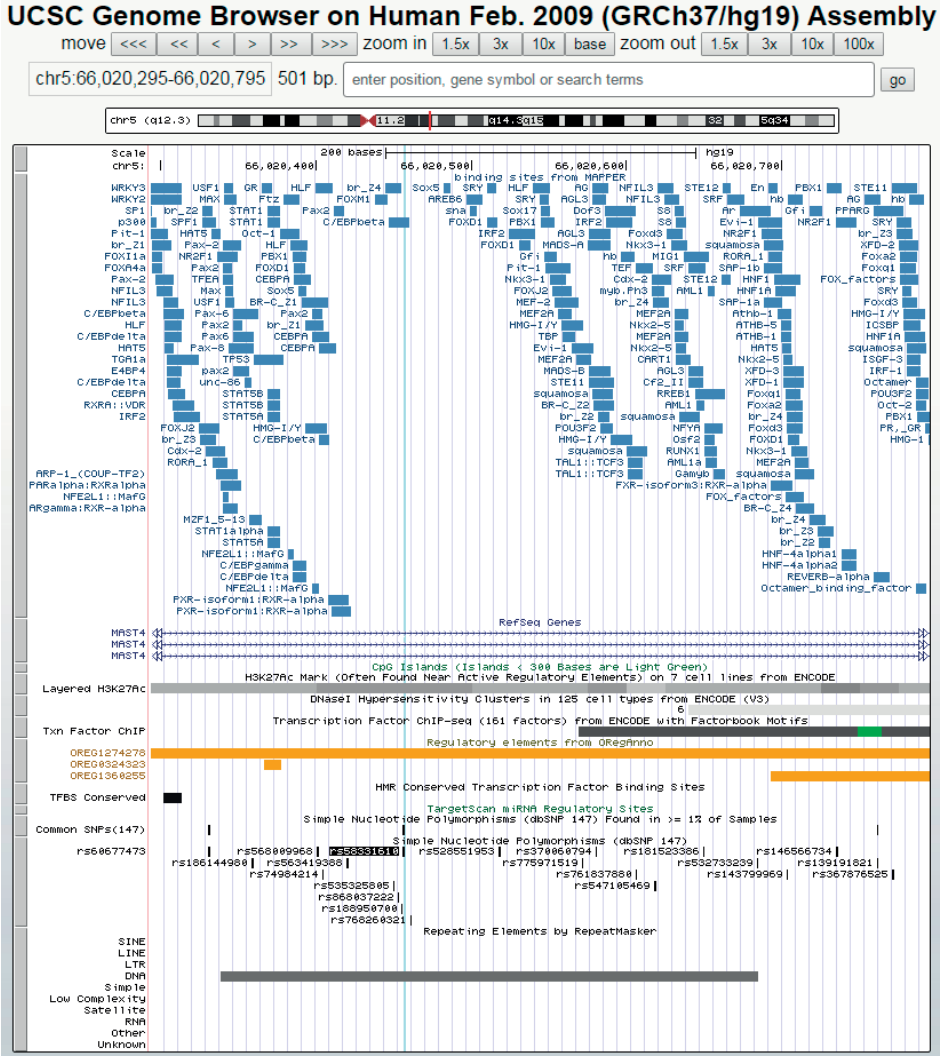
2: Chromosome, *: Genome-wide significant ($< 5 \times 10^{-8}$), +: Intergenic, A1: reference allele, A2: other allele, Freq: frequency of reference allele. The p-values were calculated by meta-analysis of the output per SNP for each RS cohort using p-values derived from a likelihood ratio test as summary statistics.



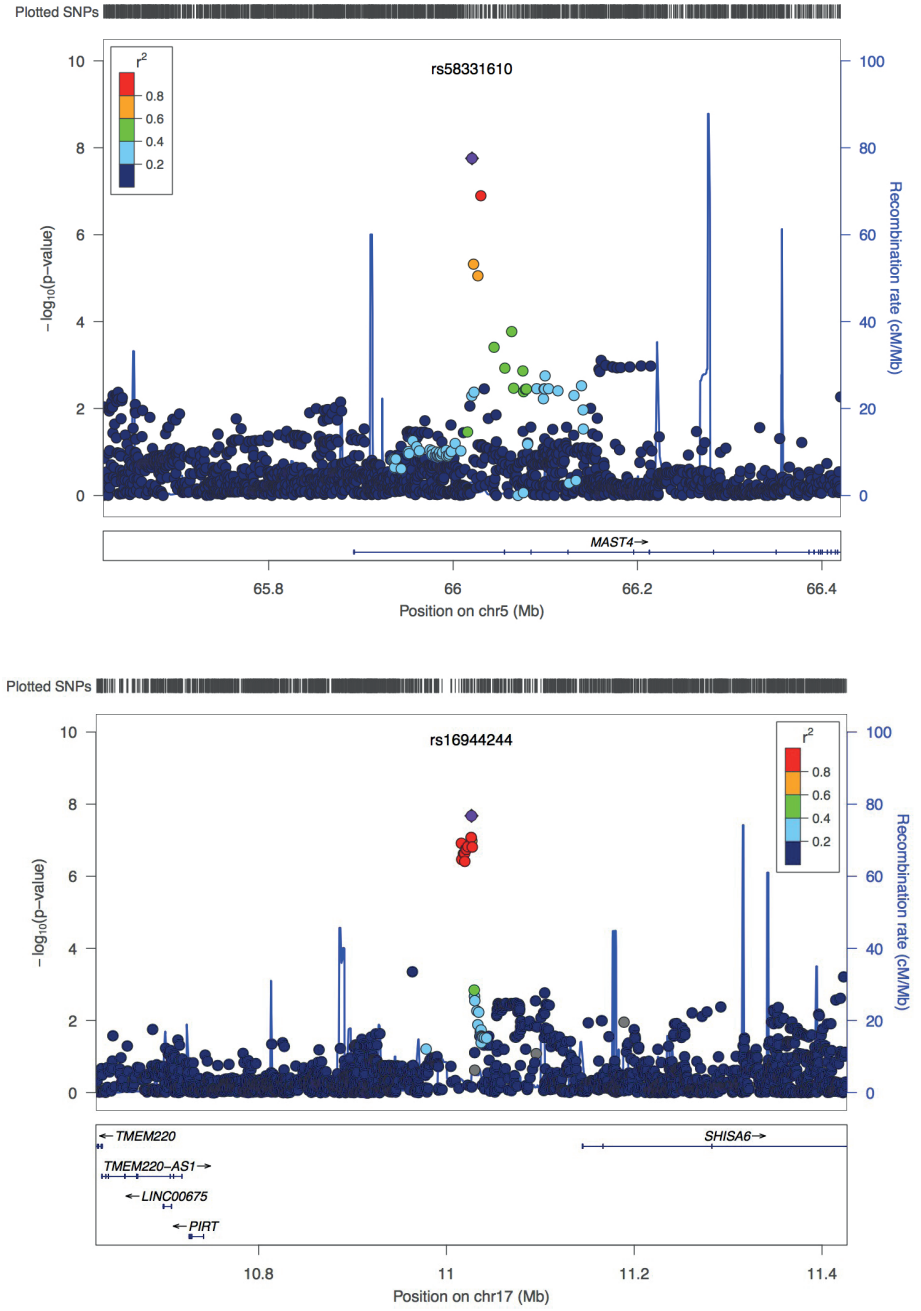
Supplementary Figure S1a. QQ-plot. Plot of the expected -log10 p-values (X-axis) and the observed -log10 p-values (Y-axis).



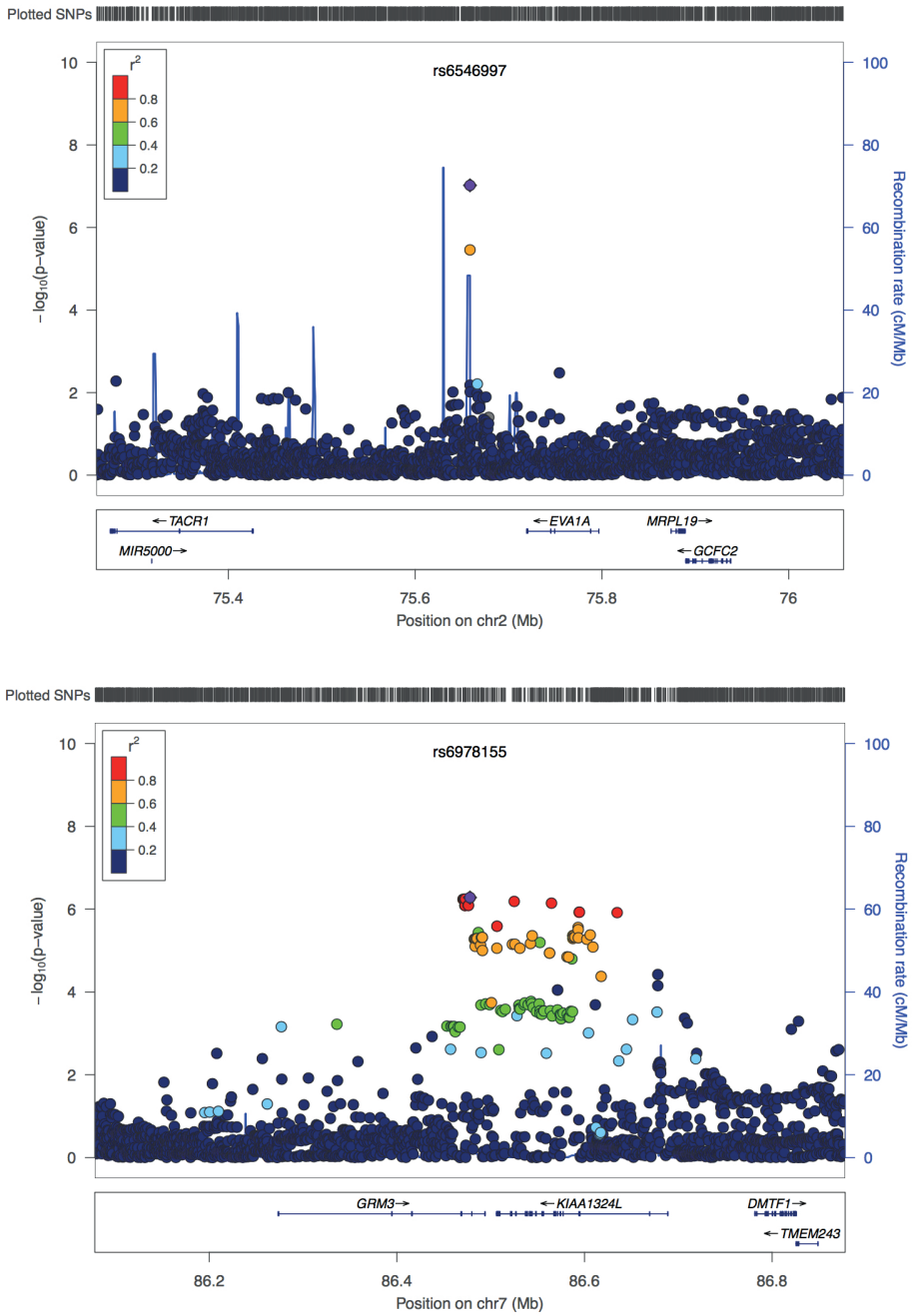
Supplementary Figure S1b. Manhattan plot of the GWAS associations. The observed -log10 p-values (Y-axis) of the association between the SNPs and susceptibility for seborrheic dermatitis are shown. All SNP are represented by dots and displayed per chromosome (X-axis).
Red line: Genome-wide significant (pvalue = 5 x 10-8)
Blue line: Genome-wide suggestive (pvalue = 5 x 10-6)



Supplementary Figure S2. Screenshot of UCSC Genome Browser showing regulatory annotation in a 501bp region around SNP rs58331610 on chr 5 (hg19 assembly). Light blue vertical line shows location of the SNP, which can be seen to be within the ORegAnno element OREG1274278 and a C/EBPbeta binding site as predicted by MAPPER. The SNP can also be seen to be within a LINE regulatory element and a h3k27ac Histone H3 enhancer mark.



Supplementary Figure S3. *(continued on next page)*



Supplementary Figure S3. Regional plot of the most significant associations between SNPs and seborrheic dermatitis. The plots represents the LD patterns of the most significant SNPs. The pairwise r2 is represented in colors. The log p-values of the associations are presented in the left Y-axis and the recombination rates are presented in the right Y-axis. The physical position of the markers are presented are presented on the X-axis. The figures was generated using LocusZoom (Pruim et al., 2010)

REFERENCES

1 Paternoster L, Standl M, Waage J *et al.* Multi-ancestry genome-wide association study of 21,000 cases and 95,000 controls identifies new risk loci for atopic dermatitis. *Nat Genet* 2015.

2 Tsoi LC, Stuart PE, Tian C *et al.* Large scale meta-analysis characterizes genetic architecture for common psoriasis associated variants. *Nat Commun* 2017; **8**: 15382.

3 Hofman A, Brusselle GG, Darwish Murad S *et al.* The Rotterdam Study: 2016 objectives and design update. *Eur J Epidemiol* 2015; **30**: 661-708.

4 Aulchenko YS, Struchalin MV, van Duijn CM. ProbABEL package for genome-wide association analysis of imputed data. *BMC Bioinformatics* 2010; **11**: 134.

5 Willer CJ, Li Y, Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. *Bioinformatics* 2010; **26**: 2190-1.

6 Sun L, Gu S, Li X *et al.* Identification of a novel human MAST4 gene, a new member of the microtubule associated serine-threonine kinase family. *Mol Biol (Mosk)* 2006; **40**: 808-15.

7 Garza LA, Yang CC, Zhao T *et al.* Bald scalp in men with androgenetic alopecia retains hair follicle stem cells but lacks CD200-rich and CD34-positive hair follicle progenitor cells. *J Clin Invest* 2011; **121**: 613-22.

8 Bidaux G, Borowiec AS, Gordienko D *et al.* Epidermal TRPM8 channel isoform controls the balance between keratinocyte proliferation and differentiation in a cold-dependent manner. *Proc Natl Acad Sci U S A* 2015; **112**: E3345-54.

9 Patel KN, Liu Q, Meeker S *et al.* Pirt, a TRPV1 modulator, is required for histamine-dependent and -independent itch. *PLoS One* 2011; **6**: e20559.

10 Tang Z, Kim A, Masuch T *et al.* Pirt functions as an endogenous regulator of TRPM8. *Nat Commun* 2013; **4**: 2179.

11 Clarke GM, Carter KW, Palmer LJ *et al.* Fine mapping versus replication in whole-genome association studies. *Am J Hum Genet* 2007; **81**: 995-1005.

12 Howie B, Fuchsberger C, Stephens M *et al.* Fast and accurate genotype imputation in genome-wide association studies through pre-phasing. *Nat Genet* 2012; **44**: 955-9.

13 Capon F, Bijlmakers MJ, Wolf N *et al.* Identification of ZNF313/RNF114 as a novel psoriasis susceptibility gene. *Hum Mol Genet* 2008; **17**: 1938-45.

14 Liu Y, Helms C, Liao W *et al.* A genome-wide association study of psoriasis and psoriatic arthritis identifies new disease loci. *PLoS Genet* 2008; **4**: e1000041.

15 Nair RP, Duffin KC, Helms C *et al.* Genome-wide scan reveals association of psoriasis with IL-23 and NF-kappaB pathways. *Nat Genet* 2009; **41**: 199-204.

16 Ellinghaus E, Ellinghaus D, Stuart PE *et al.* Genome-wide association study identifies a psoriasis susceptibility locus at TRAF3IP2. *Nat Genet* 2010; **42**: 991-5.

17 Strange A, Capon F, Spencer CC *et al.* A genome-wide association study identifies new psoriasis susceptibility loci and an interaction between HLA-C and ERAP1. *Nat Genet* 2010; **42**: 985-90.

18 Stuart PE, Nair RP, Ellinghaus E *et al.* Genome-wide association analysis identifies three psoriasis susceptibility loci. *Nat Genet* 2010; **42**: 1000-4.

19 Tsoi LC, Spain SL, Ellinghaus E *et al.* Enhanced meta-analysis and replication studies identify five new psoriasis susceptibility loci. *Nat Commun* 2015; **6**: 7001.

20 Yang J, Lee SH, Goddard ME *et al.* GCTA: a tool for genome-wide complex trait analysis. *Am J Hum Genet* 2011; **88**: 76-82.

21 Purcell S, Neale B, Todd-Brown K *et al.* PLINK: a tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 2007; **81**: 559-75.

22 Price AL, Patterson NJ, Plenge RM *et al.* Principal components analysis corrects for stratification in genome-wide association studies. *Nat Genet* 2006; **38**: 904-9.
23 Yang J, Benyamin B, McEvoy BP *et al.* Common SNPs explain a large proportion of the heritability for human height. *Nat Genet* 2010; **42**: 565-9.
24 Xing G, Lin CY, Wooding SP *et al.* Blindly using Wald's test can miss rare disease-causal variants in case-control association studies. *Ann Hum Genet* 2012; **76**: 168-77.
25 Kent WJ, Sugnet CW, Furey TS *et al.* The human genome browser at UCSC. *Genome Res* 2002; **12**: 996-1006.
26 Marinescu VD, Kohane IS, Riva A. MAPPER: a search engine for the computational identification of putative transcription factor binding sites in multiple genomes. *BMC Bioinformatics* 2005; **6**: 79.
27 Lesurf R, Cotto KC, Wang G *et al.* ORegAnno 3.0: a community-driven resource for curated regulatory annotation. *Nucleic Acids Res* 2016; **44**: D126-32.
28 Shen F, Hu Z, Goswami J *et al.* Identification of common transcriptional regulatory elements in interleukin-17 target genes. *J Biol Chem* 2006; **281**: 24138-48.
29 Antonini D, Sirico A, Aberdam E *et al.* A composite enhancer regulates p63 gene expression in epidermal morphogenesis and in keratinocyte differentiation by multiple mechanisms. *Nucleic Acids Res* 2015; **43**: 862-74.
30 Botchkarev VA. Epigenetic Regulation of Epidermal Development and Keratinocyte Differentiation. *J Investig Dermatol Symp Proc* 2015; **17**: 18-9.
31 Xiong Y, Li W, Shang C *et al.* Brg1 governs a positive feedback circuit in the hair follicle for tissue regeneration and repair. *Dev Cell* 2013; **25**: 169-81.

5 | Composition of cutaneous bacterial microbiome in seborrheic dermatitis: a cross-sectional study

Martijn G H Sanders
Tamar Nijsten
Joost Verlouw
Robert Kraaij
Luba M Pardo

Accepted, PLOS ONE

ABSTRACT

Background Seborrheic dermatitis is a chronic inflammatory skin disease with a multifactorial etiology. *Malassezia* yeasts have been associated with the disease but the role of bacterial composition in seborrheic dermatitis has not been thoroughly investigated.

