Beyond Skin Aging, Towards Health

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CONTENTS

Part I

INTRODUCTION

CHAPTER 1

General introduction

ABOUT SKIN AGING

Skin aging is the result of a complex ensemble of endogenous and exogenous factors, which influence the composition and breakdown of skin as humans age. Skin aging signs are caused by both chronological and extrinsic aging [1]. Chronological aging of skin results in both thinner dermis and epidermis, as vascularity and cellularity decrease. There is a decrease in the number of fibroblasts which produce less collagen both in terms of quality as in quantity. Less elastin is produced and subcutaneous fat diminishes, which further weakens the skin's foundations. Reactive oxygen species (ROS), a byproduct of cellular metabolism, build up and cause damage to important cellular structures, including to our deoxyribonucleic acid (DNA). These changes result in reduced elasticity and integrity of the skin [2]. Transepidermal water loss (TEWL) increases, which further declines barrier function [3]. External factors ultraviolet (UV) irradiation of the skin together with smoking, to date, are the most important known risk factors associated with skin aging [4, 5]. Degradation of collagen in the extracellular matrix is activated trough UV and Tobacco-induced increased ROS. Matrix metalloproteinases (MMPs) are activated which do not only promote dermal matrix breakdown, but also inhibit collagen synthesis by MMP-generated collagen degradation products [6]. Accumulation of ROS causes dermal damage that eventually causes skin aging phenotypes.

Facial skin aging is a complex phenotype, where different aspects contribute to an aged appearance. These aspects or phenotypes include wrinkling, sagging, pigmented spots and telangiectasia. Dry skin can also be regarded as a feature of an aging skin, although it can also be a symptom of disease in e.g. atopic dermatitis and ichthyosis. Although chronological aging promotes all of the above mentioned features of skin aging, it is unclear whether the different phenotypes have similar genetic and epidemiological determinants. At the same time, looking old for one's age raises the question whether someone's insides also age more rapidly.

HOW TO MEASURE SKIN AGING

Over the years, several scoring systems have been developed to capture different aspects and severity of skin aging. Daniell was a pioneer in the skin wrinkling epidemiology, who discovered an important association between smoking and skin wrinkling of the crow's feet in the early seventies [4]. Griffiths was one of the first to develop a photonumeric scale for the assessment of cutaneous photodamage [7]. In this nine-point scale, fine and course wrinkles, yellowness of the skin and mottled hyperpigmentation were scored. Glogau also developed a four category photoaging scale with a main focus on wrinkles where type I was, "no wrinkles"; type II, "wrinkles in motion"; type 3, "wrinkles at rest"; and type 4, "only wrinkles"[8]. These scores predominantly focus on extrinsic factors for skin aging, including UV exposure and smoking. However, both internal and external factors contribute to an aged look. The 'SCINEXA' tool was developed to

simultaneously assess both factors and comprises five items indicative of intrinsic and 18 items highly characteristic of extrinsic skin aging [9].

Aforementioned scores are compound methods, where multiple items are combined into one score. However, one can imagine that for example skin wrinkling and telangiectasia have a different etiology, since one is (partly) the result of mimicry and the other comprises the dilatation of small vessels. Therefore, it seems reasonable to investigate these phenotypes separately. This creates opportunity to dive deeper into mechanisms of skin aging and to discover possible contradictory determinants for different phenotypes.

Practically it comes down to two perspectives; either man-scored or machine-scored. Both methods have its benefits and disadvantages. Where scoring by a human panel can introduce bias, for example based on the background of the assessor, digital methods can be subject to technical errors and its interpretability can be challenging (i.e., what is the clinically important difference?).

ROTTERDAM STUDY

The Rotterdam Study (RS) is a large population-based cohort which started in 1990 in a large suburb located North-East of Rotterdam, named Ommoord. The aim of this prospective study when initiated, was to investigate the risk factors of cardiovascular, neurological, ophthalmological and endocrine diseases in the elderly. Examinations were repeated every 3-5 years in order to gain insight into early signs of developing diseases in the elderly. As the study yielded promising results into the pathophysiology and risk factors for several diseases, it expanded its scope towards other fields of medicine [10]. Dermatology joined the Rotterdam Study in 2010 [11]. All participants underwent a Full Body Skin Examination (FBSE) and were photographed. In the RS, a digital method was used for the grading of the severity of pigmented spots, wrinkles and telangiectasia. These standardized photographs were taken by trained physicians, using the Premier 3dMDface3-plus UHD camera (Atlanta, GA). In this setting, participants were instructed not to wear any jewelry, creams or make-up. From these three-dimensional pictures, two-dimensional masks were extracted using a semi-automated script in MATLAB (version 2013a, The MathWorks, Natick, MA). This method has been validated and has successfully been used in several studies [12-14]. Dry skin was visually examined during an FBSE and scored as absent, localized (limited to extensor side of arms and legs) or as generalized. Clinical dermatological diseases were scored at the center whereas dietary habits and medication use were collected via questionnaires.

PHENOTYPES OF SKIN AGING EXPLORED IN THESIS

Wrinkles

Facial wrinkles are probably the most notable feature of skin aging. They are often also one of the first signs which appear on an aging face. The most important associated epidemiological risk factors are smoking and UV exposure [4, 5]. Other parameters such as a higher body mass index (BMI) are associated with fewer wrinkles [15], probably because of the filler effect of subcutaneous tissue in wrinkle appearance. In the RS, both physiological and lifestyle factors associated with facial wrinkles were investigated. Here, they discovered and replicated several factors which were associated with facial wrinkling and that these determinants (the localization and severity of the wrinkles) differed between men and women [13, 16]. The most important risk factors are lifestyle-related parameters. However, one of the most important and variable lifestyle factors, namely our diet, has not been well investigated in association with wrinkling.

Telangiectasia

The term 'telangiectasia' is formed by the three Greek words *telos* (end), *angeion* (vessel) and *ectasis (dilatation)*, indicating that it is a widening of the small end vessels. These red to blue linear and branch-liked structures are most prevalent on the cheeks. To date, epidemiological data on risk factors for telangiectasia is scarce and genetic susceptibility relatively unknown. In one cross-sectional study, telangiectasia were associated with fair skin, male sex, increasing age, smoking and outdoor occupations [5]. Smoking has repeatedly been associated with telangiectasia [17, 18], still little is known about other determinants and lifestyle parameters associated with telangiectasia.