Objectives To profile the bacterial microbiome of seborrheic dermatitis patients and to compare this with the microbiome of individuals with no inflammatory skin disease (controls).

Methods This was a cross sectional study embedded in a population-based study. Skin swabs were taken from naso-labial fold from patients with seborrheic dermatitis (lesional skin: n=22; non-lesional skin seborrheic dermatitis: n=75) and controls (n=465). Sample collection began in 2016 at the research facility and is still ongoing. Shannon and Chao1 α- diversity metrics were calculated per group. Associations between the microbiome composition of cases and controls was calculated using multivariate statistics (permANOVA) and univariate statistics.

Results We found an increased α-diversity between seborrheic dermatitis lesional cases versus controls (Shannon diversity: Kruskal-Wallis rank sum: Chi-squared: 19.06; global p-value= 7.7×10^{-5}). Multivariate statistical analysis showed significant associations in microbiome composition when comparing lesional seborrheic dermatitis skin to controls (p-value= 0.03;R^2=0.1%). Seven out of 13 amplicon sequence variants (ASVs) that were significantly different between controls and lesional cases were members of the genus *Staphylococcus*, most of which showed increased composition in lesional cases, and were closely related to *S. capitis S. caprae* and *S. epidermidis.*

Conclusion Microbiome composition differs in patients with seborrheic dermatitis and individuals without diseases. Differences were mainly found in the genus *Staphylococcus*.

INTRODUCTION

Seborrheic dermatitis is a common chronic relapsing inflammatory skin disease occurring mainly on the sebum-rich areas of the face, scalp and chest. The disease spectrum is heterogeneous ranging from a mild form of scaling confined to the scalp to severe erythema and scaling of the scalp, face and trunk. The etiology of seborrheic dermatitis is complex. Host factors such as skin barrier dysfunction, genetic susceptibility and immune status may all influence disease risk.[1-3] Also, several life style and environmental factors increase risk for seborrheic dermatitis. In recent epidemiological studies this has been shown for male gender, white skin colour, winter climate, generalized dry skin and specific diets.[4,5]

Since the condition was first described in 1887,[6] it is often assumed that overgrowth of species of the genus *Malassezia* cause and maintain seborrheic dermatitis. The *Malassezia* yeast are more abundant on affected skin, and treatments targeting this yeast (e.g. ketoconazole) can effectively treat seborrheic dermatitis.[7] However, the genus *Malassezia* is also part of the healthy microbiome and similar species of *Malassezia* in seborrheic dermatitis patients and controls have been found. There might be a causal relationship between *Malassezia* and seborrheic dermatitis, but it is not the only factor.[8] A few more recent studies have shown that next to a higher abundances of *Malassezia* yeast, seborrheic dermatitis patient might also have bacterial dysbiosis on the scalp.[9,10] One other study investigated the microbiome on the face of 24 patients with seborrheic dermatitis and found a higher density of *Acinetobacter, Staphylococcus* and *Streptococcus* in lesional skin when compared with non-lesion skin.[11] As this study did not include healthy controls and these bacteria are also part of the normal skin microbiome, it is not clear whether they are indeed associated with seborrheic dermatitis. Also, members of the *Staphylococcus* genus can act as pathogens and thus it would be of interest to investigate which specific species are relevant in seborrheic dermatitis. In addition, as for any observational studies, initial findings need to be replicated in an independent cohort.

As stated above, most studies have focused on the role of *Malassezia* or were based on small series of cases with no controls, which are necessary to control for 'normal' variation in the microbiome. Here we profiled the bacterial microbiome of the nasolabial fold of participants with seborrheic dermatitis in a middle aged and elderly population based study in the Netherlands (the Rotterdam Study) and compared this to the same skin areas of individuals without seborrheic dermatitis. By using exact amplicon sequence variants (ASVs) instead of operational taxonomic units (OTUs), we were able to zoom in at species level and made our results easier to compare.[12]

MATERIALS AND METHODS

Study participants and sample collection

This is a cross-sectional study conducted within the Rotterdam Study. The Rotterdam Study started in 1990 and is an ongoing prospective population based cohort study in a middle aged and elderly population in the Ommoord district of Rotterdam, The Netherlands. All residents aged 45 years and above were invited to participate. At this point, the Rotterdam Study compromises over 15,000 subjects of predominantly North-European descent. Details of the study have been published before.[13] In 2016, collection of skin swabs of the nasolabial fold were introduced and are still ongoing. Participants were not allowed to use any skincare products on the day of the examination. Participants that did use skincare products were excluded (n=108). Participants who used antibiotics in the past 6 months were also excluded (n=126). Data collection for this research included participants with swabs until April 2018.

Seborrheic dermatitis was diagnosed by dermatology-trained physician during a full body skin examination. The seborrheic dermatitis diagnosis was based on a greasy scaling, erythema and a characteristic distribution in areas rich in sebaceous glands. Since the microbial swabs were taken from the nasolabial folds in the Rotterdam Study, participants with seborrheic dermatitis and involvement of the nasolabial fold were considered lesional cases. Participants with seborrheic dermatitis, but without involvement of the nasolabial fold were considered non-lesional cases. Participants in the Rotterdam Study without seborrheic dermatitis or any other skin diseases were considered as controls. No sample size calculation was performed.

Swab collection, DNA extraction and 16S rRNA gene polymerase chain reaction amplification and sequencing

A cotton wool swab, pre-moistened with three drops of 0.9% NaCl fluid, was rubbed forth and back, in parallel position along the nasolabial fold (50 times, during 30 seconds). Samples were stored at -20 °C for a maximum period of three hours and then at -140 °C before processing. Negative swabs (air swabs) were taken for quality control of the sequencing.

DNA was extracted using the DNA Extraction Kit on the Arrow pipetting instrument (DiaSorin S.P.A., Saluggia, Italy). Swabs were treated with DNA Pre-treatment Buffer 2 and Proteinase K for 30 minutes at 56°C, followed by DNA isolation on the Arrow instrument according to the manufacturer's protocol in batches of 12 samples per run. DNA concentration was measured using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Waltham, MA) and DNA was stored at -20°C.

The V1 to V3 variable regions of the 16S rRNA gene were amplified using the 27F-519R primer pair and dual indexing as previously described.[14,15] Amplicons were normalized and

pooled in batches (total number of swabs per sequencing run: 300). The pools were purified using Agencourt AMPure XP (Beckman Coulter Life Science, Indianapolis, IN) and the quantity of the pool was assessed using the Quant-iT PicoGreen dsDNA Assay Kit. PhiX Control v3 library (Illumina Inc., San Diego, CA) was spiked into (~10%) the pool prior to sequencing on an Illumina MiSeq sequencer (MiSeq Reagent Kit v3, 2 x 300 bp). Samples were sequenced in three batches.

Bioinformatic analysis

Raw reads from Illumina MiSeq were demultiplexed using a custom script to separate sample fastq files based on the dual index. Primers, barcodes and heterogeneity spacers were trimmed off using tagcleaner v0.16.[16] Trimmed fastq files were further processed using the DADA2 pipeline.[17] In contrast to standard clustering of reads that cluster sequence reads based on a similarity threshold (usually 97%), and assign them to taxa to generate operational taxonomic units (OTUs), DADA2 allows to analyse exact amplicon sequence variants (ASVs) as the unit of analysis without imposing dissimilarity thresholds.[17] This is done by inferring the biological sequences before incorporating amplification and sequencing errors. Parameters used to run DADA2 are detailed in the Supplementary Information.

Data analysis

Demographic characteristics of participants included in the study were presented as proportions and compared between cases and controls using chi-squared statistics. We excluded ASVs belonging to contaminant phyla as detailed in Salter et al.[18] Further, we included ASVs that were present in at least 5% of the total dataset and had a minimum of 1000 reads based on the percentile count distribution. Frequency plots of most abundant phyla in the dataset were calculated using relative abundances. Most abundant genera were plotted using different prevalence thresholds from (5 to 100%) using a heatmap plot.

To compare the profile of bacteria between seborrheic dermatitis patients against controls, we first calculated α-diversity per sample using Chao1 and Shannon diversity. Chao1 is an estimation of the richness of the sample (aka: how many ASV per group), while Shannon-diversity gives an estimate of the relative distribution of sequences among the ASVs. We tested for differences in Chao1 and Shannon diversity using Kruskal-Wallis tests and adjusted the p-values for pair-wise comparisons. We also calculated Shannon and Chao1 after rarefying all samples to have exactly 10000 counts. These analyses were done to evaluate whether any significant difference between the groups was due to different library sizes (details are presented in the Supplementary Material).

Next, for downstream analysis we transformed the dataset using centered log-ratio approach to account for unequal library sizes. This data transformation is recommended to analyse data

derived from Next Generation Sequencing since this data is assumed to be compositional (data conveys only relative information since the total abundance is unknown).[19,20] Because the logarithmic normalization does not handle zeros, these needed to be imputed first. The imputation of zeroes can be done under different assumptions (see Palarea-Albaladejo et al. for details[21]). Here, we assumed that any feature (ASV) observed in more than one sample could appear in another sample if sequenced with infinite depth, which can be modelled using Bayesian-Multiplicative replacement of count zeros.[21]

To visualize whether the microbiome composition clustered per sample characteristics (here, being a case or control, or other sample characteristics), we used Principal Component Analysis (PCA). Further, we tested for differences in the microbiome composition using permutational multivariate analysis of variance (permANOVA)[22] and adjusting for sex, age (as a continuous variable) and batch variables.

Finally, we performed compositional data analysis to test for differential abundance between specific ASVs for seborrheic (lesional and non-lesional) cases and controls with lesional cases used as the reference groups using the ALDEx2 package.[23] The package uses centered log-ratio transformation of the data to correct for unequal library sizes. We tested for differential composition using a generalized model, while adjusting for sex, age and batch as covariates. Significant p-values were defined with a threshold of p-value=0.05 and borderline with a p-value of 0.1. Of note, the package considers significant effects when the absolute value of the coefficients are higher than 1. However we considered estimates significant if the p-value was <0.05 after multiple testing (Benjamini-Hochberg Procedure, adjusted p-values are given by the package).

For the analysis of alpha and beta diversities, the phyloseq and microbiome packages were used (24) (https://github.com/microbiome/microbiome). The pairwise comparison and graphical display of the alpha-diversity was performed using library ("ggstatsplot") retrieved from https://cran.r-project.org/web/packages/ggstatsplot/index.html.

PermANOVA analysis was done using the function "adonis" from the vegan package (https://cran.r-project.org/web/packages/vegan/index.html). Imputation of zeroes was done using the Z compositions packages. The generation of plots and statistical analysis were done using phyloseq and microbiome packages. All the packages were implemented in Rv.3.5.2 (Eggshell Igloo version). Functions and parameters used for these analyses are detailed in the Supplementary Information.

Phylogenetic analysis

To improve the taxonomic resolution of the ASV that showed differential composition between lesional cases and controls, an exploratory phylogenetic analysis was done. We performed this by aligning the sequence reads of the ASVs that showed differential composition between cases and controls with the partial 16S rRNA sequences from different *Staphylococcus s*pecies retrieved from GenBank (reference numbers were obtained from Ghebremedhin B. et al.[25]). After the multiple sequencing alignment, a phylogenetic tree was constructed using the neighbour-joining algorithm. The phylogenetic analysis was implemented in the package MEGA.[26] Details on the programs and parameters for generation of the phylogenetic tree are presented in the Supplementary Materials.

RESULTS

Skin swabs were collected from 562 participants. Of these 75 participants were classified as non-lesional cases and 22 as lesional cases. Demographic characteristics are shown in Table 1. Most of the non-lesional and lesional cases were male. The median age was 53 years (inter-quantile range; IQR: 48-64 years) in the control group, and 56 years (IQR: 49-55 years) for non-lesional cases and 68 years (IQR: 49-55 years) for lesional cases. The quantile age distribution was significantly different with lesional cases being older than the controls.

Table 1. Descriptive statistics of participants with skin swabs

	Controls (n=465)	Non-lesional Cases (n=75)	Lesional Cases (n=22)	P-value*
Sex				<0.00004
Men	210 (77%)	44 (16%)	20 (7%)	
Women	255 (89%)	31 (11%)	2 (0.7%)	
Batch				0.042
1	215 (46%)	46 (61%)	9 (41%)	
2	250 (54%)	29 (39%)	13 (59%)	
Age quantiles (years)				0.01
40-49	130 (28%)	18 (24%)	1 (5%)	
50-54	113 (24%)	13 (17%)	5 (23%)	
55-65	116 (25%)	20 (27%)	2 (9%)	
> 65	106 (23%)	24 (32%)	14 (64%)	

* Chi-square test of the comparisons between lesional non-lesional and controls.

Bacterial community profiles of the naso-labial fold

In total, 15651 amplicon sequencing variants (ASVs) were identified in 562 individuals. The ASVs were assigned to 27 unique phyla (S1 Fig), some of which were clearly contaminant phyla

including *Planctomycetes* and *Cyanobacteria*. The total number of reads was 12277291 with a median count of 21340 (range: 0-218661) per sample. After quality control that included the filtering of contaminant ASVs and samples with low counts and outliers (Supplementary Materials and Methods), and including ASVs present in at least 5% of the total dataset, 185 ASVs in 529 samples (controls: 436; non-lesional cases:71; lesional cases:22) remained.

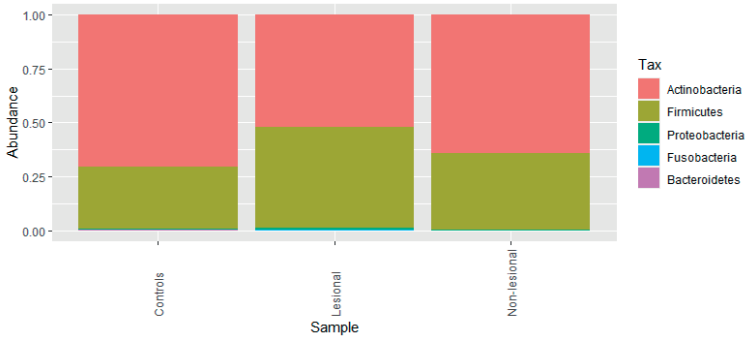


Figure 1. Barplot of relative frequency of most phyla averaged per control, non-lesional and lesional cases.

There was an apparent increase in the relative abundance of *Firmicutes* in lesional cases when compared with controls at phylum level as seen in the plot, but these differences were not significant. Fig 2 showed that several genera seemed to be increased in the lesional cases. However, after testing, only *Corynebacterium_1* was nominally significant (p-value<=0.05; Aldext text; Supplementary Table 2).

Fig 3 presents a heatmap of ASVs identified at different prevalence estimates in all samples (*y-axis*) and at different levels of detections per sample; from 0.0001% to 20% (*x-axis*). As shown in this plot, one member of the genus *Cutibacterium* (ASV1) was the most prevalent and was found in over 75% of all samples with relative abundances of 3% and higher. The second most prevalent ASV belonged to the genus *Staphylococcus* (ASV2) with a prevalence of 75% at lower abundances (<=2%). Other ASVs present in at least 50% of the samples included other ASVs belonging to *Staphylococcus* species (ASV68, ASV5, ASV18; *Staphylococcus epidermidis*), *Cutibacterium granulosum* (ASV7) *Peptoniphilus rhinitidis* and *Anaerococcus*.

To investigate whether the participants clustered based on their disease status we used PCA. S2 fig displays the first two axis of variation in the cutaneous microbiome composition. The first component explained 13% of the variation of the data and the second component explained 7%. However, there was not a clear separation between cases and controls.

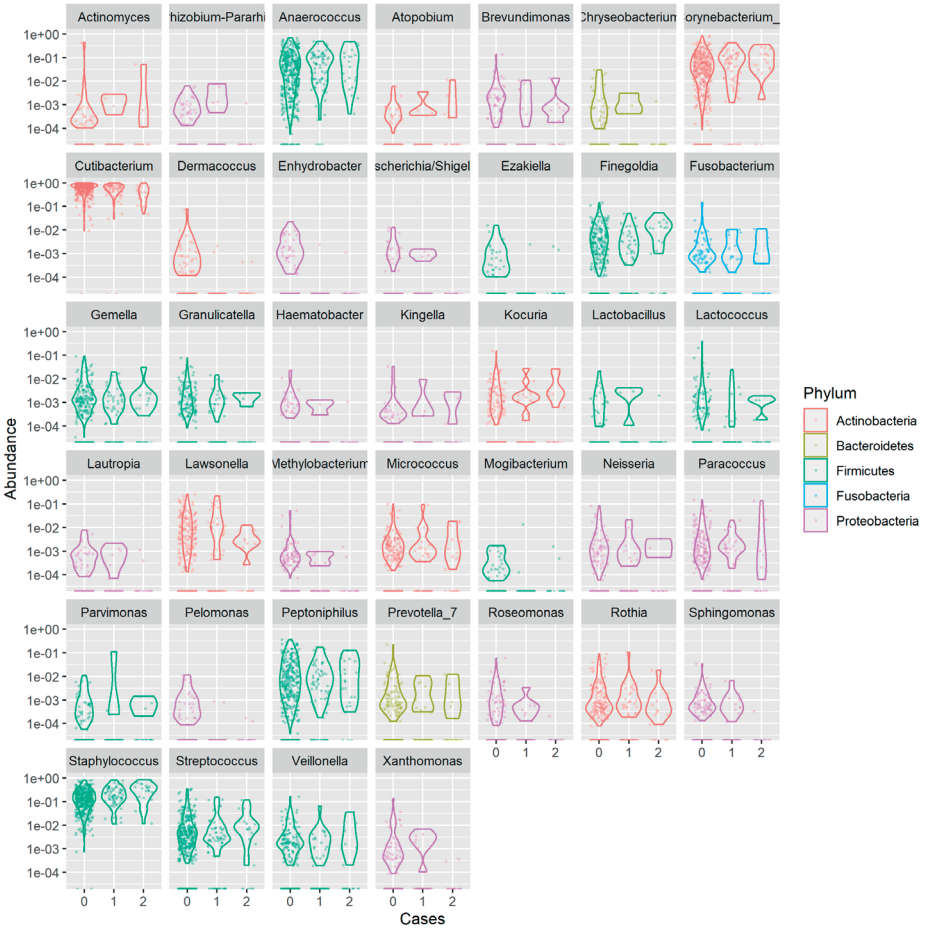


Figure 2. Barplot of relative frequency of genera per phylum. The relative abundance of main genera is presented separately, with controls =0, non-lesional cases =1 and lesional cases =2. Only *Corynebacterium_1* was nominally significantly different between lesional SD versus controls.

Comparison of the microbiome composition between seborrheic dermatitis and controls

We estimated α-diversity of our data per group (controls; non-lesional and lesional seborrheic dermatitis) using Shannon-diversity and Chao1 statistics. As shown in Fig 4A, the median Chao1 was significantly higher for lesional seborrheic dermatitis cases in comparison with both non-lesional cases and controls (Kruskal-Wallis rank sum: Chi-squared: 11.14; global p-value= 0.004; controls vs lesional cases; adjusted p-value=0.003; lesional cases vs non-lesional cases; adjusted p-value=0.0012). Shannon α-diversity estimates were also significantly different between lesional cases and controls (Kruskal-Wallis rank sum: Chi-squared: 19.06; global p-value= 7.3×10^{-5}; lesional cases vs controls; adjusted p-value=2.4×10^{-4}; lesional cases

vs non-lesional cases; adjusted p-value=0.03; non-lesional cases versus controls: adjusted p-value=0.03). Since alpha diversity parameters are sensitive to differences in sample library size, we evaluated the impact of different filtering thresholds as well as rarefaction (Supplementary Material). As shown in the Supplementary S3-S5 figs, alpha diversity estimates were significantly higher in lesional cases when compared with non-lesional cases and controls at different filtering thresholds and after rarefying (Supplementary Methods).

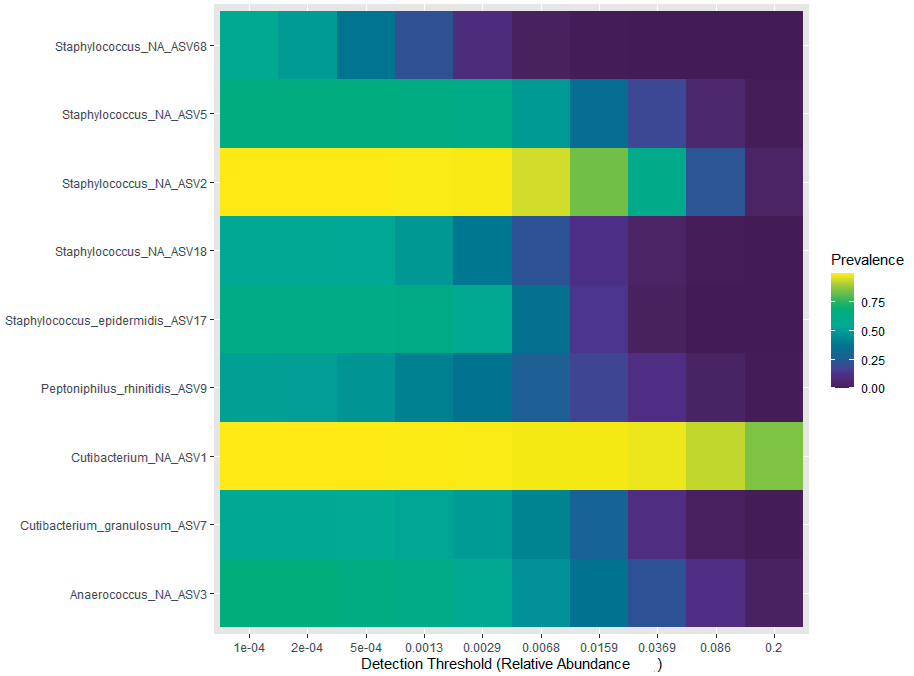


Figure 3. Heatmap plot of the most prevalent ASV identified in the sample. Figure presents the most prevalent ASVs (y-axis) identified at different detection thresholds (0.001 to 20%). Most common ASVs appear at low abundance per sample (0.01 to 1%)

Multivariate analysis. We tested for differences in the microbiome composition between seborrheic dermatitis cases and controls using permANOVA. We found that overall, the composition of the microbiome differed between cases and controls (R^2 =0.1%; p-value= 0.03). In addition, age, sex and batch effects were also significant (age: p-value=0.009; R^2=0.6%; sex: p-value: 001; R^2=2%; batch: p-value=0.001; R^2=1%).

Univariate analysis. We investigated whether there was a difference in the composition of ASVs between seborrheic dermatitis and controls adjusting for known sex, age and batch variables. We found nominally significant differences in the composition between lesional

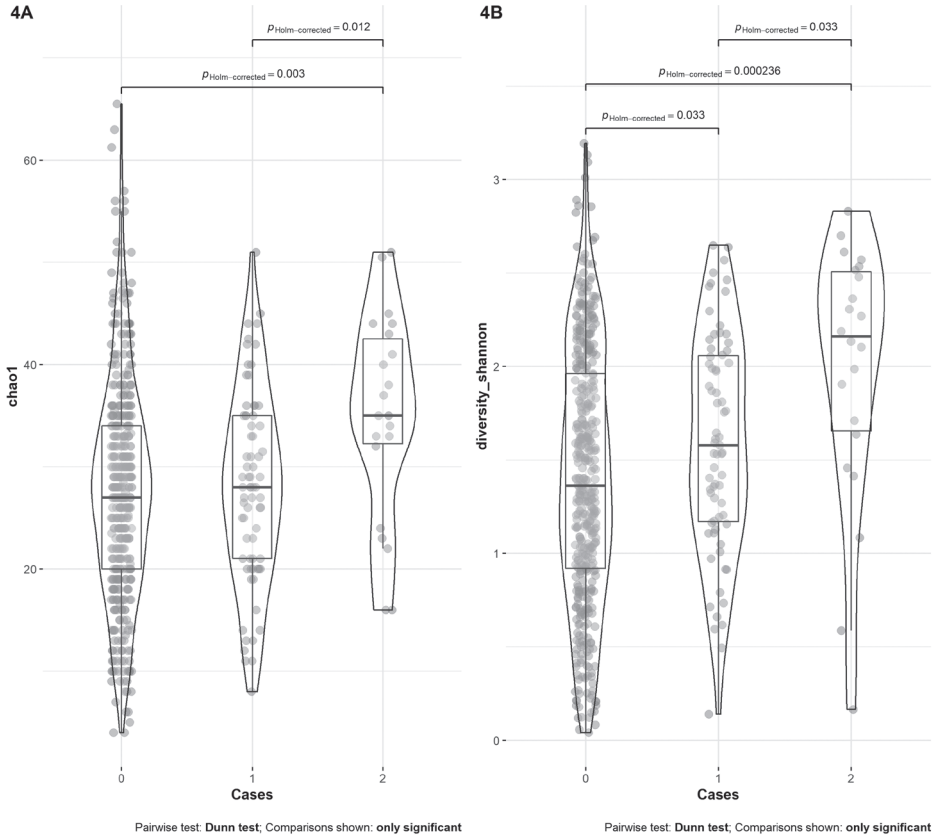


Figure 4. Boxplot of α-diversity. Fig 4A presents the violin-plot distribution of Chao1 diversity in controls, lesional and non-lesional cases. Fig 4B presents the violin-plot of Shannon diversity in controls, lesional and non-lesional cases. P-values are adjusted pair-wise comparisons. Overall Kruskal-Wallis test is described in the text.

cases compared against controls for bacteria of the genus *Staphylococcus* including ASV18 (effect size -3.37; p-value=0.01), ASV15 (effect size: -2.97 ;p-value=0.02); ASV22 (effect size -2.88; p-value=0.03), ASV13 (effect size -2.64; p-value=0.04) and ASV5 (effect size -2.41;p-value=0.05). Other bacteria with differences between lesional and controls included a member of the genus *Micrococcus* (ASV137), *Finegoldia* (ASV60), and *Lawsonell*a (ASV32). None of the differences were significant after correcting for multiple testing (Table 2). *Cutibacterium* (ASV1), which was most prevalent in this cohort, and nominally significant at genus level was only borderline associated with seborrheic dermatitis (effect size:-0.83, p-value: 0.07).

Table 2. Association analysis between ASV composition (ASV individual level) and seborrheic dermatitis (with categories controls, non-lesional cases and lesional cases)

Bacteria	Effect size[a]	P-value[a]	Effect size[b]	P-value[b]	Genus	Species
ASV18	**-3.37 (1.16)**	**0.01**	-2.22 (1.28)	0.09	Staphylococcus	NA
ASV15	**-2.97 (1.23)**	**0.02**	-2.35 (1.35)	0.1	Staphylococcus	NA
ASV22	**-2.88 (1.21)**	**0.03**	-2.39 (1.33)	0.09	Staphylococcus	NA
ASV13	**-2.64 (1.22)**	**0.04**	-1.39 (1.34)	0.32	Staphylococcus	NA
ASV137	**-2.13 (0.91)**	**0.04**	**-2.33 (1.01)**	**0.05**	Micrococcus	NA
ASV5	**-2.41 (1.19)**	**0.05**	-0.88 (1.31)	0.51	Staphylococcus	NA
ASV20	2.43 (1.15)	0.06	1.5 (1.3)	0.3	Anaerococcus	NA
ASV68	-1.54 (0.81)	0.07	-1.18 (0.9)	0.21	Staphylococcus	NA
ASV63	1.89 (0.98)	0.1	2.0 (1.1)	0.13	Staphylococcus	NA
ASV60	-1.89 (0.96)	0.11	-2.23 (1.06)	0.07	Finegoldia	NA
ASV171	-1.67 (0.89)	0.11	1.90 (0.1)	0.11	Streptococcus	sanguinis
ASV25	-1.83 (1.08)	0.12	-1.06 (1.20)	0.4	Anaerococcus	NA
ASV32	1.91 (1.08)	0.13	1.30 (1.20)	0.37	Lawsonella	NA

[a] Effect sizes and p-values of the comparison controls versus lesional cases (with negative effects meaning 'relative decrease' in controls relative to lesional cases. [b] Effect sizes and p-values of the comparison non-lesional versus lesional cases (with negative effects meaning 'relative decrease' in non-lesional cases relative to lesional cases. Effect sizes larger than 1 are considered significant.[20] Analysis were adjusted for age, sex and Batch effects.

Phylogenetic analysis of ASV with suggestive differential composition between lesional seborrheic dermatitis and controls

As shown in the Table 2, seven out of 12 ASVs that were (nominally) significantly different between controls and lesional cases were members of the genus *Staphylococcus*, most of which showed increased composition in lesional cases in comparison with controls. Of these, none of them could be annotated to species level by the DADA2 pipeline. To better understand the role of this genus in seborrheic dermatitis, we compared the DNA sequence of the reads with the 16S rRNA sequences available from different strains of staphylococci available from GenBank (Fig 5). As shown in this figure, the ASVs with differences in composition between lesional cases and controls were closely related to *S. capitis* (ASV5, ASV13, ASV15; ASV22) *S. caprae* (ASV) and *S. epidermidis* (ASV33) and were separate from the from *S. aureus* sequence.

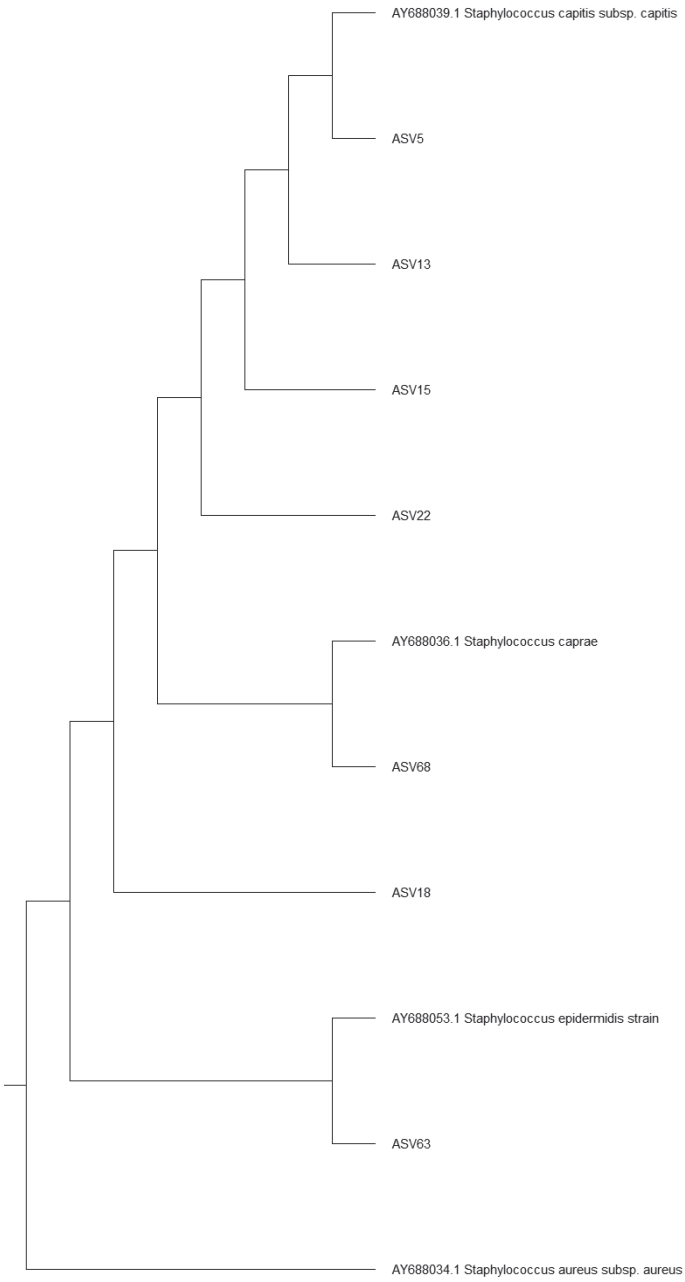


Figure 5. Phylogenetic tree of the ASVs with suggestive differences in composition between lesional SD and controls. The figure depicts the phylogenetic relationship between ASVs assigned to *Staphylococcus* species and *Staphylococcus* species obtained from GenBank (16S rRNA sequence). This sequences begin with AY*. ASVs closely related to *S. capitis* belong to the same node (ASV5, ASV13, ASV15 and ASV22). ASV68 appears to belong to *S. caprae* together with ASV18. ASV63 clustered with *S. epidermidis.* Sequence of *S. aureus* appear as outgroup (sequence with most differences).

DISCUSSION

In this large cross-sectional study comparing the cutaneous microbiome of individuals with seborrheic dermatitis with individuals with no skin disease (controls), we found a significant increase in the α-diversity between lesional cases and controls. Comparing the overall microbiome composition between cases and controls using a multivariate approach also led to significant differences between the groups, although the contribution of skin condition was small in comparison to other sample characteristics such as sex. In the design of the study, we assumed that the bacterial microbiome of non-lesional skin would also differ from healthy participants as Soares et al. showed this for people with dandruff,[27] but we did not find a clear separation between non-lesional cases and controls. In the univariate analysis we found differences in the composition of members of specific bacteria from the genus *Staphylococcus,* thought to be members of the normal skin flora, including: *S. capitis*, *S. caprae* and *S. epidermidis*, with most of them being increased in lesional cases compared to controls. No differences were found for *S. aureus* in this analysis. Other bacteria with an increased abundance in the cases were members of the genus *Micrococcus*, *Finegoldia* sp. and *Streptococcus sanguinis*. The changes found in the univariate analysis were not statistically significant after correcting for multiple testing. Though, especially for the *Staphylococcus* genus there seems to be a trend, as seen in the comparison between non-lesional and lesional seborrheic dermatitis groups (Table 2). The principal component analysis did not show a clear separation of microbiome composition between controls and cases. In the previous smaller study by Tanaka et al.,[28] a separation between cases and control was suggested, although their graph leaves room for discussion and no statistical test was performed. We replicated the finding that *Staphylococcus* species have an increased abundance in lesional skin.

In our study, we found similar distribution of bacteria from sebum rich areas in our dataset as in previous studies.[29] Using a prevalence of 50% to define a 'core' microbiome, we found that members of *Cutibacterium* species (ASV1_ *Cutibacterium _NA* and *Cutibacterium granulossum*) and *Staphylococcus* species (*Staphylococcus epidermidis* and *Staphylococcus _NA*) were the most abundant, which serves as an internal validation of our dataset. Of this 'core' bacteria only one member of the *Cutibacterium* species seemed to have differences in their 'abundance' between seborrheic dermatitis cases and controls as shown in the univariate analysis (Table 2). The other ASVs with suggestive differences in the abundance between cases and controls were not amongst the core set, which suggests that the most differences between lesional seborrheic dermatitis and controls occur for bacteria that have relatively low (relative) abundances between 5% to 10%.

We found differences in the relative composition of members of the *Staphylococcus* genus, which supports previous findings of a differential composition of this genus in seborrheic

dermatitis based on smaller series of cases.[11] *Staphylococcus* species include different species known to be part of normal skin flora, such as *S. epidermidis, S. capitis* and *S. caprae,* as well as pathogens such as *S. aureus*. Because in most studies on seborrheic dermatitis the analysis of bacterial microbiome is done at genus level, it is difficult to compare our results with these from previous studies since we chose to analyse exact ASV instead of grouping ASVs into genera. In a recent study an increased abundance of *S. epidermidis* in patients with impaired skin barrier was found.[30] Because commensal bacteria can also act as opportunistic pathogens under special circumstances (e.g. impaired barrier function), the previous study as well as our results supports the hypothesis that commensal bacteria rather than pathogens may be associated with seborrheic dermatitis.

The higher alpha diversity in seborrheic dermatitis cases (both lesional and non lesional) in comparison to the controls is in contrast with studies on bacterial microbiome in other chronic inflammatory skin conditions such as atopic dermatitis, where a decreased alpha diversity in lesional skin has been observed. Although both diseases are associated with a skin barrier impairment, it is likely that the role of microbial dysbiosis is different for the two skin conditions. In atopic dermatitis, alpha diversity is lower due to the predominance of one bacterium, namely: *S. aureus*, which is considered more of a pathogen than a commensal.[31] Higher *S. aureus* colonization has been associated with immune dysfunction and a decrease of the production of antimicrobial peptides, which will favor the overgrowth of these bacteria while inhibiting the growth of competing bacteria.[32] We looked at the total number of 47 ASVs related to *Staphylococcus* species in our data and did a phylogenetic analysis with all sequences available at GenBank for 16S rRNA for a total of 75 sequences. Interestingly, only one of 47 ASVs in our data was phylogenetically closer to *S. aureus* than to the rest (ASV109: S6 fig), which suggests that *S. aureus* is not a major player in seborrheic dermatitis. It could also be argued that an affected nasolabial skin could be a niche for bacteria from nearby areas such as mouth (e.g. *Streptococcus sanguinis*) or nose (*Peptonilus rinitidis*). ASVs associated with these bacteria seemed to be increased in seborrheic dermatitis (when compared with controls), although the differences were not statistically significant. This also may explain the increase in the diversity seen in our dataset. Thus, one could argue that in seborrheic dermatitis subjects the increased diversity is secondary to an impaired skin barrier, which leads to the colonization of otherwise commensal bacteria.