Dry skin

One of the most common skin conditions in aging populations is dry skin or xerosis cutis. Epidemiological studies have shown that 29-85% of the world population suffer from a form of xerosis, which affects roughly every other individual [19-23]. The most important function of the skin is forming a two-way barrier. Meaning it prevents pathogens and other possibly harmful influences from entering our body, but more importantly, it prevents excessive fluid loss. As we age this barrier deteriorates. Although it is not the first notable feature of skin aging, xerosis can be considered part of the physiological aging of skin or it can co-occur with or be part of different (skin) diseases. Since previous research on epidemiological risk factors mainly comprised small nursing home populations, evidence on dry skin determinants and its associations with disease for the general population is lacking.

Perceived age

Physicians are inclined to evaluate patients by their looks in order to make a better judgement about their health status. Perceived age, or how old someone looks, can be considered the

product of multiple aging characters. It represents how old someone looks, not knowing his or her calendar age. This measure is calculated as the mean of grades from a large panel of independent graders, to reduce inter observer bias. Perceived age is a fairly easy obtainable measure, which can reliably be used across different countries when using standardized images [24]. Scoring methods for perceived age were previously validated and successfully used in association studies [24, 25].

Perceived age has shown to be a good clinical marker of health and to predict survival, even after correction for calendar age, sex and rearing environment [26]. Furthermore, perceived age associated with both functional as well as biological longevity parameters such as cognitive function and telomere length [26]. Studies in monozygotic twin pairs showed that the older looking twin is likely to die on average 1.4 years younger than his/her younger looking sibling [27]. A few studies have investigated how looking older associates with specific morbidities. In 460 Dutch women a higher perceived age associated with lower measures of bone health [28] and in 273 Japanese women a higher perceived age was associated with a higher carotid intima media thickness [29]. Aforementioned studies, however, are often performed in relatively small sub-samples of the population and mainly focused on only one organ system. How perceived age exactly correlates with aging of multiple organ systems and their morbidities is yet to be determined.

GENETICS OF SKIN AGING

When the skin aging process starts, which signs are first to be noticed and how fast one ages is partly genetically determined. When looking at the properties of the skin, there are large ethnic differences, for example in lipid and water content, but also in melanosome induction and sebum production. Likewise, signs of chronological skin aging and photoaging also differ between groups with different genetic backgrounds [30]. Because populations of different descent genetically are too different, research is done per population separately. This makes it difficult to draw conclusions regarding differences found between studies of different descent and whether these are attributed to the studied phenotype or to the different population. In the Rotterdam Study, a predominantly West-European population was investigated.

The heritability of different skin aging phenotypes has been studied previously. Twin studies showed that the heritability of facial wrinkles is estimated to be 55%, which suggests that one's genetic basis is the strongest predictor for having a wrinkled appearance [31]. However, it remains a tedious and challenging task to identify which genes are the culprits. Genome-wide research into facial wrinkles using a large group of men and women from the Rotterdam Study, meta-analyzed with a smaller group from the Leiden Longevity Study (LLS), found an intergenic genome-wide significant hit, rs10476781, 628 kilo base pairs downstream of the *NMUR2* gene[16]. This gene has previously been associated with increased bodyweight [32], however sensitivity

analysis revealed that these signals were independent of BMI. Replication of novel signals and understanding of the genomic context in GWAS analyses is key and may additionally reduce false positive findings.

A GWAS into facial pigmented spots identified the skin color genes *IRF4*, *MC1R*, *ASIP* and *BNC2* influencing facial pigmented spots [14]. This association was independent of skin color. Skin color genes are more often linked with signs of skin aging. The melanocortin-1 receptor (*MC1R)* gene is one of these genes, which is additionally very well known for its' association with skin aging. Research shows that individuals carrying a homozygote *MC1R* risk haplotype looked on average up to 2 years older than non-carriers [33]. In addition to its known skin color functionalities, *MC1R* variants are also found to be associated with loss of fine skin patterning [34], sleep lines [35], severe photoaging [36] and even with increased melanoma risk [37].

Populations with different genetic constitutions can be difficult to compare. Single Nucleotide Polymorphisms (SNPs), which are used as a marker for genomic regions, have various prevalence rates in different populations. A recent GWAS in 1543 Han Chinese female discovered six genomic regions associated with different skin aging traits [38]. In aforementioned study, the first SNP-based association with facial telangiectasia has been reported*;* rs191497052 (located in the promotor of the *KIDINS220* gene). Unfortunately this association could not be replicated in two independent Caucasian populations since the SNP was not present. In Western populations, the genetic basis for having facial telangiectasia remains to be unraveled.

The *FLG* gene located on chromosome 1 is the best known gene involved in dermatological conditions which co-occur with dry skin. Most well-known conditions associated with loss-of-function mutations of the *FLG* gene include atopic dermatitis and ichthyosis vulgaris [39]. However, the genetic basis for having dry skin in the general population without signs of accompanying inflammation or skin disease and outside the *FLG* region is not yet well established.

ASSOCIATIONS WITH HEALTH AND AGING

Today, we live in a society where longevity and healthy aging are becoming more important as the average life-span keeps increasing and maintaining a high quality of life is a priority for many. Maintenance of youthful appearance contributes to this quality of life, as skin aging also has an impact on psychosocial health [40]. In addition, we know that adopting a healthy lifestyle helps to preserve health. This knowledge is reflected in several global campaigns and correlated lifestyle trends. Tobacco smoking is recognized as one of the largest threats to global health and legislative smoking bans have shown to improve cardiovascular health outcomes and reduce mortality for smoking related illnesses [41]. Physical exercise is identified as an essential activity to maintain not only physical but also mental health [42].

Change of dietary habits is also an important part of these twenty first century trends. Where half a century ago the intake of sufficient calories was the primary concern which needed to

be addressed, today the opposite is often true. High caloric foods are easily available at any time of the day. This, together with the often lack of physical activity of our generation, results in almost half of the European population being overweight or obese [43]. There is an increased public attention for prevention of overweight and obesity related morbidity and -costs through promoting healthy diet on a population level. Adherence to a healthy diet and maintenance of a healthy BMI are known to reduce morbidity and stimulate longevity. Simultaneously, there is a rise of functional foods claiming various skin benefits, which suggests that there are foods that can prevent skin aging and enhance cosmesis [44]. Several small studies have investigated the effects of dietary supplements on skin aging [45-47], and three previous studies have investigated features of skin aging in association with diet [48-50]. However, sample-sizes were limited and the food groups were often assessed separately, whereas in epidemiological nutritional research studying complete dietary patterns is preferred above studying single nutrients, since people do not eat isolated nutrients and there is a high level of intercorrelation among many of them [51].