As mentioned above, we chose exact ASVs as the unit of analysis instead of OTUs in an attempt to gain taxonomic resolution beyond genus level, since the pipeline can annotate 1 to 2 nucleotides difference between ASVs. However, because the DADA2 bioinformatic pipeline uses a strict threshold to annotate ASVs to bacteria from reference databases such as SILVA (100% match; in other words identical sequences), many of the ASVs could not be annotated to species level, and were left as 'unknown' species. In most studies, this

issue is solved by grouping ASVs or OTUs into genera as unit of analysis, which may lead to spurious results, because different species with different roles in skin biology or pathology (*e.g. S. aureus* versus *S. epidermidis*) are then assumed to be the same. With the methods we used, we could have agglomerated similar ASVs into species level (*e.g.* different ASVs associated with *S. epidermidis* into one single *S. epidermidis*), but due to the high proportion of ASVs that were unknown at species level, we would not have enough data to analyse. With this, we showed that trying to separate species from the same genus might be valuable to understand the role of specific bacteria in seborrheic dermatitis. Because whole genome sequencing is still expensive for large studies, especially when analyzing microbiome from low to medium biomass, a better annotation of reference datasets and perhaps sequencing of another region or even the whole gene could improve the resolution in future studies.

Our study has some limitations. First, this study includes a middle aged and elderly population, and as the microbiome composition changes with age, this might limit the generalizability to younger patients. Second, the bacterial biomass of the skin is lower than that from other human niches.[33] This has implications for sequencing since samples with low to medium levels of extracted DNA are more prone to contamination by other bacteria, which can lead to spurious results. Here we excluded obvious contaminant phyla based on previous publications and filtered ASVs based on prevalence. Third, although our sample of cases and controls is much larger than most studies on skin microbiome, the number of lesional cases was probably too low in comparison with the number of controls, which had much larger variation and made it difficult to separate cases from controls in the PCA. The low density of skin bacterial DNA also creates problems in the statistical analysis since the data were highly sparse. We used methods that alleviated this issue (imputing zeroes), but still the analysis may have been too conservative to survive multiple testing. Further, most methods assume that rare species are not important in determining variation but this may not be true as shown here in our study. To alleviate this, larger sample sizes will be needed to confirm our results.

To conclude, we found differences in the microbiome composition in a large sample of patients with seborrheic dermatitis when compared with controls. There was a trend towards higher prevalence of members of the genus *Staphylococcus*, most of which showed increased composition in lesional cases, and were closely related to *S. capitis S. caprae* and *S. epidermidis.* The associations were not significant when correcting for multiple testing. Future studies are required to validate these findings.

SUPPLEMENTARY INFORMATION

SUPPLEMENTARY MATERIALS AND METHODS

Bioinformatic pipeline

Quality filtering was performed in DADA2 using the following criteria: trim=0, maxEE=c(2,2), truncQ=2, rm.phix=TRUE. Filtered reads were run through the DADA2 Amplicon Sequence Variant (ASV) assignment and ASVs were assigned a taxonomy from the SILVA version 132 rRNA database using the RDP naïve Bayesian classifier.[35] Data tables were combined into a phyloseq object using Phyloseq.[24]

Phylogenetic analysis

For the phylogenetic analysis we used the MEGA-X software.[36] This was done in several steps. First, we uploaded fasta sequence reads of the ASVs with suggestive differences in composition between lesional cases and controls into the software. Then, we aligned the sequences using the MUSCLE program [37] with neighbour joining method. Next, the phylogenetic tree was constructed using the aligned sequences using the neighbour joining method.[38] The evolutionary distances were computed using the Maximum Composite Likelihood method.[39] All ambiguous positions were removed for each sequence pair (pairwise deletion option). A phylogenetic tree can be interpreted as follows: branches (sequences) from the same node are more closely related to each other than branches from other nodes. The longer the branches the more differences between the sequences there are.

SUPPLEMENTARY RESULTS

Descriptive statistics of the microbiome data: Filtering steps

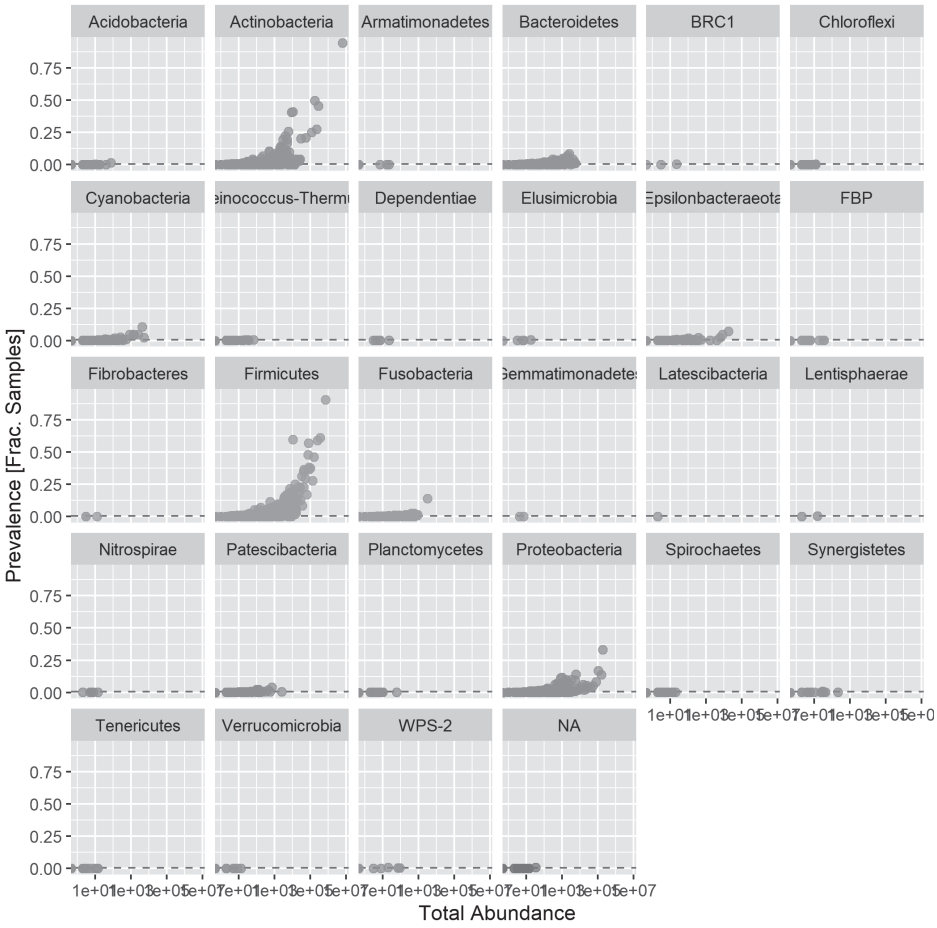
After the sequencing and bioinformatic steps, the table with amplicon sequence variants (ASVs) found in samples are imported into the package phyloseq for further downstream analysis. The total dataset consisted of a total of 15980 amplicon sequence variants identified in 895 samples, of which 25 were negative controls (air swabs taking during the sampling collection at the research centre).

We first excluded obvious contaminant phyla based on literature. These included *Planctomycetes*, *Epsilonbacteraeota*, *Cyanobacteria*, *Chloroflexi* , Ambiguous_taxa, *BRC1*, *WPS-2*, *FBP*, *Deinococcus-Thermus*, *Cyanobacteria* and *Epsilonbacteroraeota*.

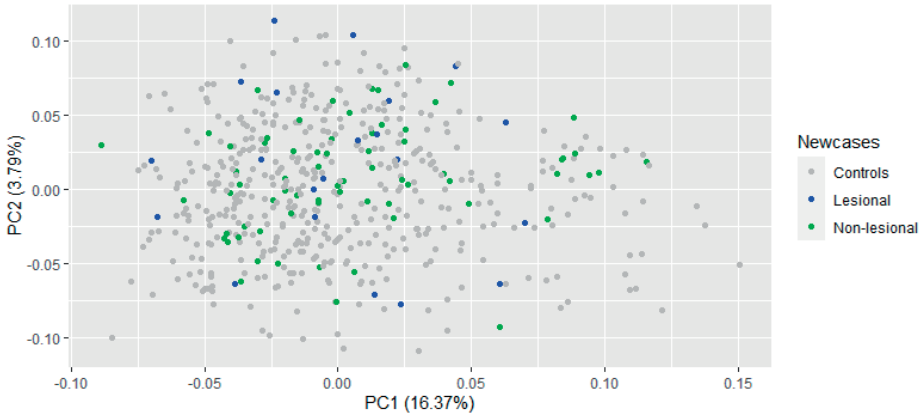
Further, we excluded samples with less than total 1000 reads, which were mostly negative controls. Next, we also removed a sample with an unusual high count since it is also likely to be the result on contamination. After these filtering steps we had 15111 ASVs in 533 samples including four negative controls, which were further removed. Finally, we used 5% prevalence filtering,[40] meaning that we included ASVs that were present in at least 5% of all samples. This left a remaining of 185 taxa in 529 samples, of which 436 were controls, 71 were non-lesional cases and 22 were lesional cases. This dataset was then used for the downstream analysis described in the main manuscript.

Analysis of alpha diversity

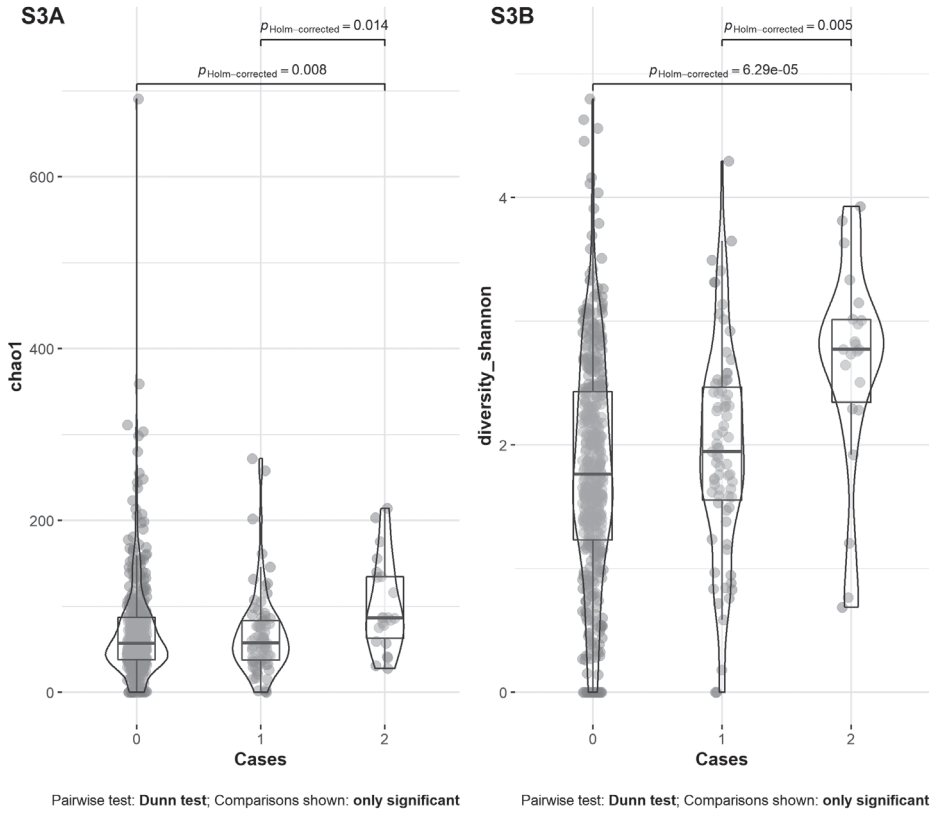
Since measures of alpha diversity are sensitive to different library sizes, we estimated Chao1 and Shannon diversities in our dataset at different filtering thresholds, namely: minimal filtering (only contaminant phyla were left out), before 5% prevalence and after 5% filtering prevalence. In addition, we also rarefied the original dataset to have exactly 10000 counts per sample. For this we used the function 'rarefy_even_depth' with sample size of 10000. S3-S5 figs showed the pairwise comparison of the Chao1 and Shannon diversity per lesional, non-lesional and control groups. In all cases the lesional cases showed the highest diversity confirming our results.



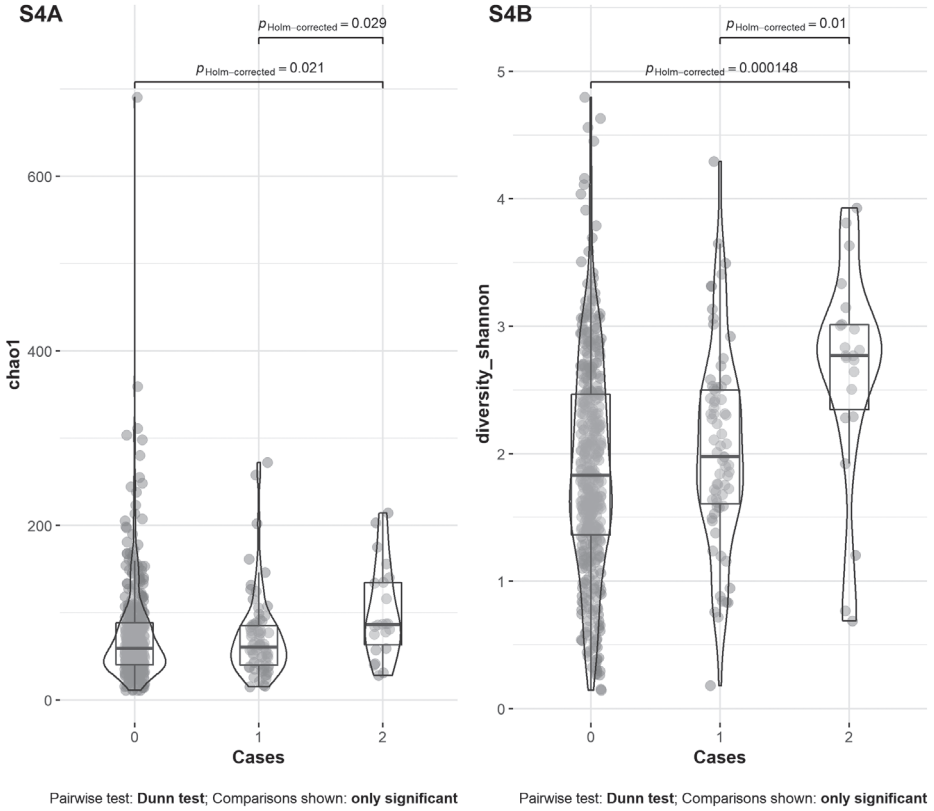
S1 fig. Frequency of 27 unique phyla presented. The graphs present the taxa prevalence against total counts, with each dot representing a different taxa.



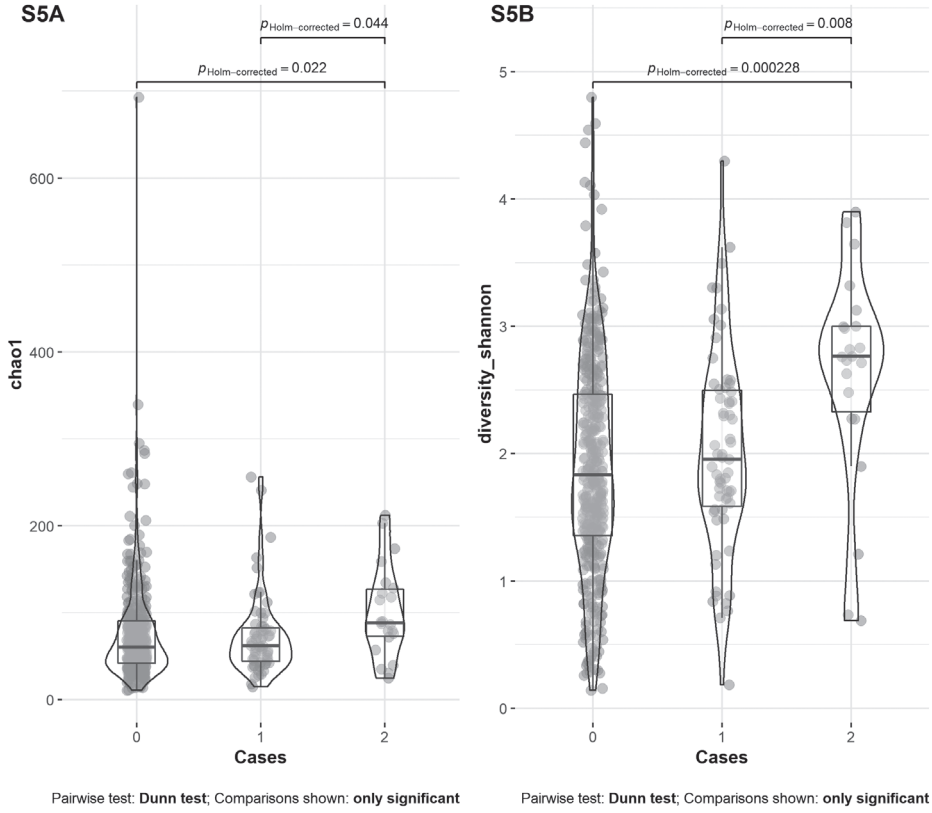
S2 fig. Principal component analysis (PCA) of microbiome composition (the centered log- ratios). No clear separation of microbiome composition was observed between controls (grey dots) and cases separated in non-lesional (blue dots) and lesional SD cases (green dots)



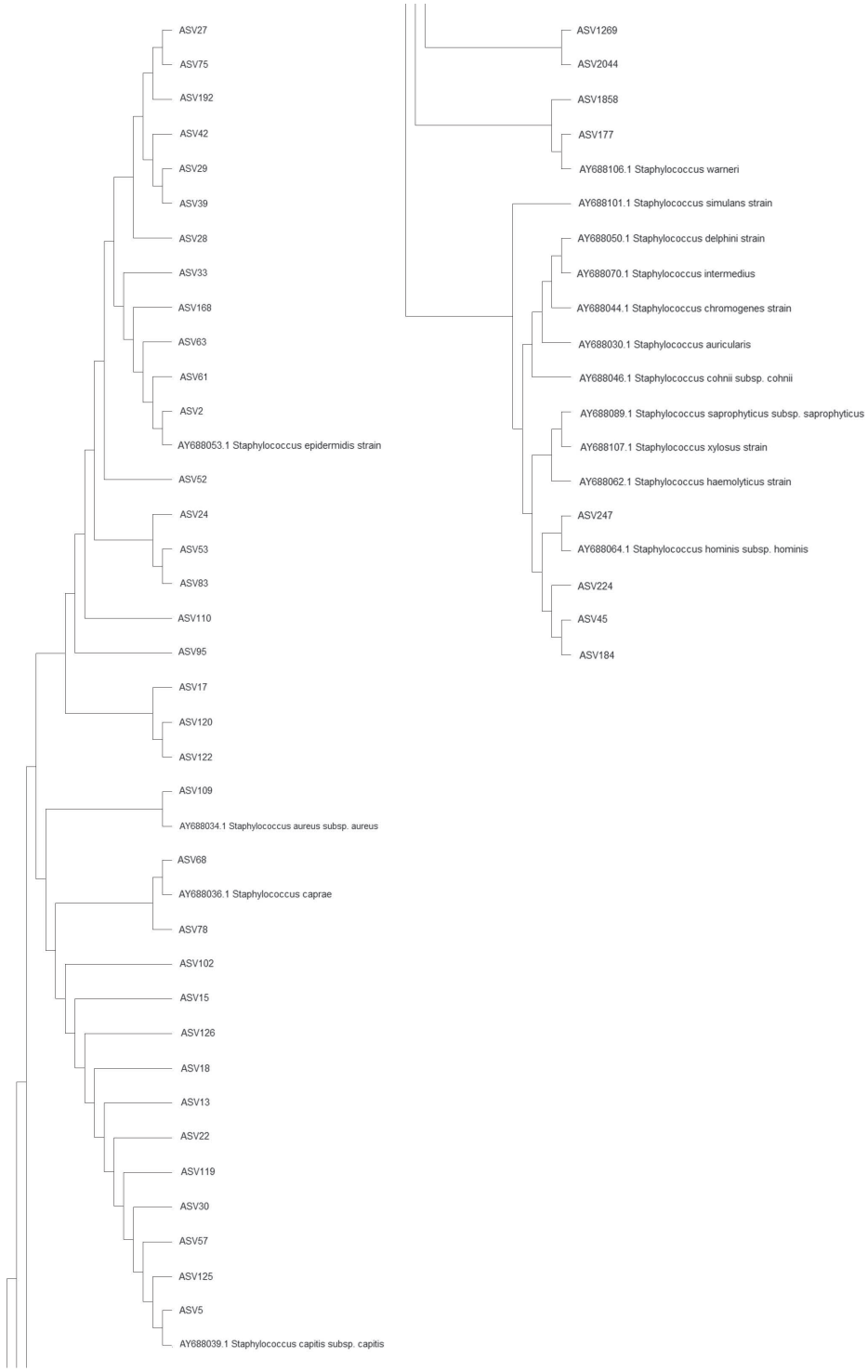
S3 fig. Boxplot of α-diversity with minimal filtering. Fig S3A presents the violin-plot of Chao1 diversity in controls, lesional and non-lesional cases. Fig S3B presents the violin-plot of Shannon diversity in controls, lesional and non-lesional cases. P-values are adjusted pair-wise comparisons.



S4 fig. Boxplot of α-diversity before 5% prevalence filtering. Fig S4A presents the violin-plot distribution of Chao1 diversity in controls, lesional and non-lesional cases. Fig S4B presents the violin-plot of Shannon diversity in controls, lesional and non-lesional cases. P-values are adjusted pair-wise comparisons



S5 fig. Boxplot of α-diversity after rarefying. Fig S5A presents the violin-plot of Chao1 diversity in controls, lesional and non-lesional cases. Fig S5B presents the violin-plot of Shannon diversity in controls, lesional and non-lesional cases. P-values are adjusted pair-wise comparisons.


ASV27
ASV75
ASV192
ASV42
ASV29
ASV39
ASV28
ASV33
ASV168
ASV63
ASV61
ASV2
AY688053.1 Staphylococcus epidermidis strain
ASV52
ASV24
ASV53
ASV83
ASV110
ASV95
ASV17
ASV120
ASV122
ASV109
AY688034.1 Staphylococcus aureus subsp. aureus
ASV68
AY688036.1 Staphylococcus caprae
ASV78
ASV102
ASV15
ASV126
ASV18
ASV13
ASV22
ASV119
ASV30
ASV57
ASV125
ASV5
AY688039.1 Staphylococcus capitis subsp. capitis
ASV1269
ASV2044
ASV1858
ASV177
AY688106.1 Staphylococcus warneri
AY688101.1 Staphylococcus simulans strain
AY688050.1 Staphylococcus delphini strain
AY688070.1 Staphylococcus intermedius
AY688044.1 Staphylococcus chromogenes strain
AY688030.1 Staphylococcus auricularis
AY688046.1 Staphylococcus cohnii subsp. cohnii
AY688089.1 Staphylococcus saprophyticus subsp. saprophyticus
AY688107.1 Staphylococcus xylosus strain
AY688062.1 Staphylococcus haemolyticus strain
ASV247
AY688064.1 Staphylococcus hominis subsp. hominis
ASV224
ASV45
ASV184

← **S6 fig. Phylogenetic tree of the ASVs belonging to the genus *Staphylococcus*.** The figure depicts the phylogenetic relationship between ASVs assigned to *Staphylococcus* species and *Staphylococcus* species obtained from GenBank (16S rRNA sequence). This sequences begin with AY*. Only ASV109 was phylogenetically closer to *S. aureus*

Supplementary Table 1. Association analysis between microbiome composition (phylum level) and seborrheic dermatitis (with categories controls, non-lesional cases and lesional cases)

Bacteria	Effect size[a]	P-value[a]	Effect size[b]	P-value[b]
Actinobacteria	-0.24 (0.26)	0.39	-0.76 (0.45)	0.12
Firmicutes	0.45 (0.27)	0.12	0.65 (0.46)	0.19
Proteobacteria	-0.15 (0.44)	0.67	0.04 (0.76)	0.75
Fusobacteria	0.50 (0.42)	0.30	0.31 (0.72)	0.53
Bacteroidetes	-0.57 (0.44)	0.28	-0.25 (0.75)	0.60

[a] Effect sizes and p-values of the comparison: controls versus lesional cases. [b] Effect sizes and p-values of the comparison: non-lesional cases versus lesional cases.

Supplementary Table 2. Association analysis between microbiome composition (genus level) with seborrheic dermatitis (with categories controls, non-lesional and lesional cases) and with lesional cases as reference groups

Genus	Effect size[a]	P-value[a]	Effect size[b]	P-value[b]
Cutibacterium	1.001 (0.468)	0.04	0.371 (0.522)	0.49
Staphylococcus	-0.797 (0.417)	0.06	-0.312 (0.466)	0.51
Peptoniphilus	-1.849 (1.149)	0.12	-1.446 (1.284)	0.28
Anaerococcus	-1.403 (0.945)	0.14	-0.435 (1.055)	0.68
Finegoldia	-1.549 (1.061)	0.18	-1.527 (1.185)	0.24
Sphingomonas	1.246 (0.812)	0.22	1.027 (0.906)	0.33
Micrococcus	-1.245 (0.929)	0.23	-1.727 (1.037)	0.15
Granulicatella	1.157 (0.881)	0.27	1.244 (0.983)	0.28

[a] Effect sizes and p-values of the comparison: controls versus lesional cases. [b] Effect sizes and p-values of the comparison: non-lesional cases versus lesional cases.

REFERENCES

1. Chatzikokkinou P, Sotiropoulos K, Katoulis A, Luzzati R, Trevisan G. Seborrheic dermatitis - an early and common skin manifestation in HIV patients. Acta Dermatovenerol Croat. 2008;16(4):226-30.
2. Lally A, Casabonne D, Newton R, Wojnarowska F. Seborrheic dermatitis among Oxford renal transplant recipients. J Eur Acad Dermatol Venereol. 2010;24(5):561-4.
3. Sanders MGH, Pardo LM, Uitterlinden AG, Smith AM, Ginger RS, Nijsten T. The Genetics of Seborrheic Dermatitis: A Candidate Gene Approach and Pilot Genome-Wide Association Study. J Invest Dermatol. 2018;138(4):991-3.
4. Sanders MGH, Pardo LM, Franco OH, Ginger RS, Nijsten T. Prevalence and determinants of seborrhoeic dermatitis in a middle-aged and elderly population: the Rotterdam Study. Br J Dermatol. 2018;178(1):148-53.
5. Sanders MGH, Pardo LM, Ginger RS, Kiefte-de Jong JC, Nijsten T. Association between diet and seborrheic dermatitis: a cross-sectional study. J Invest Dermatol. 2018.
6. Unna PG. Das seborrhoische Ekzem. Monatsh Prakt Der-matol. 1887;6:827–846.
7. Kastarinen H, Oksanen T, Okokon EO, Kiviniemi VV, Airola K, Jyrkka J, et al. Topical anti-inflammatory agents for seborrhoeic dermatitis of the face or scalp. Cochrane Database Syst Rev. 2014;5:CD009446.
8. DeAngelis YM, Gemmer CM, Kaczvinsky JR, Kenneally DC, Schwartz JR, Dawson TL, Jr. Three etiologic facets of dandruff and seborrheic dermatitis: Malassezia fungi, sebaceous lipids, and individual sensitivity. J Investig Dermatol Symp Proc. 2005;10(3):295-7.
9. Park T, Kim HJ, Myeong NR, Lee HG, Kwack I, Lee J, et al. Collapse of human scalp microbiome network in dandruff and seborrhoeic dermatitis. Exp Dermatol. 2017.
10. Lin Q, Panchamukhi A, Li P, Shan W, Zhou H, Hou L, et al. Malassezia and Staphylococcus dominate scalp microbiome for seborrheic dermatitis. Bioprocess Biosyst Eng. 2020.
11. Tanaka A, Cho O, Saito C, Saito M, Tsuboi R, Sugita T. Comprehensive pyrosequencing analysis of the bacterial microbiota of the skin of patients with seborrheic dermatitis. Microbiology and Immunology. 2016;60(8):521-6.
12. Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. ISME J. 2017;11(12):2639-43.
13. Ikram MA, Brusselle GGO, Murad SD, van Duijn CM, Franco OH, Goedegebure A, et al. The Rotterdam Study: 2018 update on objectives, design and main results. Eur J Epidemiol. 2017;32(9):807-50.
14. Fadrosh DW, Ma B, Gajer P, Sengamalay N, Ott S, Brotman RM, et al. An improved dual-indexing approach for multiplexed 16S rRNA gene sequencing on the Illumina MiSeq platform. Microbiome. 2014;2(1):6.
15. Meisel JS, Hannigan GD, Tyldsley AS, SanMiguel AJ, Hodkinson BP, Zheng Q, et al. Skin Microbiome Surveys Are Strongly Influenced by Experimental Design. J Invest Dermatol. 2016;136(5):947-56.
16. Schmieder R, Lim YW, Rohwer F, Edwards R. TagCleaner: Identification and removal of tag sequences from genomic and metagenomic datasets. BMC Bioinformatics. 2010;11(1):341.
17. Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. The ISME Journal. 2017;11(12):2639-43.
18. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC Biol. 2014;12:87-.

19. Gloor GB, Wu JR, Pawlowsky-Glahn V, Egozcue JJ. It's all relative: analyzing microbiome data as compositions. Ann Epidemiol. 2016;26(5):322-9.
20. Gloor GB, Macklaim JM, Pawlowsky-Glahn V, Egozcue JJ. Microbiome Datasets Are Compositional: And This Is Not Optional. Frontiers in Microbiology. 2017;8(2224).
21. Palarea-Albaladejo J, Martín-Fernández JA. zCompositions — R package for multivariate imputation of left-censored data under a compositional approach. Chemometrics and Intelligent Laboratory Systems. 2015;143:85-96.
22. Kelly BJ, Gross R, Bittinger K, Sherrill-Mix S, Lewis JD, Collman RG, et al. Power and sample-size estimation for microbiome studies using pairwise distances and PERMANOVA. Bioinformatics. 2015;31(15):2461-8.
23. Fernandes AD, Macklaim JM, Linn TG, Reid G, Gloor GB. ANOVA-like differential expression (ALDEx) analysis for mixed population RNA-Seq. PloS one. 2013;8(7):e67019-e.
24. McMurdie PJ, Holmes S. phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One. 2013;8(4):e61217.
25. Ghebremedhin B, Layer F, König W, König B. Genetic classification and distinguishing of Staphylococcus species based on different partial gap, 16S rRNA, hsp60, rpoB, sodA, and tuf gene sequences. Journal of clinical microbiology. 2008;46(3):1019-25.
26. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. Mol Biol Evol. 2013;30(12):2725-9.
27. Soares RC, Camargo-Penna PH, de Moraes VC, De Vecchi R, Clavaud C, Breton L, et al. Dysbiotic Bacterial and Fungal Communities Not Restricted to Clinically Affected Skin Sites in Dandruff. Front Cell Infect Microbiol. 2016;6:157.
28. Tanaka A, Cho O, Saito C, Saito M, Tsuboi R, Sugita T. Comprehensive pyrosequencing analysis of the bacterial microbiota of the skin of patients with seborrheic dermatitis. Microbiol Immunol. 2016;60(8):521-6.
29. A Grice E, Kong H, Conlan S, Deming C, Davis J, Young A, et al. Topographical and Temporal Diversity of the Human Skin Microbiome2009. 1190-2 p.
30. An Q, Sun M, Qi RQ, Zhang L, Zhai JL, Hong YX, et al. High Staphylococcus epidermidis Colonization and Impaired Permeability Barrier in Facial Seborrheic Dermatitis. Chin Med J (Engl). 2017;130(14):1662-9.
31. Otto M. Staphylococci in the human microbiome: the role of host and interbacterial interactions. Curr Opin Microbiol. 2020;53:71-7.
32. Nakatsuji T, Chen TH, Narala S, Chun KA, Two AM, Yun T, et al. Antimicrobials from human skin commensal bacteria protect against Staphylococcus aureus and are deficient in atopic dermatitis. Sci Transl Med. 2017;9(378).
33. Ferretti P, Farina S, Cristofolini M, Girolomoni G, Tett A, Segata N. Experimental metagenomics and ribosomal profiling of the human skin microbiome. Exp Dermatol. 2017;26(3):211-9.
34. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Research. 2012;41(D1):D590-D6.
35. Wang Q, Garrity GM, Tiedje JM, Cole JR. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Appl Environ Microbiol. 2007;73(16):5261-7.
36. Kumar S, Stecher G, Tamura K. MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for Bigger Datasets. Mol Biol Evol. 2016;33(7):1870-4.
37. Edgar RC. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research. 2004;32(5):1792-7.

38. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol Biol Evol. 1987;4(4):406-25.
39. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc Natl Acad Sci U S A. 2004;101(30):11030-5.
40. Dawson TL, Jr. Malassezia globosa and restricta: breakthrough understanding of the etiology and treatment of dandruff and seborrheic dermatitis through whole-genome analysis. J Investig Dermatol Symp Proc. 2007;12(2):15-9.

6 General discussion

The very common but understudied disease

Seborrheic dermatitis is a very common skin disease with a poorly understood pathophysiology. There might be several reasons for the lack of interest in seborrheic dermatitis. Seborrheic dermatitis flares are often mild, can easily be suppressed, and are usually present for a short period of time.[1] Patients might therefore not always visit a health professional. Some might even argue that seborrheic dermatitis is a personal hygiene problem or a skin feature, rather than a disease. However, seborrheic dermatitis can severely affect quality of life because it is extremely visible, may cause symptoms, and may have diagnostic and therapeutic difficulties.[2-4] Although it can be suppressed successfully, there is no definitive cure and it tends to relapse frequently. Therefore, a better understanding of this common skin disease is warranted.

Both host and environmental determinants have been implicated in the pathogenesis of seborrheic dermatitis. However, most of these studies were small (less than 100 participants), or conducted in a selected population (e.g. dermatology outpatient clinics).[5-19] During the past few years we have investigated associations between several determinants and seborrheic dermatitis - including lifestyle, genetic susceptibility and cutaneous microbiome - and provided additional insights into the role of existing and new risk factors in disease susceptibility (Figure 1). However, many questions regarding the etiology of seborrheic dermatitis remain. Considering it is one of the most common skin diseases, many patients may benefit from new insights in this disease.

How epidemiological research helps to move the field forward

Validating previous associations and identifying new ones

The studies described in this thesis were all conducted within the Rotterdam Study, a prospective cohort study of middle aged and elderly people living in Ommoord, the Netherlands.[20] In 2010, full body skin examinations were introduced in the Rotterdam Study, which made it possible to investigate prevalent diseases and phenotypes across a wide spectrum of severity, including mild cases that do not always warrant a visit to a doctor, such as seborrheic dermatitis. The prevalence of seborrheic dermatitis in the Rotterdam Study was 14.3%, which is substantially higher than in studies using hospital or insurance data.[5,21,22] In part, this can be explained by the fact that a large proportion of affected people have mild forms of the condition and therefore do not seek medical care. In addition, we focused on middle aged and elderly people and seborrheic dermatitis prevalence increases with age (i.e., selection bias).[23,24] At the same time, seborrheic dermatitis is a chronic relapsing disease and participants were only cross-sectionally selected as case when seborrheic dermatitis was diagnosed during full body skin examination. Therefore, we missed participants that had seborrheic dermatitis but just not at that particular moment (i.e., ascertainment bias).

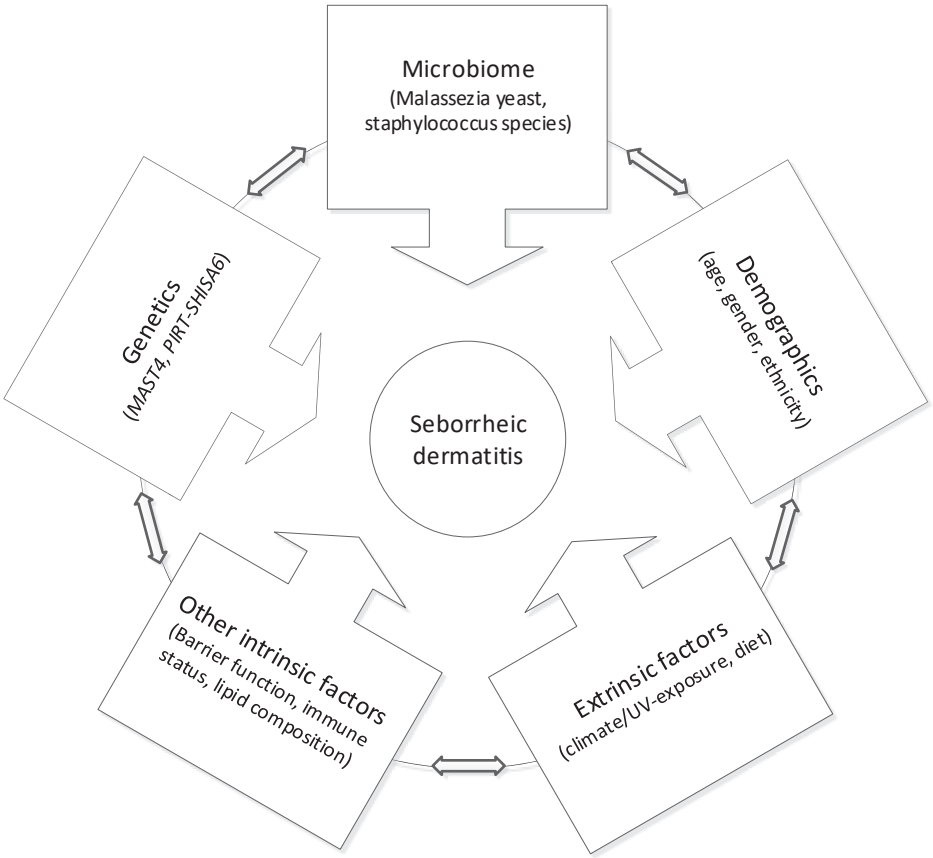


Figure 1. Factors that influence seborrheic dermatitis.