AIMS OF THIS THESIS

Wrinkling is the most studied skin aging phenotype. Risk factors and determinants for facial telangiectasia, however, are relatively under investigated. Existing studies are limited in sample size or focus on a subsample of the population. Xerosis cutis, now is also recognized as an aging entity rather than only being a feature of skin disease. A deteriorated skin barrier might also serve as an indicator of a person's health. In a time where links between health, aging and disease are being unraveled, healthy aging is one of the most important topics of conversation.

This thesis aims to continue where the population-based skin aging research has stopped, by investigating and exploring less well-known skin aging subtypes, and by diving into the links with health-related behavior and disease with aging of the skin in a large sample of the population. This thesis therefore aimed to investigate the following research questions:

1. What are genetic and epidemiological determinants of facial telangiectasia?
2. What are genetic and epidemiological risk factors for having dry skin and how does this correlate with (skin) disease?
3. How does dietary pattern correlate with facial wrinkles?
4. How do youthful looks associate with morbidities of the elderly?

REFERENCES

1. Gilchrest BA. Skin aging and photoaging: an overview. J Am Acad Dermatol 1989;21:610-3.
2. Farage MA, Miller KW, Elsner P , Maibach HI. Characteristics of the Aging Skin. Adv Wound Care (New Rochelle) 2013;2:5-10.
3. Wilhelm KP, Cua AB , Maibach HI. Skin aging. Effect on transepidermal water loss, stratum corneum hydration, skin surface pH, and casual sebum content. Arch Dermatol 1991;127:1806-9.
4. Daniell HW. Smoker's wrinkles. A study in the epidemiology of "crow's feet". Ann Intern Med 1971;75:873-80.
5. Green AC, Hughes MC, McBride P , Fourtanier A. Factors associated with premature skin aging (photoaging) before the age of 55: a population-based study. Dermatology 2011;222:74-80.
6. Fisher GJ, Kang S, Varani J, Bata-Csorgo Z, Wan Y, Datta S et al. Mechanisms of Photoaging and Chronological Skin Aging. Archives of Dermatology 2002;138:1462-70.
7. Griffiths CEM, Wang TS, Hamilton TA, Voorhees JJ , Ellis CN. A Photonumeric Scale for the Assessment of Cutaneous Photodamage. JAMA Dermatology 1992;128:347-51.
8. Glogau RG. Aesthetic and anatomic analysis of the aging skin. Semin Cutan Med Surg 1996;15:134-8.
9. Vierkotter A, Ranft U, Kramer U, Sugiri D, Reimann V , Krutmann J. The SCINEXA: a novel, validated score to simultaneously assess and differentiate between intrinsic and extrinsic skin ageing. J Dermatol Sci 2009;53:207-11.
10. 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;32:807-50.
11. 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;177:e98-e100.
12. Hamer MA, Jacobs LC, Lall JS, Wollstein A, Hollestein LM, Rae AR et al. Validation of image analysis techniques to measure skin aging features from facial photographs. Skin Research and Technology 2015;21:392-402.
13. Hamer MA, Pardo LM, Jacobs LC, Ikram MA, Laven JS, Kayser M et al. Lifestyle and physiological factors associated with facial wrinkling in men and women. J Invest Dermatol 2017.
14. Jacobs LC, Hamer MA, Gunn DA, Deelen J, Lall JS, van Heemst D et al. A Genome-Wide Association Study Identifies the Skin Color Genes IRF4, MC1R, ASIP, and BNC2 Influencing Facial Pigmented Spots. J Invest Dermatol 2015;135:1735-42.
15. Ernster VL, Grady D, Miike R, Black D, Selby J , Kerlikowske K. Facial wrinkling in men and women, by smoking status. Am J Public Health 1995;85:78-82.
16. Hamer MA, Pardo LM, Jacobs LC, Deelen J, Uitterlinden AG, Slagboom E et al. Facial Wrinkles in Europeans: A Genome-Wide Association Study. J Invest Dermatol 2018;138:1877-80.
17. Kennedy C, Bastiaens MT, Bajdik CD, Willemze R, Westendorp RG, Bouwes Bavinck JN et al. Effect of smoking and sun on the aging skin. J Invest Dermatol 2003;120:548-54.
18. Isik B, Gurel MS, Erdemir AT , Kesmezacar O. Development of skin aging scale by using dermoscopy. Skin Res Technol 2013;19:69-74.
19. Hahnel E, Lichterfeld A, Blume-Peytavi U , Kottner J. The epidemiology of skin conditions in the aged: A systematic review. J Tissue Viability 2017;26:20-8.
20. Lichterfeld A, Lahmann N, Blume-Peytavi U , Kottner J. Dry skin in nursing care receivers: A multicentre cross-sectional prevalence study in hospitals and nursing homes. International Journal of Nursing Studies 2016;56:37-44.