Previous studies and results from this thesis show that many factors influence the susceptibility for seborrheic dermatitis. Our results based on a large sample showed that seborrheic dermatitis is significantly more prevalent in men and in people with a light skin colour. Although this might be because of underdiagnosing in dark-skinned individuals, because erythema is less striking, it might also be the result of a superior barrier function in more pigmented skin.[8,25,26] Seasonality also seems to have a great impact on seborrheic dermatitis prevalence; during the winter, seborrheic dermatitis is about 1.6 times more prevalent than during the summer.[25] Possibly, this is due to the cutaneous immunosuppressive effects of ultraviolet light (UV), temperature and humidity of the environment during summer.

In chapter 3, we found that seborrheic dermatitis was more common in participants with a dry skin. From a prevention and management perspective, maintaining an optimal skin barrier by applying moisturizers might by itself be a target in seborrheic dermatitis. For now, no clinical study has been designed to answer this specific question. However, there are several

randomized clinical studies comparing a vehicle with a calcineurin inhibitor, corticosteroid or anti-fungal treatment. All these treatments are found to be more effective than the placebo cream, but great improvements in both objective as subjective measurements were also seen with placebo cream alone. This supports the positive effect of optimizing the skin barrier in the management of seborrheic dermatitis.[27] In a future study, the relapse rate in patients that are randomized in using daily emollients versus no follow up treatment should be compared to further assess the impact of moisturizers in the long-term control of seborrheic dermatitis.

Western diet has been implicated to have a role in chronic systemic inflammation. Adherence to a healthy diet has been associated with a lower incidence of chronic diseases and a lower mortality.[28] We hypothesized that diet might also influence chronic inflammation in seborrheic dermatitis. Several skin diseases have been associated with diet as well and diet modification is popular among dermatological patients, especially among those with chronic inflammatory diseases.[29,30] The role of diet in seborrheic dermatitis was assessed in Chapter 3.2. A high fruit intake was associated with less seborrheic dermatitis. In women, a western diet was associated with a higher seborrheic dermatitis prevalence. Many patients ask about the effect of diet on (skin) health. Although exact dietary recommendations are not sufficiently supported by the study, a healthy lifestyle including a healthy diet may result in additional health benefits and possibly affect seborrheic dermatitis. In clinical practice, this would mean advising patients to follow national guidelines regarding diet and emphasizing the protective role of fruit. We hypothesized that psoralens in citrus fruits, leading to additional immunosuppression in combination with UV light, might explain the positive effects of diet on seborrheic dermatitis. This hypothesis is in line with other observational research that shows an association between citrus fruit intake and development of skin cancer.[31] However, it could also be that the diet influences the skin immune system through the gut microbiome. The gut microbiome has an effect on systemic immunity, but may also interfere in skin homeostasis more directly through metabolites passed though the bloodstream to the skin.[32] In the past decade, much has been discovered about the links between diet, the (gut) microbiome and disease (i.e., obesity, inflammatory bowel disease and rheumatoid arthritis), which suggests that we are able to modify our microbiome through diet and influence disease risk.[33]

Given the large sample size from the Rotterdam Study, we were able to validate previously observed associations in smaller studies and create the opportunity to investigate new hypotheses. However, the study design provides associations, rather than causal relations. Several aspects of associations should be considered. The Bradford Hill criteria are a set of principles that may be used to distinguish causal from non-causal associations in epidemiological studies. It states that strong associations are more likely to be casual than weak associations and causality is more probable if associations show consistency, specificity,

temporality and a biological gradient. Furthermore, a plausible explanation for the effect, which is not in conflict with what is already known about the disease or which is substantiated by experimental evidence, might give an additional argument.[34] In our studies, we found small to moderate effect sizes for the risk of seborrheic dermatitis, and were not (yet) able to demonstrate temporality due to the cross-sectional design. However, the findings are mainly consistent with previous observations and were adjusted for known confounders. In studying diet, previous observations were not available, but a dose-response effect was observed. Many biases, as discussed in the separate studies, might have affected the results. Also, participants of the Rotterdam study are mostly of European ascent and the vast majority has skin type I or II according to the Fitzpatrick classification, which might limit the external validity. Therefore, it is important to replicate findings in different populations and in different study designs.

Not all risk factors of interest could be investigated in the Rotterdam study. The possible association between seborrheic dermatitis and Parkinson's disease for example, as there were very few participants with Parkinson's disease that underwent a full body skin examination. An increased sebum production, induced by an increased α-melanocyte stimulation hormone in Parkinson's patients, might be the cause for this previously found association.[19] The occurrence of seborrheic dermatitis in areas with a high sebaceous gland density in combination with the fatty squamae suggests that sebaceous glands play a role in the development of seborrheic dermatitis. However, some studies show that the sebum production in seborrheic dermatitis patients is not higher compared to healthy controls.[35,36] Also, the association between seborrheic dermatitis and Parkinson's disease might also be related to a higher prevalence of seborrheic dermatitis in elderly people.[37] A long-term follow up of participants of Rotterdam Study with a full body skin examination could give a definitive answer. Possibly, seborrheic dermatitis might then even be used as a potential biomarker for Parkinson's disease, as some studies suggest.[38]

Innovations in seborrheic dermatitis research

Genetic susceptibility

Genetic associations assisted us to investigate whether seborrheic dermatitis is part of a spectrum of other diseases. In Chapter 4, we investigated genetic associations between Single Nucleotide Polymorphisms (SNPs) and seborrheic dermatitis. As seborrheic dermatitis was associated with dry skin, might even resemble eczema and has been suggested as a clinical variant of psoriasis, we started the genetic exploration with a candidate gene approach. Despite the similarities, no robust evidence for a shared genetic background between seborrheic dermatitis and atopic dermatitis or psoriasis was observed. It suggests, as most clinicians think, that seborrheic dermatitis is a separate disease entity. However, the lack of overlap may also be due to modest sample size. On the contrary, in a relatively small genome

wide association study (GWAS), we found two genome-wide significant associations for seborrheic dermatitis, indicating that genetic susceptibility probably plays a role in seborrheic dermatitis.

Bioinformatics analysis of the region with the highest genome-wide significant association (rs58331610, *MAST4*, p-value: 1.75 x 10-8) suggested a potential role for *MAST4* in epidermal integrity, which supports the epidemiological observation that barrier integrity is pivotal in seborrheic etiology. However, even though bioinformatics analyses could explain the significant associations, a false positive association cannot be ruled out without a replication of the findings.

In the candidate gene approach, we did not find significant associations after adjusting for multiple testing. Nevertheless, *IL12B,* top hit of the SNP based CGA might be an interesting candidate to investigate in addition to the genome wide significant loci. *IL12* is mainly secreted by antigen presenting cells and has an important function in regulating immune response during infection, which is also disturbed in HIV infected patients.

Due to the large amounts of statistical tests accompanying GWAS, the level of genome-wide significance is set at < 5 x 10-8. Most GWAS studies, including the GWAS described in this thesis, are therefore underpowered to detect all heritability explained by SNPs. Increasing sample size is one method to cope with the multiple testing and as samples sizes have risen above the 100,000 patients in other GWAS, an increasing number of associations are being found.[45] Also, the genetic associations found in this thesis need replication in an independent cohort and/or functional studies of the affected genes need to be performed. Functional or interventional studies will still be necessary to determine the biological meaning of a genetic variation.[46] The development of ex vivo or in vivo models, as Wikramanayake et al. pursuit in their mouse models, will enhance the understanding of the of the observed genetic associations.[47] To replicate our findings, a candidate gene approach in a patient series could be used to replicate the top SNPs from the GWAS. It would be even better to perform a new GWAS in a larger independent cohort with physician diagnosed seborrheic dermatitis, which could replicate our findings and might find additional genetic variations in seborrheic dermatitis. Questionnaire-based phenotypes could be an option, but differentiation from dandruff, dry skin of the scalp or scalp psoriasis is problematic.

The bacterial microbiome

Recent studies showed that not only *Malassezia* yeasts, but also the bacterial microbiome might influence seborrheic dermatitis.[39,40] In Chapter 5 of this thesis, we investigated the role of the bacterial microbiome in seborrheic dermatitis in the largest series of cases and controls so far. In our study, we analyzed the V1 to V3 variable regions of the 16S rRNA gene in order to

investigate the bacterial microbiome and chose to analyse exact amplicon sequence variants (ASVs) instead of operational taxonomic units (OTUs). There are many definitions of an OTU, but in essence an OTU exists of reads that are very similar to each other and are clustered into one unit. For example, reads are clustered if they differ less than 3%. This clustering is done to minimize the effect of sequencing errors, but can induce reference bias or spurious results.[41] An ASVs describes a single exact sequence (reads are not clustered), which can lead to a higher resolution as there is no risk of potentially combining species with different roles in skin biology or pathology in one group (e.g. *S. aureus* versus *S. epidermidis*). Furthermore, ASVs analysis allows comparison across studies more easily.[42] In our study, we found higher abundances of *Staphylococcus* species in lesional skin and were able to separate different species of the genus *Staphylococcus* after a careful phylogenetic analysis. However, not all the ASVs could be called to species level by the bioinformatics pipeline. This was because the thresholds to annotate AVSs by the pipeline to bacteria were very strict in order to overcome sampling errors, which led to many 'unknown' species. The difficulty of identifying bacteria at species and strain resolution is a general issue in skin microbiome studies. First, the density of skin bacterial DNA is much lower than that of for example gut microbiome. A lower density can lead to a higher contamination background, where the DNA of contaminant bacteria is higher than this of the interested target. Adding negative controls at every step of the study, from the sampling collection to the PCR and sequencing reactions, can be used to check this.[43] Secondly, the choice of primers for amplicon-based sequencing is crucial to interpret the output of the sequencing. From previous studies, we know sequencing the V1 to V3 variable regions of the 16S rRNA gene is best for profiling skin bacteria. However, V1-3 sequencing falls short compared to whole genome sequencing, which is still the gold standard, but a lot more expensive.[44] Also, reference panels and analysis pipelines are less extensive and reliable for skin microbiome, as most were developed for gut microbiome.

Future perspectives

More studies to understand the role of microbiome

The bacterial microbiome composition of patients with seborrheic dermatitis differs from individuals without disease and these differences were mainly found in the genus *Staphylococcus*. Separating staphylococci to species level is interesting as we found that not *Staphylococcus aureus*, but other *Staphylococcus* species are associated with seborrheic dermatitis. Coagulase-negative staphylococci, including *S. epidermidis*, *S. capitis,* and *S. caprae,* can prime the skin immune system, which limits the colonization of *S. aureus* and other potential invaders, by shaping the skins CD4+ T cell network and influencing the expression of anti-microbial peptides.[57] However, coagulase-negative staphylococci might also act as pathogens, in particular when the skin barrier is disrupted and in elderly and immunocompromised people.[58,59] Analysis of skin microbial communities combined with global patterns of cutaneous gene expression, as done recently by Fyhrquist et al., seems the next

step forward in understanding these microbe–host interactions.[60] Furthermore, we should study the interaction between *Malassezia* yeast and the bacterial microbiome and how treatment affects the interaction and/or restores a microbial balance. As new treatments that target specific bacteria develop, this information might lead to treatment options.[61] Applying antimicrobial peptides in creams or possibly even applying probiotics in topical therapies may be important themes in future trials.

The human microbiome is seen as the second genome by many researchers. A human compromises more bacterial cells than it has cells of its own.[62] In addition, these microbes produce thousands of metabolites that can interact with human tissue and immunological cells and potentially influence health. An interesting example of microbiome-host interactions is the recent study that showed that the gut microbiome modulates the response to immunotherapy in melanoma patients.[63] These interaction studies of gut microbiome and treatment response show that integration of data is pivotal to make it clinically relevant. Other forms of interaction in microbiome research would be the interaction with host characteristics (including genetics) and lifestyle habits. Starting our research on seborrheic dermatitis, we tried to unravel different aspects of seborrheic dermatitis in a fairly straightforward approach, but we feel that the future lies in multilevel data integration analysis. As demonstrated in Fig 1 of the discussion, seborrheic dermatitis is a complex disease and data of the different research fields should be integrated in future studies.

Host immunity

Observational studies showed that seborrheic dermatitis is more prevalent in populations with a compromised immune system (e.g. due to immunosuppressive medication or HIV), suggesting that immune dysfunction increases the risk and severity of seborrheic dermatitis. The higher prevalence might be explained by an altered immune response to *Malassezia* yeasts, as previously suggested, or more directly due to immune dysregulation.[48,49] The few immunohistological studies of seborrheic dermatitis affected skin demonstrated a pro-inflammatory switch. However, these studies evaluated only a very limited number of markers, or used tape-stripping methods that had not been validated in seborrheic dermatitis. Therefore, conclusions drawn from these studies should be interpreted with caution. An increase of IL1, IL2 and INFy are the only inflammatory cytokines that are documented in more than one study with human subjects.[50-52] In *Mpzl3* knock out mice with a seborrheic-like skin condition a central role for IL-17 was seen. However, IL-17 was not tested in the other studies.[47]

With the upcoming non-invasive samples methods, it would be interesting to create a molecular map of immune and barrier alterations in seborrheic dermatitis skin as done by Hu et. al. for psoriasis and atopic dermatitis.[54] Is seborrheic dermatitis driven by IL-17 and IL-23 pathways similar to psoriasis? Which epidermal barrier genes might be involved and what

abnormalities in lipid biosynthesis/metabolism may be found? These methods could also measure antimicrobial peptides (AMPs), which play a role in the adaptive and innate immune system and have a direct anti-microbial effect. In psoriasis an upregulation of AMPs has been found, whereas in atopic dermatitis there is a downregulation.[55] As treatments targeting AMPs are being developed, this might also be beneficial for seborrheic dermatitis patients.[56]

Collaborations

This discussion started with the statement that seborrheic dermatitis is understudied. In the studies of this thesis, several associations found in previously smaller studies have been replicated. However, there is a need for replication/validation of many new found associations. Future studies would benefit from an international collaborative network that could generate sufficient statistical power to come to more definite conclusions and might also generate new hypotheses or insights in the understanding of seborrheic dermatitis. This thesis might be seen as an invitation for future collaborations.

Concluding remarks

In order to prevent seborrheic dermatitis relapses and to develop a long time cure, a broader understanding of the seborrheic dermatitis etiology is necessary. In this thesis, we gave more insights in possible risk factors for seborrheic dermatitis, laid the foundation for further genetic research and provided more insight in the bacterial microbiome of seborrheic dermatitis. Future research should focus on a better understanding of immune and barrier alterations, replication of genetic variations and multi-level integration of these data.

REFERENCES

1 Bolognia JL, Schaffer JV, Cerroni L. Dermatology, 4th edition. *London: Elsevier.* 2017.
2 Abbas Z, Ghodsi SZ, Abedeni R. Effect of itraconazole on the quality of life in patients with moderate to severe seborrheic dermatitis: a randomized, placebo-controlled trial. *Dermatol Pract Concept* 2016; **6**: 11-6.
3 Szepietowski JC, Reich A, Wesolowska-Szepietowska E *et al.* Quality of life in patients suffering from seborrheic dermatitis: influence of age, gender and education level. *Mycoses* 2009; **52**: 357-63.
4 Xuan M, Lu C, He Z. Clinical characteristics and quality of life in seborrheic dermatitis patients: a cross-sectional study in China. *Health Qual Life Outcomes* 2020; **18**: 308.
5 Palamaras I, Kyriakis KP, Stavrianeas NG. Seborrheic dermatitis: lifetime detection rates. *J Eur Acad Dermatol Venereol* 2012; **26**: 524-6.
6 Johnson MT, Roberts J. Skin conditions and related need for medical care among persons 1-74 years. United States, 1971-1974. *Vital Health Stat 11* 1978: i-v, 1-72.
7 Plunkett A, Merlin K, Gill D *et al.* The frequency of common nonmalignant skin conditions in adults in central Victoria, Australia. *Int J Dermatol* 1999; **38**: 901-8.
8 Breunig Jde A, de Almeida HL, Jr., Duquia RP *et al.* Scalp seborrheic dermatitis: prevalence and associated factors in male adolescents. *Int J Dermatol* 2012; **51**: 46-9.
9 Linder D, Dreiher J, Zampetti A *et al.* Seborrheic dermatitis and hypertension in adults: a cross-sectional study. *J Eur Acad Dermatol Venereol* 2014; **28**: 1450-5.
10 Oztas P, Calikoglu E, Cetin I. Psychiatric tests in seborrhoeic dermatitis. *Acta Derm Venereol* 2005; **85**: 68-9.
11 Misery L, Touboul S, Vincot C *et al.* [Stress and seborrheic dermatitis] Stress et dermatite seborrheique. *Ann Dermatol Venereol* 2007; **134**: 833-7.
12 Bae JM, Ha B, Lee H *et al.* Prevalence of common skin diseases and their associated factors among military personnel in Korea: a cross-sectional study. *J Korean Med Sci* 2012; **27**: 1248-54.
13 Maietta G, Fornaro P, Rongioletti F *et al.* Patients with mood depression have a high prevalence of seborrhoeic dermatitis. *Acta Derm Venereol* 1990; **70**: 432-4.
14 Bergbrant IM. Seborrhoeic dermatitis: A clinical survey. *J EUR ACAD DERMATOL VENEREOL* 1992; **1**: 285-90.
15 Hancox JG, Sheridan SC, Feldman SR *et al.* Seasonal variation of dermatologic disease in the USA: a study of office visits from 1990 to 1998. *Int J Dermatol* 2004; **43**: 6-11.
16 Dinis-Oliveira RJ, Magalhaes T, Moreira R *et al.* Clinical and forensic signs related to ethanol abuse: a mechanistic approach. *Toxicol Mech Methods* 2014; **24**: 81-110.
17 Marino CT, McDonald E, Romano JF. Seborrheic dermatitis in acquired immunodeficiency syndrome. *Cutis* 1991; **48**: 217-8.
18 Binder RL, Jonelis FJ. Seborrheic dermatitis in neuroleptic-induced parkinsonism. *Arch Dermatol* 1983; **119**: 473-5.
19 Mastrolonardo M, Diaferio A, Logroscino G. Seborrheic dermatitis, increased sebum excretion, and Parkinson's disease: a survey of (im)possible links. *Med Hypotheses* 2003; **60**: 907-11.
20 Ikram MA, Brusselle GGO, Murad SD *et al.* The Rotterdam Study: 2018 update on objectives, design and main results. *Eur J Epidemiol* 2017; **32**: 807-50.
21 Gupta AK, Bluhm R. Seborrheic dermatitis. *J Eur Acad Dermatol* 2004; **18**: 13-26.
22 Dessinioti C, Katsambas A. Seborrheic dermatitis: Etiology, risk factors, and treatments: Facts and controversies. *Clin Dermatol* 2013; **31**: 343-51.

23 Farage MA, Miller KW, Berardesca E *et al.* Clinical implications of aging skin: cutaneous disorders in the elderly. *Am J Clin Dermatol* 2009; **10**: 73-86.

24 Mastrolonardo M, Diaferio A, Vendemiale G *et al.* Seborrhoeic dermatitis in the elderly: inferences on the possible role of disability and loss of self-sufficiency. *Acta Derm Venereol* 2004; **84**: 285-7.

25 Sanders MGH, Pardo LM, Franco OH *et al.* Prevalence and determinants of seborrhoeic dermatitis in a middle-aged and elderly population: the Rotterdam Study. *Br J Dermatol* 2018; **178**: 148-53.

26 Man MQ, Lin TK, Santiago JL *et al.* Basis for enhanced barrier function of pigmented skin. *J Invest Dermatol* 2014; **134**: 2399-407.

27 Warshaw EM, Wohlhuter RJ, Liu A *et al.* Results of a randomized, double-blind, vehicle-controlled efficacy trial of pimecrolimus cream 1% for the treatment of moderate to severe facial seborrheic dermatitis. *J Am Acad Dermatol* 2007; **57**: 257-64.

28 Voortman T, Kiefte-de Jong JC, Ikram MA *et al.* Adherence to the 2015 Dutch dietary guidelines and risk of non-communicable diseases and mortality in the Rotterdam Study. *Eur J Epidemiol* 2017; **32**: 993-1005.

29 Lakdawala N, Babalola O, 3rd, Fedeles F *et al.* The role of nutrition in dermatologic diseases: facts and controversies. *Clin Dermatol* 2013; **31**: 677-700.

30 Bronsnick T, Murzaku EC, Rao BK. Diet in dermatology: Part I. Atopic dermatitis, acne, and nonmelanoma skin cancer. *J Am Acad Dermatol* 2014; **71**: 1039 e1- e12.

31 Mahamat-Saleh Y, Cervenka I, Al-Rahmoun M *et al.* Citrus intake and risk of skin cancer in the European Prospective Investigation into Cancer and Nutrition cohort (EPIC). *Eur J Epidemiol* 2020; **35**: 1057-67.

32 Salem I, Ramser A, Isham N *et al.* The Gut Microbiome as a Major Regulator of the Gut-Skin Axis. *Front Microbiol* 2018; **9**: 1459.

33 Singh RK, Chang HW, Yan D *et al.* Influence of diet on the gut microbiome and implications for human health. *J Transl Med* 2017; **15**: 73.

34 Rothman KJ, Greenland S. Causation and causal inference in epidemiology. *Am J Public Health* 2005; **95 Suppl 1**: S144-50.

35 Burton JL, Pye RJ. Seborrhoea is not a feature of seborrhoeic dermatitis. *Br Med J (Clin Res Ed)* 1983; **286**: 1169-70.

36 Ro BI, Dawson TL. The role of sebaceous gland activity and scalp microfloral metabolism in the etiology of seborrheic dermatitis and dandruff. *J Investig Dermatol Symp Proc* 2005; **10**: 194-7.

37 Zander N, Sommer R, Schafer I *et al.* Epidemiology and Dermatological Comorbidity of Seborrhoeic Dermatitis - Population-based Study in 161,000 Employees. *Br J Dermatol* 2019.

38 Ravn AH, Thyssen JP, Egeberg A. Skin disorders in Parkinson's disease: potential biomarkers and risk factors. *Clin Cosmet Investig Dermatol* 2017; **10**: 87-92.

39 Park T, Kim HJ, Myeong NR *et al.* Collapse of human scalp microbiome network in dandruff and seborrhoeic dermatitis. *Exp Dermatol* 2017.

40 Tanaka A, Cho O, Saito C *et al.* Comprehensive pyrosequencing analysis of the bacterial microbiota of the skin of patients with seborrheic dermatitis. *Microbiol Immunol* 2016; **60**: 521-6.

41 Prodan A, Tremaroli V, Brolin H *et al.* Comparing bioinformatic pipelines for microbial 16S rRNA amplicon sequencing. *PLoS One* 2020; **15**: e0227434.

42 Callahan BJ, McMurdie PJ, Holmes SP. Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *ISME J* 2017; **11**: 2639-43.

43 Salter SJ, Cox MJ, Turek EM *et al.* Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. *BMC Biol* 2014; **12**: 87.

44 Meisel JS, Hannigan GD, Tyldsley AS *et al.* Skin Microbiome Surveys Are Strongly Influenced by Experimental Design. *J Invest Dermatol* 2016; **136**: 947-56.

45 Manolio TA. In Retrospect: A decade of shared genomic associations. *Nature* 2017; **546**: 360-1.

46 Huffman JE. Examining the current standards for genetic discovery and replication in the era of mega-biobanks. *Nat Commun* 2018; **9**: 5054.

47 Wikramanayake TC, Hirt P, Almastadi M *et al.* Increased IL-17-expressing gammadelta T cells in seborrhoeic dermatitis-like lesions of the Mpzl3 knockout mice. *Exp Dermatol* 2018; **27**: 1408-11.

48 Tragiannidis A, Bisping G, Koehler G *et al.* Minireview: Malassezia infections in immunocompromised patients. *Mycoses* 2010; **53**: 187-95.

49 Wikramanayake TC, Borda LJ, Miteva M *et al.* Seborrheic dermatitis-Looking beyond Malassezia. *Exp Dermatol* 2019; **28**: 991-1001.

50 Bergbrant IM, Johansson S, Robbins D *et al.* An immunological study in patients with seborrhoeic dermatitis. *Clin Exp Dermatol* 1991; **16**: 331-8.

51 Schwartz JR, Messenger AG, Tosti A *et al.* A comprehensive pathophysiology of dandruff and seborrheic dermatitis - towards a more precise definition of scalp health. *Acta Derm Venereol* 2013; **93**: 131-7.

52 Trznadel-Grodzka E, Blaszkowski M, Rotsztejn H. Investigations of seborrheic dermatitis. Part I. The role of selected cytokines in the pathogenesis of seborrheic dermatitis. *Postepy Hig Med Dosw (Online)* 2012; **66**: 843-7.

53 Donnarumma G, Perfetto B, Paoletti I *et al.* Analysis of the response of human keratinocytes to Malassezia globosa and restricta strains. *Arch Dermatol Res* 2014; **306**: 763-8.

54 He H, Bissonnette R, Wu J *et al.* Tape strips detect distinct immune and barrier profiles in atopic dermatitis and psoriasis. *J Allergy Clin Immunol* 2020.

55 Clausen ML, Slotved HC, Krogfelt KA *et al.* In vivo expression of antimicrobial peptides in atopic dermatitis. *Exp Dermatol* 2016; **25**: 3-9.

56 Pfalzgraff A, Brandenburg K, Weindl G. Antimicrobial Peptides and Their Therapeutic Potential for Bacterial Skin Infections and Wounds. *Front Pharmacol* 2018; **9**: 281.

57 Parlet CP, Brown MM, Horswill AR. Commensal Staphylococci Influence Staphylococcus aureus Skin Colonization and Disease. *Trends Microbiol* 2019; **27**: 497-507.

58 Natsis NE, Cohen PR. Coagulase-Negative Staphylococcus Skin and Soft Tissue Infections. *Am J Clin Dermatol* 2018; **19**: 671-7.

59 Brown MM, Horswill AR. Staphylococcus epidermidis-Skin friend or foe? *PLoS Pathog* 2020; **16**: e1009026.

60 Fyhrquist N, Muirhead G, Prast-Nielsen S *et al.* Microbe-host interplay in atopic dermatitis and psoriasis. *Nat Commun* 2019; **10**: 4703.

61 de Wit J, Totte JEE, van Mierlo MMF *et al.* Endolysin treatment against Staphylococcus aureus in adults with atopic dermatitis: A randomized controlled trial. *J Allergy Clin Immunol* 2019; **144**: 860-3.

62 Sender R, Fuchs S, Milo R. Are We Really Vastly Outnumbered? Revisiting the Ratio of Bacterial to Host Cells in Humans. *Cell* 2016; **164**: 337-40.

63 Gopalakrishnan V, Spencer CN, Nezi L *et al.* Gut microbiome modulates response to anti-PD-1 immunotherapy in melanoma patients. *Science* 2018; **359**: 97-103.

7 Summary
Samenvatting

SUMMARY

Chapter 1 gives the introduction to the thesis. It contains general information about the clinical spectrum, diagnoses and treatment of seborrheic dermatitis and the Rotterdam Study, an ongoing large population based cohort study of a middle aged and elderly population, is discussed. Also, it gives the main aim of this thesis; In this thesis we aimed to provide more insight in the common skin disease seborrheic dermatitis by investigating the occurrence and determinants of seborrheic dermatitis.

Many skin diseases, including seborrheic dermatitis, are not identified or neglected in the general population. Therefore, in **Chapter** 2 we aimed to provide more insight in the occurrence of skin diseases, we estimated the point prevalence and age- and sex-adjusted standardized prevalence rates of common inflammatory and (pre)malignant skin diseases in the Rotterdam study. After analyses of the full body skin examinations, actinic keratosis was the most common skin disorder found in the population (26.1%), followed by seborrheic dermatitis (13.3%). Further, we found at least one cutaneous malignance in about one in twenty-five skin examination. Although the most of the prevalent skin diseases have a low mortality, the can severely impact quality of life. We concluded that diagnosis, treatment and prevention of skin diseases should get sufficient priority in general healthcare education and policies.

In **Chapter 3** we aimed to establish which lifestyle and physiological factors are associated with seborrheic dermatitis. Knowledge about determinants of seborrheic dermatitis may give new insights in the pathophysiology, possibly leading to new interventions. The chapter consists of two parts.

In **Part 1**, we investigated determinants that had been suggested to influence disease risk in 5,498 participants of the Rotterdam Study, of which 788 had seborrheic dermatitis. We conducted a multivariable logistic regression including: age, sex, obesity, skin colour, stress, depression, education level, hypertension, climate, xerosis cutis, alcohol and tobacco use. We found that male sex, light skin colour, winter season and a generalized xerosis cutis were associated with seborrheic dermatitis, while obesity, depression, hypertension, alcohol consumption and tobacco use were not. Further, we investigated if total testosterone in men and the free androgen index in woman might influence disease risk, which might explain the difference in prevalence between genders, but we did not find a significant difference.

In **Part 2** we looked into the effects of diet on seborrheic dermatitis. The objective of this study was to determine whether specific dietary patterns or total antioxidant capacity were associated with the disease. In this study, we used the data of 4,379 participants of the Rot-

terdam Study who underwent a full body skin examination and filled out a food frequency questionnaire. We defined four dietary patterns by means of a principal component analysis and characterized these as a vegetables dietary pattern, a Western pattern (driven by meat, potato, and alcohol consumption), a fruit pattern and a fat pattern (driven by olive oil and other healthy and unhealthy fats). We concluded that a high fruit intake was associated with less seborrheic dermatitis, whereas high adherence to a "Western" dietary pattern in females was associated with more seborrheic dermatitis. Further, we assessed the total antioxidant capacity of the diet on the basis of ferric reducing antioxidant potential of each food item, but we did not find evidence for an association between antioxidant intake and seborrheic dermatitis.

In **Chapter 4**, we searched for genetic susceptibility in seborrheic dermatitis patients. First, we investigated genes that are implicated in the susceptibility of psoriasis and atopic dermatitis by means of a candidate-gene approach (CGA) study. We did not find robust evidence for a shared genetic background between seborrheic dermatitis and psoriasis or atopic dermatitis. However, some loci (e.g. *LCE3* and *MICB*) might be interesting to investigate further. In addition, we performed a case-control genome-wide association study (GWAS) in 4,050 participants, of which 609 were diagnosed with seborrheic dermatitis. We found two significant SNPs in the GWAS: r58331610 (p-value: 1.75 x 10-8) that mapped to the *MAST4* gene and rs16944244 (p-value: 2.10 x 10-8) that mapped to an intergenic region between genes *PIRT* and *SHISA*. This study gives a basis for further genetic research on seborrheic dermatitis and could lead to a next step in the understanding of the seborrheic dermatitis pathogenesis.

In **Chapter 5** we profiled the bacterial microbiome of the nasolabial fold of participants with seborrheic dermatitis and compared this to the same skin areas of individuals without seborrheic dermatitis (lesional skin: n=22; non-lesional skin SD: n=75; controls: n=200). We characterized the bacterial microbiome using the 16S rRNA V1-V3 regions and estimated the α- diversity between cases and controls. In addition, we tested for associations between the microbiome composition between cases and controls using multivariate statistics (permANOVA) and univariate statistics. We found an increased α-diversity between seborrheic dermatitis lesional cases versus controls (Shannon diversity: Kruskal-Wallis rank sum: Chi-squared: 19.06; global p-value= 7.7×10^{-5}). Multivariable statistical analysis showed significant associations in microbiome composition when comparing lesional SD skin to controls (p-value=0.03;R2=0.1%). Seven out of 12 amplicon sequence variant (ASVs) that were different between controls and lesional cases were members of the genus *Staphylococcus sp*, most of which showed increased composition in lesional cases, and were closely related to *S. capitis S. caprae* and *S. epidermidis*. Future studies are required to validate these findings.

Chapter 6 consists of the general overview of the main findings. Furthermore, our view on future perspectives is presented. In order to prevent seborrheic dermatitis relapses and to develop a long time cure, a broader understanding of the seborrheic dermatitis etiology is necessary. In this thesis, we gave more insights in possible risk factors for seborrheic dermatitis, laid the foundation for further genetic research and provided more insight in the bacterial microbiome of seborrheic dermatitis. Future research should focus on a better understanding of immune and barrier alterations, replication of genetic variations and multilevel integration of these data.

NEDERLANDSE SAMENVATTING

Hoofdstuk 1 van dit proefstuk bestaat uit de algemene introductie. Het beslaat onder andere het klinische spectrum van seborrhoisch eczeem, de differentiaal diagnose en de behandeling. Verder wordt er uitleg gegeven over de Rotterdam Studie, de prospectieve populatie-gebaseerde studie welke de basis vormt van de beschreven studies. Daarnaast wordt er verder ingegaan op het doel van het proefschrift; We pogen nieuwe inzichten te verkrijgen in deze veel voorkomende huidaandoening, door zowel de prevalentie te onderzoeken, als het onderzoek naar een verscheidenheid van mogelijke determinanten voor seborrhoisch eczeem.

De prevalentie van vele huidaandoeningen is niet duidelijk in de algemene bevolking en seborrhoisch eczeem wordt vaak niet herkend of genegeerd. In **hoofdstuk 2** van dit proefschrift geven we meer inzicht in de prevalentie van veel voorkomende huidaandoeningen. Dit hebben we gedaan door de puntprevalentie en de naar leeftijd en geslacht gestandaardiseerde prevalenties te berekenen van veel voorkomende huidaandoeningen van de deelnemers van de Rotterdam Studie. Actinische keratoses werden het vaakst gedocumenteerd tijdens het volledige lichamelijk onderzoek (26,1%), gevolgd door seborrhoisch eczeem (13,3%). Verder vonden we ten minste één vorm van huidkanker bij 1 op de 25 deelnemers. Ook al hebben de meeste veel voorkomende huidaandoeningen een lage mortaliteit, ze kunnen wel degelijk de kwaliteit van leven aantasten. Daarom zijn wij van mening dat diagnose, behandeling en preventie van huidaandoeningen genoeg prioriteit moet krijgen bij het opleiden van artsen en in de landelijke richtlijnen.

In **hoofdstuk 3** onderzochten we welke levensstijl en fysiologische factoren mogelijk een rol spelen in seborrhoisch eczeem. Deze kennis kan mogelijk bijdragen aan nieuwe inzichten in de pathofysiologie en dat kan op zijn beurt weer bijdragen aan mogelijke nieuwe behandelingen of adviezen. Dit hoofdstuk bestaat uit twee delen.

In **deel 1** onderzochten we associaties welke eerder werden gevonden in andere studies. Dit deden we middels een multivariabele logistische regressie in 5.498 deelnemers van de Rotterdam Studie van wie er 788 seborrhoisch eczeem hadden. We includeerde de volgende variabelen: leeftijd, geslacht, BMI, huidskleur, stress, depressie, opleidingsniveau, hypertensie, klimaat, droge huid, alcohol gebruik en roken. Het mannelijk geslacht, een lichte huidskleur, het winter seizoen en een droge huid bleken risicofactoren in ons model. De andere factoren bleken geen significante associatie te hebben. Verder onderzochten we of het verschil in risico tussen man en vrouw misschien door hormonen werd veroorzaakt. We keken naar het totale testosteron in mannen en de vrije androgene index in vrouwen, maar hier hebben we geen bewijs voor gevonden.

In **deel 2** van dit hoofdstuk hebben we gekeken naar het dieet van de deelnemers van de Rotterdam Studie. We onderzochten of een specifiek dieet of de antioxidanten in een dieet mogelijk geassocieerd zouden zijn met seborrhoisch eczeem. In deze studie includeerden we deelnemers van de Rotterdam Studie die zowel de vragenlijsten met betrekking tot voeding hadden ingevuld, als een volledig lichamelijk onderzoek hebben gehad, dit waren in totaal 4.378 deelnemers. De antwoorden op de vragenlijsten werden gecategoriseerd in een dieetpatroon door middel van een principale component analyse. Hieruit volgden 4 dieetpatronen, het eerste patroon werd gekarakteriseerd door groente, de tweede door fruit, de derde door vlees, aardappelen en alcohol (het "westers dieet") en de vierde door vetten en oliën. Na analyse van deze patronen concludeerden we dat een hoge fruit consumptie geassocieerd was met een lagere kans op het hebben van seborrhoisch eczeem. Een "westers dieet" was geassocieerd met een hogere kans, maar alleen voor vrouwen. Een maat voor de totale hoeveelheid antioxidanten in het dieet was niet significant geassocieerd met de ziekte.