21. Paul C, Maumus-Robert S, Mazereeuw-Hautier J, Guyen CN, Saudez X , Schmitt AM. Prevalence and risk factors for xerosis in the elderly: a cross-sectional epidemiological study in primary care. Dermatology 2011;223:260-5.
22. Smith DR, Atkinson R, Tang S , Yamagata Z. A survey of skin disease among patients in an Australian nursing home. J Epidemiol 2002;12:336-40.
23. Augustin M, Kirsten N, Körber A, Wilsmann-Theis D, Itschert G, Staubach-Renz P et al. Prevalence, predictors and comorbidity of dry skin in the general population. Journal of the European Academy of Dermatology and Venereology;0.
24. Gunn DA, Murray PG, Tomlin CC, Rexbye H, Christensen K , Mayes AE. Perceived age as a biomarker of ageing: a clinical methodology. Biogerontology 2008;9:357.
25. Gunn DA, Dick JL, van Heemst D, Griffiths CEM, Tomlin CC, Murray PG et al. Lifestyle and youthful looks. British Journal of Dermatology 2015;172:1338-45.
26. Christensen K, Thinggaard M, McGue M, Rexbye H, Hjelmborg JvB, Aviv A et al. Perceived age as clinically useful biomarker of ageing: cohort study. Bmj 2009;339:b5262.
27. Gunn DA, Larsen LA, Lall JS, Rexbye H , Christensen K. Mortality is Written on the Face. J Gerontol A Biol Sci Med Sci 2016;71:72-7.
28. Nielsen BR, Linneberg A, Christensen K , Schwarz P. Perceived age is associated with bone status in women aged 25-93 years. Age (Dordr) 2015;37:106.
29. Kido M, Kohara K, Miyawaki S, Tabara Y, Igase M , Miki T. Perceived age of facial features is a significant diagnosis criterion for age-related carotid atherosclerosis in Japanese subjects: J-SHIPP study. Geriatr Gerontol Int 2012;12:733-40.
30. Venkatesh S, Maymone MBC , Vashi NA. Aging in skin of color. Clinics in Dermatology 2019;37:351-7.
31. Gunn DA, Rexbye H, Griffiths CE, Murray PG, Fereday A, Catt SD et al. Why some women look young for their age. PLoS One 2009;4:e8021.
32. Benzon CR, Johnson SB, McCue DL, Li D, Green TA , Hommel JD. Neuromedin U receptor 2 knockdown in the paraventricular nucleus modifies behavioral responses to obesogenic high-fat food and leads to increased body weight. Neuroscience 2014;258:270-9.
33. Liu F, Hamer MA, Deelen J, Lall JS, Jacobs L, van Heemst D et al. The MC1R Gene and Youthful Looks. Curr Biol 2016;26:1213-20.
34. Law MH, Medland SE, Zhu G, Yazar S, Vinuela A, Wallace L et al. Genome-Wide Association Shows that Pigmentation Genes Play a Role in Skin Aging. J Invest Dermatol 2017;137:1887-94.
35. Jdid R, Ezzedine K, Latreille J, Galan P, Hercberg S, Malvy D et al. MC1R major variants are a risk factor of sleep lines in Caucasian women. J Eur Acad Dermatol Venereol 2014;28:805-9.
36. Elfakir A, Ezzedine K, Latreille J, Ambroisine L, Jdid R, Galan P et al. Functional MC1R-gene variants are associated with increased risk for severe photoaging of facial skin. J Invest Dermatol 2010;130:1107-15.
37. Duffy DL, Lee KJ, Jagirdar K, Pflugfelder A, Stark MS, McMeniman EK et al. Naevus count and MC1R R alleles contribute to melanoma risk. Br J Dermatol 2019;181:e119-e42.
38. Liu Y, Gao W, Koellmann C, Le Clerc S, Huls A, Li B et al. Genome-wide scan identified genetic variants associated with skin aging in a Chinese female population. J Dermatol Sci 2019;96:42-9.
39. Akiyama M. FLG mutations in ichthyosis vulgaris and atopic eczema: spectrum of mutations and population genetics. Br J Dermatol 2010;162:472-7.
40. Gupta MA , Gilchrest BA. Psychosocial aspects of aging skin. Dermatol Clin 2005;23:643-8.

41. Frazer K, Callinan JE, McHugh J, van Baarsel S, Clarke A, Doherty K et al. Legislative smoking bans for reducing harms from secondhand smoke exposure, smoking prevalence and tobacco consumption. Cochrane Database Syst Rev 2016;2:CD005992.
42. Carek PJ, Laibstain SE , Carek SM. Exercise for the treatment of depression and anxiety. Int J Psychiatry Med 2011;41:15-28.
43. Gallus S, Lugo A, Murisic B, Bosetti C, Boffetta P , La Vecchia C. Overweight and obesity in 16 European countries. Eur J Nutr 2015;54:679-89.
44. Cho S. The Role of Functional Foods in Cutaneous Anti-aging. Journal of Lifestyle Medicine 2014;4:8-16.
45. Yoon H-S, Kim JR, Park GY, Kim J-E, Lee DH, Lee KW et al. Cocoa Flavanol Supplementation Influences Skin Conditions of Photo-Aged Women: A 24-Week Double-Blind, Randomized, Controlled Trial. The Journal of Nutrition 2016;146:46-50.
46. Schwartz S, Frank E, Gierhart D, Simpson P , Frumento R. Zeaxanthin-based dietary supplement and topical serum improve hydration and reduce wrinkle count in female subjects. Journal of Cosmetic Dermatology 2016;15:e13-e20.
47. Žmitek K, Pogačnik T, Mervic L, Žmitek J , Pravst I. The effect of dietary intake of coenzyme Q10 on skin parameters and condition: Results of a randomised, placebo-controlled, double-blind study. BioFactors 2017;43:132-40.
48. Purba MB, Kouris-Blazos A, Wattanapenpaiboon N, Lukito W, Rothenberg EM, Steen BC et al. Skin wrinkling: can food make a difference? J Am Coll Nutr 2001;20:71-80.
49. Cosgrove MC, Franco OH, Granger SP, Murray PG , Mayes AE. Dietary nutrient intakes and skin-aging appearance among middle-aged American women. The American Journal of Clinical Nutrition 2007;86:1225-31.
50. Nagata C, Nakamura K, Wada K, Oba S, Hayashi M, Takeda N et al. Association of dietary fat, vegetables and antioxidant micronutrients with skin ageing in Japanese women. British Journal of Nutrition 2010;103:1493-8.
51. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002;13:3-9.

Part II

TELANGIECTASIA

CHAPTER 2.1

Epidemiology and determinants of facial telangiectasia: a cross-sectional study

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ABSTRACT

Background: Telangiectasia or red veins are one of the prominent features of facial skin aging. To date, there are few studies investigating the determinants of telangiectasia.

Objectives: We investigated lifestyle and physiological factors associated with facial telangiectasia in a large prospective Dutch cohort study.

Methods: Telangiectasia were quantified digitally from standardized facial photographs of 2842 northwestern European participants (56.8% female, median age 66.9) from the Rotterdam Study, collected in 2010-2013. Effect estimates from multivariable linear regressions are presented as the percentage difference in the mean value of telangiectasia area per unit increase of a determinant (%Δ) with corresponding 95% CI.

Results: Significant determinants were older age (1.7%Δ per year, 95%CI 1.4 to 2.0), female sex (18.3%Δ, 95%CI 13.2 to 23.6), smoking (current versus never 38.4%Δ, 95%CI 30.3 to 47.0; former versus never 11.6%Δ, 95%CI 6.6 to 16.9), a high susceptibility to sunburn (10.2%Δ, 95%CI 5.4 to 15.3), and light skin color (pale versus white-to-olive 31.4%Δ, 95%CI 19.7 to 44.1; white vs. white-to-olive 9.2%Δ, 95%CI 2.8 to 16.0).

Conclusions: In this large cohort study, we confirmed known and described new determinants of facial telangiectasia.

INTRODUCTION

Facial telangiectasia are a feature of skin aging, alongside wrinkling, pigmented spots, and sagging. Most skin aging studies have focused on aging as a compound phenotype, predominantly using manual photonumeric scales[1-3]. This makes it difficult to make inference on the role of lifestyle and physiological factors associated with specific features such as telangiectasia, if they have varying influence on different skin aging features.

In line with this, recent skin aging research into pigmented spots, wrinkles, and sagging eyelids showed differences in genetic background as well as different environmental risk factors per subtype[4-6]. This highlights the need for separate analysis of risk factors for telangiectasia.

To date, few studies have specifically focused on telangiectasia. In one cross-sectional study of 1400 subjects (aged 20-54 years), telangiectasia were associated with increasing age, male sex, fair skin, smoking, and mainly outdoor occupations[7]. Smoking has repeatedly been associated with telangiectasia[8, 9], but little is known about other lifestyle and physiological factors associated with red veins in the middle-aged to elderly.

In the Rotterdam Study, a large population-based cohort study, we investigated multiple lifestyle and physiological factors associated with facial telangiectasia in 2842 northwestern European elderly, using multiple linear regression.

METHODS

Study design, setting and participants

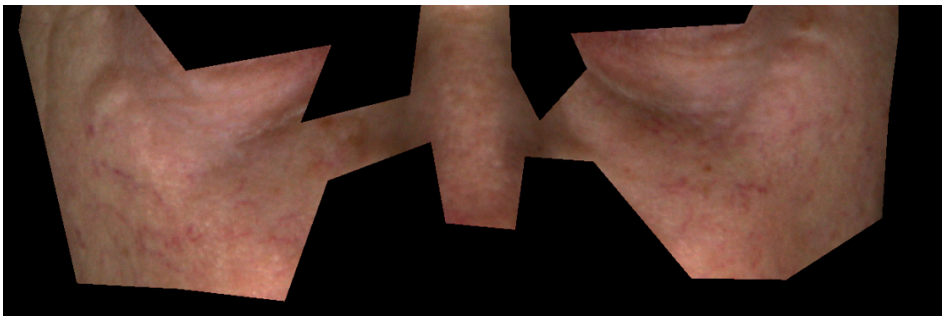
The Rotterdam Study (RS) is an ongoing prospective population-based cohort study of middle-aged to elderly (≥45 years of age) inhabitants of Ommoord, a suburb of Rotterdam in the Netherlands. Since 2010, skin examinations have been conducted by trained physicians, focusing on the most common skin diseases. In addition, standardized high-resolution digital facial photographs (Premier 3dMDface3-plus UHD, Atlanta, GA, USA) are collected of participants not wearing make-up, cream, or jewelry. The present study aimed to include all participants who visited the dermatological screening at the research center between September 2010 and July 2013. For this study, a cross-sectional design was applied where data were measured at a single moment. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus University Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports[10]. All participants provided written informed consent to participate in the study.

Telangiectasia assessment

The presence of telangiectasia was digitally quantified using semi-automated image analysis of high-resolution facial frontal photographs. The algorithms, digital rendering, measurement,

and validation with numerical grading have been described in detail previously[11]. In short, the analysis detects areas that are colored red to purple and linear or branch-like in shape (Figure 1). It subsequently calculates the percentage of skin area detected as telangiectasia.

a



b

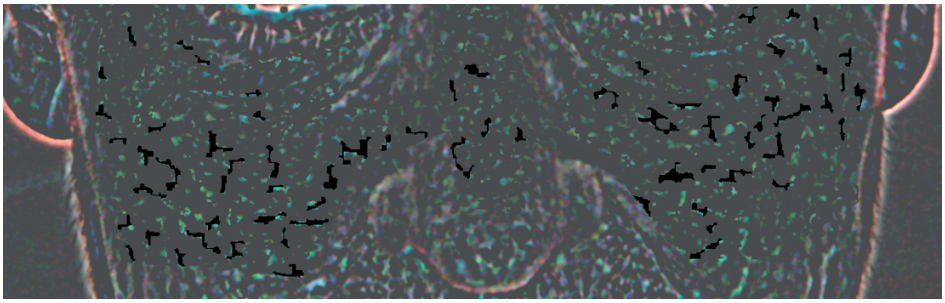


Figure 1 (**a**). Example of masked image. (**b**) Example of image analysis technique where the black structures are picked up as telangiectasia.

Determinants

Variables were selected based on known literature and biologically plausible associations. Level of education, smoking habit, alcohol consumption, and UV-related questions were collected through interview[12]. Variables collected by physical examination were body mass index (BMI), presence of dry skin, validated constitutional skin color assessment at sun-protected sites (pale, white, and white-to-olive)[13], rosacea (graded as centrofacial redness and red papules), and baldness. We used the Norwood-Hamilton scale[14, 15] for baldness in men and the Ludwig scale[16] for baldness in women and classified these into none to minimal, moderate, and extensive baldness. Serum estradiol, testosterone and sex hormone binding globulin (SHBG) were measured on average 5.6 years before photograph collection. For women, the free androgen index (FAI) was calculated: (total testosterone / SHBG * 100)[17]. Details of all variables have been previously published[4].

Statistical analysis

We excluded variables with >35% missing values, namely the UV variables "outdoor work history" and "frequency of tanning bed visits". For the other missing values (maximum per variable: 16.4%), we performed multiple imputation based on all available variables shown in Table 1, with 20 iterations. To investigate the associations between lifestyle and physiological factors and telangiectasia, we used multivariable linear regressions, where all these variables are adjusted for one another in one model. Additionally, we adjusted for two technical variables in all analyses: one which accounted for possible variations in resolutions and another which accounted for variation in flash light[11, 13]. Interaction terms for age, sex, smoking, and UV variables were tested. They were not significant or did not change the beta's significantly and hence not added to our model.

Because the residuals of the linear regression of telangiectasia area did not fit a normal distribution, we transformed the outcome using the natural logarithm (ln), resulting in an approximately normal distribution of the regression residuals. To interpret the effect estimates (regression betas), we transformed the betas back, using the formula: $(\exp^{\beta}-1) * 100\%$. This outcome is interpreted as the percentage change (%Δ): the percentage increase in the mean value of telangiectasia area per unit increase of the independent variable, e.g. 3% increase in telangiectasia area per 1 year of age. There was no statistical interaction between sex and other variables (data not shown); therefore, all analyses were performed for men and women together. FAI, estradiol, and testosterone, hence, were excluded from this analysis. All analyses were performed using SPSS for Windows version 21.0 (SPSS, Chicago, IL) and software package R. A two-sided P-value of <0.05 was considered statistically significant.