In **hoofdstuk 4** onderzochten we of er een genetische predispositie bestaat voor het krijgen van seborrhoisch eczeem. In eerste instantie hebben we dit gedaan door te kijken of genen die een rol spelen in atopisch eczeem of psoriasis, ook een rol spelen in seborrhoisch eczeem. Dit deden we in een zogenoemde kandidaatgen benadering in 4.050 deelnemers, waarvan 609 met seborrhoisch eczeem. Echter, we vonden geen duidelijk bewijs voor een rol van deze genen in seborrhoisch eczeem. Wel waren er enkele bevindingen welke mogelijk interessant zijn om verder te onderzoeken, bijvoorbeeld in de genen *LCE3* en *MICB*. In dezelfde groep voerden we daarna een genoom wijde associatie studie uit. Hierin vonden we twee significante variaties: r58331610 (p-waarde: 1,75 x 10-8) welke gelegen is in het *MAST4* gen en rs16944244 (p-waarde: 2,10 x 10-8) welke gelegen was tussen de genen *PIRT* en *SHISA*.

In **hoofdstuk 5** hebben we onderzoek gedaan naar het microbioom van mensen met seborrhoisch eczeem. Uit eerdere studies blijkt dat Malassezia gisten een rol lijken te spelen in het ziekte proces. In deze studie onderzochten we het bacteriële microbioom in mensen met seborrhoisch eczeem. Het bacteriële microbioom van de neusplooi van deelnemers met en zonder seborrhoisch eczeem werd met elkaar vergeleken (leasionale huid: n=22; niet leasionale huid: n=75; controles: n=200). Het bacteriële microboom werd bepaald door het analyseren van de V1-V3 regio's van het 16S-rRNA. We vonden dat de α-diversiteit in leasionale huid was toegenomen ten opzichte van controles (Shannon diversity: Kruskal-Wallis rank sum: Chi-squared: 19.06; global p-value= 7.7x10-5). In de multivariabele analyse vonden we tevens een verschil tussen aangedane huid en controles (p-waarde= 0,03;R2=0.1%). Van de 12 meest significante amplicon sequence variants (ASVs), behoorden er 7 tot de *Staphylococcus sp.* Deze waren voornamelijk toegenomen in leasionale huid en het dichts gerelateerd aan *S. capitis S. caprae* en *S. epidermidis.* Een ASV welke behoorde tot het genus *Cutibacterium* liet juist een verminderde hoeveelheid zien ten opzichten van gezonde huid.

Hoofdstuk 6 geeft een overzicht weer van de belangrijkste bevindingen van dit proefschrift. Daarnaast wordt besproken wat de implicaties zijn van deze bevindingen en wat dit mogelijk betekent voor de toekomst. Meer onderzoek is nodig om de etiologie beter in kaart te brengen, zodat we patiënten met seborrhoisch eczeem beter kunnen behandelen. In dit proefschrift lieten we zien welke risicofactoren mogelijk een rol spelen in seborrhoisch eczeem, onderzochten we de rol van genetica, en deden we onderzoek naar het bacteriële microbioom. Toekomstige studies zouden zich onder andere kunnen richten op de replicatie van onze bevindingen, of op de rol van het immuunsysteem en de barrière functie van de huid.

8

Abbreviations
List of co-authors
List of publications
Curriculum vitae
PhD Portfolio
Dankwoord

ABBREVIATIONS

A1	reference allele
A2	other allele
AD	atopic dermatitis
AK	actinic keratosis
ASVs	amplicon sequencing variants
BCC	basal cell carcinoma
BMI	body mass index
CESD	Center for Epidemiologic Studies Depression Scale
CGA	candidate gene approach
Chr	chromosome
CI	confidence interval
FAI	free androgen index
FBSE	full body skin examination
FFQ	food frequency questionnaire
FRAP	ferric-reducing ability of plasma
GWAS	genome-wide association study
IQR	interquartile range
MET	metabolic equivalent
OR	odds ratio
OTUs	operational taxonomic units
PCA	principal component analysis
PR	prevalence ratio
PSO	psoriasis
RS	Rotterdam Study
SCC	squamous cell carcinoma
SD	seborrheic dermatitis
SNP	single-nucleotide polymorphism
UV	ultraviolet

LIST OF CO-AUTHORS

Affiliations at the time at which the research was conducted

Oscar H. Franco
Department of Epidemiology, Erasmus Medical Center, Rotterdam, the Netherlands.

Rebecca S. Ginger
Unilever Research and Development, Colworth Science Park, Sharnbrook, U.K.

Sterre A.S. Hamann
Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands.

Merel A. Hamer
Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands.

Jessica C. Kiefte-de Jong
Department of Epidemiology, Erasmus MC, Rotterdam, The Netherlands;
Leiden University College, The Hague, The Netherlands.

Robert Kraaij
Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands

Tamar Nijsten
Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands.

Luba M. Pardo Cortes
Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands.

Adrian M. Smith
Unilever Research and Development, Colworth Science Park, Sharnbrook, U.K.

André G. Uitterlinden
Department of Internal Medicine, Erasmus Medical Center, Rotterdam, The Netherlands
Department of Epidemiology, Erasmus Medical Center, Rotterdam, The Netherlands.

Joris A.C. Verkouteren
Department of Dermatology, Erasmus Medical Center, Rotterdam, the Netherlands.

Joost A.M. Verlouw
Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands

PUBLICATIONS

In this thesis

Sanders MGH, Pardo LM, Verkouteren JAC, Hamann SAS, Hamer MA, Nijsten T. Dermatological screening of a middle-aged and elderly population: the Rotterdam Study. Br J Dermatol. 2017 Oct;177(4):e98-e100. doi: 10.1111/bjd.15359. Epub 2017Aug 13. PubMed PMID: 28145062.

Sanders MGH, Pardo LM, Uitterlinden AG, Smith AM, Ginger RS, Nijsten T. The Genetics of Seborrheic Dermatitis: A Candidate Gene Approach and Pilot Genome-Wide Association Study. J Invest Dermatol. 2018 Apr;138(4):991-993. doi: 10.1016/j.jid.2017.11.020. Epub 2017 Dec 2. PubMed PMID: 29203360.

Sanders MGH, Pardo LM, Franco OH, Ginger RS, Nijsten T. Prevalence and determinants of seborrhoeic dermatitis in a middle-aged and elderly population: the Rotterdam Study. Br J Dermatol. 2018 Jan;178(1):148-153. doi: 10.1111/bjd.15908. Epub 2017 Dec 8. PubMed PMID: 28856679.

Sanders MGH, Pardo LM, Ginger RS, Kiefte-de Jong JC, Nijsten T. Association between Diet and Seborrheic Dermatitis: A Cross-Sectional Study. J Invest Dermatol. 2019 Jan;139(1):108-114. doi: 10.1016/j.jid.2018.07.027. Epub 2018 Aug 18. PubMed PMID: 30130619.

Sanders MGH, Nijsten T, Verlouw J, Kraaij R, Pardo LM. Composition of cutaneous bacterial microbiome in seborrheic dermatitis patients: a cross-sectional study
Accepted, PLOS ONE

Sanders MGH, Pardo LM, Nijsten T. Seborrheic dermatitis: clinical management, epidemiology and pathophysiology.
Submitted

CURRICULUM VITAE

Martijn Sanders werd geboren op 18 oktober 1989 in Almelo, waar hij ook opgroeide. Na het behalen van zijn VWO diploma op de openbare scholengemeenschap Het Erasmus in 2008, startte hij hetzelfde jaar aan de studie geneeskunde aan het Erasmus MC te Rotterdam. Tijdens zijn studie beklede hij meerdere functies in commissies binnen de Rotterdamse Studentenvereniging Sanctus Laurentius. In 2012 bracht hij een half jaar door op Aruba, waar hij onderzoek deed naar dengue ter behoeve van zijn masterscriptie. Tijdens zijn coschappen werd zijn interesse in de dermatologie steeds groter, waarop hij besloot zijn tijd van zowel zijn keuze- als zijn oudste coschappen binnen de dermatologie te besteden in respectievelijk Het Franciscus Gasthuis en het Amphia Ziekenhuis. In 2015 gaf prof. Tamar Nijsten hem de kans om aansluitend aan zijn coschappen fulltime te beginnen aan zijn promotieonderzoek, mede onder begeleiding van dr. Luba Pardo Cortes. De drie daaropvolgende jaren werden fulltime besteed aan dit onderzoek, maar ook zijn klinische interesse in de dermatologie begon meer en meer op te spelen. In 2018 startte hij naast zijn promotieonderzoek aan de opleiding tot dermatoloog binnen het Erasmus MC, waar hij op dit moment nog werkzaam is.

PHD PORTFOLIO

Name PhD student:	M.G.H. Sanders
Erasmus MC Department:	Dermatology
Research schools:	NIHES / MolMed
PhD period:	2015 – 2021
Promotor:	Prof. T.E.C. Nijsten
Copromotor:	Dr. L.M. Pardo Cortes

Activity	Year	Workload
Courses / workshops / seminars		
CPO Course: Patient Oriented Research: design, conduct, analysis and clinical implications	2015	0.3 ECTS
Biostatistics for Clinicians	2015	0.7 ECTS
Principles of Epidemiologic Data-analysis	2015	0.7 ECTS
Basic Course on 'R'	2015	2 ECTS
Integrity in Research	2016	0.3 ECTS
Biostatistical Methods 1: Basic principles	2016	5.7 ECTS
Biomedical English Writing Course for MSc and PhD-students	2016	2 ECTS
Workshop on Microsoft Excel 2010: Basic and Advanced	2016	0.7 ECTS
Course Microbiomics I	2016	0.1 ECTS
Regelgeving en organisatie (BROK), Erasmus MC	2017	1.5 ECTS
Presenting Skills for junior researchers	2017	1 ECTS
English biomedical writing and communication	2017	2 ECTS
Unilever ICP studentship workshop, Colworth, Engeland	2018	1 ECTS
DOO-course Teach the Teacher	2019	1 ECTS
Conferences – Oral presentations		
Dermatological screening of a middle-aged and elderly population: the Rotterdam Study. IDEA-KeraCon, Aurora, Colorado	2016	1 ECTS
Prevalence and determinants of seborrhoeic dermatitis in a middle-aged and elderly population: the Rotterdam Study. EDEN, Madrid, Spain	2017	1 ECTS
The epidemiology and genetics of seborrheic dermatitis. Skintermezzo, Rotterdam, Netherlands	2017	1 ECTS

The Genetics of Seborrheic Dermatitis: A Candidate Gene Approach and Pilot Genome-Wide Association. NVED, Lunteren.	2018	1 ECTS
Case presentation. SNNDV Themadag. Antwerp, Belgium	2019	1 ECTS
Seborrheic dermatitis: new perspectives. EADV, online.	2020	1 ECTS
Conferences - attending		
KNAW symposium Genetic Screening, Amsterdam, Netherlands	2015	1 ECTS
45th European Society for Dermatological Research Meeting, Rotterdam, Netherlands	2015	1 ECTS
Gut day (focus on microbiome), Rotterdam, Netherlands	2015	1 ECTS
PhD-Day 2016, balance your career	2016	1 ECTS
Symposium "Microbiome and skin", Paris, France	2016	1 ECTS
Department of Dermatology Annual PhD weekend	2015-2018	4 ECTS
Weekly journal clubs and research meeting, Erasmus MC, Rotterdam	2015-2018	2 ECTS
SNNDV, Maastricht, Netherlands	2019	1 ECTS
Skintermezzo meetings, Erasmus MC, Rotterdam	2015-2020	1 ECTS
Teachings		
Big Data. PhD-weekend, Department of Dermatology, Erasmus MC	2017	0.5 ECTS
EADV course on Clinical Research and Epidemiology, Erasmus MC	2017	0.5 ECTS
Education for medical interns: surgical skills, STDs , inflammatory diseases	2016-2019	2 ECTS
Basic dermatology for nurses (anesthetists)	2018-2019	1 ECTS
Other		
Organizing committee PhD weekend, Department of Dermatology, Erasmus MC, Rotterdam	2017	1 ECTS

DANKWOORD

En dan zit ik op onze werkkamer ineens het dankwoord te schrijven, het voelt onwerkelijk.

Zes jaar later, twee jaar klinische ervaring in het EMC, een jaar in Dordrecht, een flinke verbouwing en verhuizing verder, vier nichtjes erbij, nieuwe vriendschappen gemaakt en bestaande vriendschappen nog hechter geworden, nog meer collega's mogen ontmoeten, een jaar Covid-19 en heel wat pieken en dalen in het gehele promotietraject.

Het is een feest om op dit punt te zijn beland, en ik wil dan ook graag iedereen bedanken die mij heeft geholpen, zowel op de werkvloer als daarbuiten.

Prof. dr. Nijsten, Beste Tamar, ik ben je ontzettend dankbaar voor de kansen die je mij hebt gegeven de afgelopen jaren. In de oudbouw kwam je regelmatig langslopen om er voor te zorgen dat ik niet met vragen bleef zitten en om het promotie traject in goede banen te leiden. Je zorgde dat vraagstukken waar ik soms een week mee zat, ineens een makkie leken. De afgelopen jaren stonden voor mij regelmatig in het teken van mijn klinische werkzaamheden en spraken we elkaar minder vaak, maar toch heb je me weten te stimuleren om dit proefschrift tot een goed einde te brengen. Onze beklimming (en afdaling!) van de Pikes Peak in de Rocky Mountains is ook een ervaring om niet te vergeten!

Dear Luba, without your critical view and your extensive knowledge on statistics, genetics and microbiome the publications in this thesis would not have been possible. Most of the times you are a serious person, who thought me to be very critical on my research and on that of others, but when I think of you, I immediately think of your humour! Thank you very much for guiding me as my co-promotor!

Prof. dr. Prens, beste Errol, bedankt dat u de rol van secretaris in mijn leescommissie op u wilde nemen. Ik ben ervan onder de indruk dat u voor elk probleem een oplossing heeft, of er anders een bedenkt met uw buitengewone kennis van de wetenschap. Beste prof. dr. Uitterlinden en prof. dr. Lambert, heel erg bedankt voor het doornemen van mijn proefschrift en het plaatsnemen in mijn leescommissie. Ik zie er naar uit u te treffen bij de promotie.

Beste leden van de grote commissie, prof. dr. De Jong, prof. dr. Pasmans, dr. Gunn, prof. dr. Kiefte – de Jong en dr. Reeder. Dank voor de bereidheid om deel te nemen aan de commissie. Beste Suzan, afgelopen jaar heb jij er samen met de andere collega's uit het ASZ voor gezorgd dat ik een ontzettend prettig en leerzaam jaar heb gehad. Het was mede de energie die ik van dit jaar heb gekregen, die mij het doorzettingsvermogen gaf om alles af te ronden. Ik ben erg blij dat jullie nu ook onderdeel zijn van mijn promotie.

Aan alle coauteurs die hebben meegewerkt aan de artikelen en aan alle medewerkers op het ERGO-centrum, ik heb veel goede herinneringen aan onze samenwerking. Heel erg bedankt! Ook bedank ik natuurlijk alle deelnemers van de Rotterdam Studie die naar het ERGO-centrum kwamen. Zonder jullie was mijn proefschrift, en die van vele anderen, niet mogelijk geweest.

Het voelt al weer een eeuwigheid geleden dat we dagelijks met elkaar in GK-026 zaten. Merel, Joan, Kirtie en Joris, ik heb heel veel aan jullie gehad die eerste jaren en vond het ook ontzettend gezellig! Merel en Joris, zonder jullie tips waren de maanden die het kostte om alle genetica analyses te snappen waarschijnlijk een jaar geworden, daarnaast ook bedankt voor het doorlezen van mijn proefschrift voordat het naar de drukker ging!

Sven en Selma, jullie natuurlijk in het bijzonder bedankt voor het aanjagen van de vrijdag-middagborrels, maar ook voor het proofreaden van delen van mijn proefschrift. Viola, dank voor alle waardevolle hulp bij het proofreaden, je weet me te vinden als het bij jou zo ver is! Tristan, bedank voor alle lol op en naast het werk en natuurlijk ook voor je sponsoring van dit boekje. Ik kom het nog bij je innen.

Aan alle AIOS, ANIOS en onderzoekers, door jullie is het een genot om op onze afdeling te werken en ook buiten de werkvloer was het vaak een feest. Hopelijk kan dit allemaal snel weer worden opgepakt. Als een feest meteen na mijn promotie niet mogelijk is, houden jullie het zeker van mij tegoed!

Lieve Saar. Ik vind het een eer dat jouw werk op de omslag van dit boekje staat, ik ben er zo ontzettend blij mee!

Lieve vrienden en (schoon)familie, bedankt dat jullie er altijd voor mij zijn. Er zijn te veel mensen om op te noemen die mij hebben gesteund de afgelopen jaren, ook door soms juist niet naar mijn proefschrift te vragen ;) Een aantal mensen wil ik graag extra bedanken.

Mijn paranimfen Fons en Xavier. Fons, er valt altijd wat met jou te beleven en ik zal me geen moment vervelen met jou in de buurt. Je staat bekend om je enorme kennis van medicijnen en je bent een geweldige arts geworden. Xav, nadat je ons eerste studentenhuis verliet, was het niet vanzelfsprekend dat we elkaar later nog weer zo zouden opzoeken, maar ik ben ontzettend blij dat we dat hebben gedaan! Je bent één van mijn dierbaarste vrienden en ik hoop dat dit ook altijd zo zal blijven.

Lieve pap en mam, ik ben ontzettend trots dat jullie mijn ouders zijn, met al jullie positiviteit, doorzettingsvermogen en liefde kan ik mij geen betere ouders wensen. Met al jullie steun sta

ik vandaag waar ik sta en ben ik blij met de persoon die ik ben geworden. Ik vind het ontzettend fijn dat jullie sinds kort ook voor een gedeelte in Rotterdam wonen en dat we elkaar nu een stuk vaker kunnen zien! Lieve Jo en Steijn, jullie staan altijd voor mij en Floor klaar en het voelt ontzettend fijn en vertrouwd om jullie zo dicht bij mij te hebben. Jullie hebben mij door het gehele promotietraject, zowel de pieken als de dalen, ontzettend gesteund en aangemoedigd en daar ben ik jullie heel dankbaar voor! Ik ben zo blij voor jullie dat alles nu zo de goede kant op gaat!

Allerliefste Floor, jou moet ik natuurlijk het meest bedanken van iedereen. Ik denk niet dat ik het boekje af had gekregen zonder al je steun de afgelopen jaren. Naast dat je altijd geweldig lief voor me bent, heb je me ontzettend geholpen met het nalezen van stukken, maar vooral ook met je management talenten (en lijstjes ;)). Het is een feest om met jou samen te zijn en ik kan me geen leven zonder jou voorstellen! Ik hou van je.