Sensitivity and additional analyses

The missing UV variables ("outdoor work history" and "tanning bed use") which were missing for ≥35% of the participants, were analyzed for association with telangiectasia in an exploratory complete-case analysis. Rosacea could have falsely been detected as telangiectasia, although people with rosacea do not necessarily show telangiectasia. In the RS cohort, we manually graded 54 individuals as having rosacea. To show their relationship, we calculated the correlation coefficient between rosacea and telangiectasia area. Lastly, we also retrieved data on telangiectasia from another cohort, the German SALIA cohort of elderly women. Because this cohort was a lot smaller, contained only women, and was different in terms of telangiectasia assessment and studied determinants, information on methods and results of these analyses is presented separately in the Supplementary Material. In an attempt to make a comparison between the RS and the SALIA cohort, we performed a linear regression analysis in RS women only, also including the variables FAI and estradiol.

Table 1. Characteristics of 2842 participants of the Rotterdam Study with telangiectasia measurements

Characteristic	Men N=1321	Women N=1521
Telangiectasia % - median [IQR]	0.77 [0.49 to 1.21]	0.96 [0.62 to 1.41]
Age at photo in years - median [IQR]	66.8 [61.3 to 72.0]	66.39 [61.0 to 71.3]
BMI in kg/m^2 - mean (SD)	27.7 (3.70)	27.56 (4.76)
Skin color		
Pale (%)	100 (7.57)	141 (9.27)
White (%)	1014 (76.76)	1196 (78.63)
White-to-olive (%)	207 (15.67)	184 (12.10)
Baldness[a]		
No/mild baldness (%)	656 (49.66)	1013 (66.60)
Moderate (%)	299 (22.63)	365 (24.00)
Extensive (%)	365 (27.63)	111 (7.30)
Tendency to develop sunburn		
Low (%)	870 (65.86)	921 (60.55)
High (%)	414 (31.34)	528 (34.71)
Outdoor work history		
No (%)	536 (40.58)	717 (47.14)
Yes (%)	244 (18.47)	140 (9.20)
Missing (%)	541 (40.95)	664 (43.66)
History of living in a sunny country >1 year		
No (%)	1178 (89.17)	1399 (91.98)
Yes (%)	118 (8.93)	67 (4.40)
Sun-protective behavior[b]		
Never/almost never (%)	482 (36.49)	485 (31.89)
Often/almost always/always (%)	814 (61.62)	980 (64.43)
Tanning bed use		
Never or less than 10x (%)	631 (47.77)	717 (47.14)
More than 10x (%)	74 (5.60)	140 (9.20)
Missing (%)	616 (46.63)	664 (43.66)
Spend winter in sunny country		
No or less than 1 month (%)	1169 (88.49)	1366 (89.81)
Yes, ≥1 month/yr (%)	61 (4.62)	69 (4.54)
Missing (%)	91 (6.89)	86 (5.52)
Smoking history[c]		
Current (%)	275 (19.45)	241 (15.84)
Former (%)	766 (57.99)	695 (45.69)
Never (%)	280 (21.20)	583 (38.33)
Education level[d]		
Low (%)	91 (6.89)	139 (9.14)
Medium (%)	745 (56.40)	1021 (67.13)
High (%)	469 (35.50)	349 (22.95)

Table 1. (continued)

Characteristic	Men N=1321	Women N=1521
Alcohol		
Median use in glasses/day [IQR]	1.24 [0.31 - 2.42]	0.45 [0.05 - 1.40]
Missing (%)	242 (18.32)	225 (14.79)
Dry skin		
No (%)	444 (33.61)	388 (25.51)
Yes (%)	877 (66.39)	1132 (74.42)
Testosterone in nmol/l – median [IQR]	16.58 [13.09 - 20.48]	na
Free androgen index[e] - median [IQR]	na	1.34 [0.89 - 1.93]
Missing (%)		76 (5.00)
Estradiol in pmol/l - median [IQR]	na	39.72 [18.35 - 73.09]

Abbreviatons: BMI, body mass index; IQR, interquartile range; na, not applicable; SD, standard deviation. [a] Based on the Norwood-Hamilton (NH) scale for men and the Ludwig scale for women; None or minimal: NH score 1, 2, 3, 9, 10, 11 and Ludwig scale score none. Moderate: NH score 4, 5, 6, 12 and Ludwig scale score 1. Extensive: NH score 7, 8 and Ludwig scale score 2, 3; [b] Wearing sunglasses and/or a brimmed hat in the sunshine; [c] Cigars, cigarettes or pipe; [d] Low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [e] Free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l).

RESULTS

Study population

Between September 2010 and July 2013, a total of 3831 participants visited the dermatological examination of the RS. We excluded individuals due to non-northwestern European origin, poor image quality, and make-up, leaving 2842 participants with eligible 3D photographs used to measure facial telangiectasia area. There were slightly more women than men (N=1521; 53.5%), and the median age was 66.6 years old (Table 1). The median telangiectasia area was higher in women than in men (men: 0.77%, IQR 0.49 to 1.21; women: 0.96%, IQR 0.62 to 1.41).

Determinants for facial telangiectasia area

With higher age, telangiectasia area increased 1.7% per year (95%CI 1.4 to 2.0). Women had 18.3% (95%CI 13.2 to 23.6) more telangiectasia than men, and the lighter the skin color, the higher the risk for more red veins was. Having a white skin color associated with a 9.2% (95%CI 2.8 to 16.0) larger telangiectasia area and having a pale skin color with 31.4% more red veins, compared to white- to-olive skinned participants. Interestingly, not only did current smokers have 38.4% (95%CI 30.3 to 47.0) more telangiectasia than non-smokers, but former smokers also had 11.6% (95%CI 6.6 to 16.9) more telangiectasia than non-smokers. Finally, participants with a tendency to develop sunburn also showed a 10.2% (95%CI 5.4 to 15.3) larger telangiectasia area than those not susceptible to sunburn (Table 2).

Table 2. Multivariable linear regression of facial telangiectasia: determinants of facial telangiectasia among 2842 participants of the Rotterdam Study

Determinant	%Δ telangiectasia area[a]	95% CI	P-value
Sex			
Male	ref	ref	ref
Female	18.3	**[13.2 to 23.6]**	**<0.001**
Age (per year)	1.7	**[1.4 to 2.0]**	**<0.001**
BMI (per point)	0.2	[-0.2 to 0.7]	0.405
Skin color			
White-to- olive	ref	ref	ref
White	9.2	**[2.8 to 16.0]**	**0.004**
Pale	31.4	**[19.7 to 44.1]**	**<0.001**
Baldness			
No/mild baldness	ref	ref	ref
Moderate	1.7	[-3.1 to 6.8]	0.500
Extensive	$-1.1*10^{-2}$	[-5.7 to 11.0]	0.997
Tendency to develop sunburn	10.2	**[5.4 to 15.3]**	**<0.001**
History of living in a sunny country	0.5	[-7.3 to 8.9]	0.905
Sun-protective behavior[b]	-0.6	[-4.8 to 3.7]	0.772
Spending winter in sunny country	-8.1	[-16.4 to 0.9]	0.076
Smoking history[c]			
Never	ref	ref	ref
Former	11.6	**[6.6 to 16.9]**	**<0.001**
Current	38.4	**[30.3 to 47.0]**	**<0.001**
Education level[d]			
Low	ref	ref	ref
Medium	4.31	[-3.23 to 12.4]	0.270
High	2.26	[-5.76 to 11.0]	0.592
Alcohol (per glass per day)	-0.8	[-2.2 to 0.7]	0.291
Dry skin			
No	ref	ref	ref
Yes	-1.5	[-5.8 to 3.1]	0.519
Batch[e]	32.26	**[23.4 to 41.7]**	**<0.001**
Residual[f]	2.27	**[2.0 to 2,5]**	**<0.001**

Abbreviations: 95% CI, 95% conference interval; BMI, body mass index; ref, reference variable. [a] %Δ: the percentage change in telangiectasia area (the % increase in the mean value of telangiectasia area per unit increase of the independent variable, calculated by the formula: $(\exp^{\beta}-1) * 100\%$. E.g. 1.7% increase in telangiectasia area per 1 year of age; [b] Wearing sunglasses and/or a brimmed hat in the sunshine; [c] Cigars, cigarettes or pipe; [d] Low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [e] Technical variable which accounts for possible changes in resolution; [f] Technical variable which accounts for possible changes in flash light variability. R^2 total model: 0.354. **Boldface** indicates statistically significant determinants.

Sensitivity and additional analyses

In a complete case analysis including the two additional UV variables "outdoor work history" and "tanning bed use", both were not significantly associated (Supplementary Table S1). Additionally, the effect estimates of the significant determinants remained similar to the previous analysis, indicating there was no meaningful association between these two UV variables and telangiectasia. However, the variable "spending winter in a sunny country" showed a negative association (-20.8%, 95%CI -31.4 to -8.5) instead of no association in the previous analysis. Spearman's rho correlation coefficient between rosacea and teleangiectasia area was 0.04, indicating no correlation between the two conditions. In the SALIA cohort, age, light skin color type, and smoking were significantly associated determinants (Supplementary Material & Supplementary Tables S3 and S4).

When analyzing RS women only (N=1521), we found similar results to those in the analysis of men and women together. The only meaningful difference was that BMI was associated with more telangiectasia area in women (0.6%Δ per 1 point BMI increase, 95%CI 0.1 to 1.2) (Supplementary Table S2).

DISCUSSION

In this large cross-sectional study, the most important variables associated with facial telangiectasia were light skin color type and smoking. Increasing age was also significantly associated with more facial red veins, although with a smaller effect size. Additionally, we found that female sex and tendency to develop sunburn were significant determinants for telangiectasia in the RS. We replicated our associations in a smaller cohort of women from European ancestry, showing the relevance of our findings. Although the cohort was smaller and only assessed telangiectasia in women with a different assessment, we demonstrated that main determinants were indeed associated with telangiectasia.

Smoking was the most important determinant for telangiectasia with the largest effect size. Current smokers had more than a third extra telangiectasia compared with non-smokers. This is not surprising, as we know that smoking is one of the most important lifestyle factors inducing premature skin aging[9, 18-20]. It might even be the most important risk factor for telangiectasia since it is repeatedly replicated in all telangiectasia studies. Even former smoking had a significant effect in our cohort. The underlying mechanism on how smoking could lead to more red veins is not yet known; however, smoking induces DNA damage, elastosis, and more atrophy of the skin, which could make red veins more visible[21]. Smoking has also been associated with dilated venules in other human organs such as the retina[22]. Alternatively, smoking causes vasoconstriction of the small vessels which leads to a chronic hypoxemic state in the skin[23]. This could result in proliferation of new red veins, visible as more telangiectasia.

Pale skin color type was associated with more telangiectasia, as previously reported[7]. Similar to smoking, the underlying mechanism is not yet elucidated, but we hypothesize that UV- induced DNA damage will play a role, as in other types of skin aging. Alternatively, telangiectasia might be more visible on lighter skin.

Female sex was associated with more telangiectasia, which was opposite to what has been previously reported[7]. This could be explained in part by the higher average age in the RS population compared to the age of the participants in the previous report. Men tend to show signs of skin wrinkling earlier in life, with women showing similar wrinkling prevalence as men later on in life [4]. Hence, men could also develop telangiectasia earlier in life. Additionally, male skin is 10-20% thicker than female skin and therefore might be less susceptible to thinning and showing red veins[24].

Light skin color type and current smoking were also significant determinants for telangiectasia in the SALIA cohort. Unexpectedly, in this relatively small cohort, older age was associated with less telangiectasia. However, the age range of the replication cohort is much smaller than in the RS and lies within the ages in which the RS also showed a decline in telangiectasia (Supplementary Figures S1 and S2). This phenomenon has not been described earlier, which indicates it is probably a coincidental finding. However, unknown confounders might also have a part in this. In the sensitivity analysis in the women of the RS, we found that increasing BMI associates with more telangiectasia but this was not found in the SALIA cohort. A higher BMI has previously been linked with fewer facial wrinkles, which probably has to do with the filler effect of facial fat[4]. Research into skin circulation showed that with increasing BMI, oxygenation in skin increased[25]. Furthermore, dermal microvascular dysfunction is common in diabetes patients who often have a higher BMI than healthy subjects[26]. However, how BMI exactly associates with telangiectasia remains to be fully understood.

The results of this study confirm the hypothesis that the different features of skin aging have different determinants. Age and sun exposure are the exception and are important risk factors for all skin aging phenotypes (i.e., wrinkling, pigmented spots, and telangiectasia). However, skin color, for example, is different. Pale skinned individuals are more at risk for having telangiectasia and pigmented spots while they have less wrinkles[4, 5]. Smoking is the major lifestyle risk factor for wrinkling and telangiectasia, and although it can cause smokers' melanosis in the oral cavity[27], it has not been proven to stimulate facial pigmented spots. This clustering of specific risk factors could be of use in the risk stratification and personalized approach of skin aging prevention strategies.

There are several limitations of this study. Firstly, the cross-sectional nature of the associations prevents from determining causal inferences. Secondly, we used a digital method to measure telangiectasia where most previously performed studies used photonumeric grading. However, validation of our digital method[11] has shown that there is a moderate to good correlation between digital and photonumeric measurement of telangiectasia (Spearman's rho 0.60 in women and 0.75 in men), which suggests this will not have a large effect in our conclusions.

Also, we found only one of our UV variables to be associated with telangiectasia. This illustrates that the quality of our used questions for sun exposure was suboptimal and that it remains a difficult variable to capture by questionnaire. Furthermore, besides in telangiectatic aging, facial erythema and telangiectasia are also often associated with the erythematotelangiectatic subtype of rosacea (ETR, besides the other three types of rosacea: papulopustular, phymatous and ocular). It is therefore important to recognize the differences between ETR and telangiectatic aging[28]. However, in our data, the number of rosacea patients was low and rosacea correlated poorly with telangiectasia. Looking more carefully into these rosacea cases, there was a substantial proportion with the papulopustular subtype and telangiectasia were poorly picked up in the ETR group. The latter is a limitation of our image analysis technique where it seems to pick up telangiectasia less well in an erythematous environment, probably due to lack of contrast. Lastly, our findings hold for a predominantly northwestern European population. It is not clear to which extent these can be extrapolated to other populations.

In conclusion, this large study confirmed some of the earlier found risk factors for telangiectasia such as pale skin and smoking which are similar in men and women, while identifying potential new associations such as BMI. These results support the evidence that different skin colors show varying prevalence of specific skin aging features. The correlated factors of telangiectasia can help future studies to unravel causal versus consequence determinants as more insight into etiology of telangiectasia is gained, and longitudinal or experimental studies are added to this field of research.

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REFERENCES

1. Griffiths CE, Wang TS, Hamilton TA, Voorhees JJ , Ellis CN. A photonumeric scale for the assessment of cutaneous photodamage. Arch Dermatol 1992;128:347-51.
2. Guinot C, Malvy DJ, Ambroisine L, Latreille J, Mauger E, Tenenhaus M et al. Relative contribution of intrinsic vs extrinsic factors to skin aging as determined by a validated skin age score. Arch Dermatol 2002;138:1454-60.
3. Larnier C, Ortonne JP, Venot A, Faivre B, Beani JC, Thomas P et al. Evaluation of cutaneous photodamage using a photographic scale. Br J Dermatol 1994;130:167-73.
4. Hamer MA, Pardo LM, Jacobs LC, Ikram MA, Laven JS, Kayser M et al. Lifestyle and physiological factors associated with facial wrinkling in men and women. J Invest Dermatol 2017.
5. Jacobs LC, Hamer MA, Gunn DA, Deelen J, Lall JS, van Heemst D et al. A Genome-Wide Association Study Identifies the Skin Color Genes IRF4, MC1R, ASIP, and BNC2 Influencing Facial Pigmented Spots. J Invest Dermatol 2015;135:1735-42.
6. Jacobs LC, Liu F, Bleyen I, Gunn DA, Hofman A, Klaver CC et al. Intrinsic and extrinsic risk factors for sagging eyelids. JAMA Dermatol 2014;150:836-43.
7. Green AC, Hughes MC, McBride P , Fourtanier A. Factors associated with premature skin aging (photoaging) before the age of 55: a population-based study. Dermatology 2011;222:74-80.
8. Isik B, Gurel MS, Erdemir AT , Kesmezacar O. Development of skin aging scale by using dermoscopy. Skin Res Technol 2013;19:69-74.
9. Kennedy C, Bastiaens MT, Bajdik CD, Willemze R, Westendorp RG, Bouwes Bavinck JN et al. Effect of smoking and sun on the aging skin. J Invest Dermatol 2003;120:548-54.
10. 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;32:807-50.
11. Hamer MA, Jacobs LC, Lall JS, Wollstein A, Hollestein LM, Rae AR et al. Validation of image analysis techniques to measure skin aging features from facial photographs. Skin Res Technol 2015;21:392-402.
12. Hofman A, Brusselle GG, Darwish Murad S, van Duijn CM, Franco OH, Goedegebure A et al. The Rotterdam Study: 2016 objectives and design update. Eur J Epidemiol 2015;30:661-708.
13. Jacobs LC, Hamer MA, Verkouteren JA, Pardo LM, Liu F , Nijsten T. Perceived skin colour seems a swift, valid and reliable measurement. Br J Dermatol 2015;173:1084-6.
14. Norwood OT. Male pattern baldness: classification and incidence. South Med J 1975;68:1359-65.
15. Taylor R, Matassa J, Leavy JE , Fritschi L. Validity of self reported male balding patterns in epidemiological studies. BMC Public Health 2004;4:60.
16. Ludwig E. Classification of the types of androgenetic alopecia (common baldness) occurring in the female sex. Br J Dermatol 1977;97:247-54.
17. Rosner W, Auchus RJ, Azziz R, Sluss PM , Raff H. Position statement: Utility, limitations, and pitfalls in measuring testosterone: an Endocrine Society position statement. J Clin Endocrinol Metab 2007;92:405-13.
18. Daniell HW. Smoker's wrinkles. A study in the epidemiology of "crow's feet". Ann Intern Med 1971;75:873-80.
19. Rexbye H, Petersen I, Johansens M, Klitkou L, Jeune B , Christensen K. Influence of environmental factors on facial ageing. Age Ageing 2006;35:110-5.
20. Yin L, Morita A , Tsuji T. Skin aging induced by ultraviolet exposure and tobacco smoking: evidence from epidemiological and molecular studies. Photodermatol Photoimmunol Photomed 2001;17:178-83.

21. Ortiz A , Grando SA. Smoking and the skin. Int J Dermatol 2012;51:250-62.
22. Ikram MK, de Jong FJ, Vingerling JR, Witteman JC, Hofman A, Breteler MM et al. Are retinal arteriolar or venular diameters associated with markers for cardiovascular disorders? The Rotterdam Study. Invest Ophthalmol Vis Sci 2004;45:2129-34.
23. Reus WF, Robson MC, Zachary L , Heggers JP. Acute effects of tobacco smoking on blood flow in the cutaneous micro-circulation. British Journal of Plastic Surgery 1984;37:213-5.
24. Bailey SH, Oni G, Brown SA, Kashefi N, Cheriyan S, Maxted M et al. The use of non-invasive instruments in characterizing human facial and abdominal skin. Lasers Surg Med 2012;44:131-42.
25. Kuliga KZ, McDonald EF, Gush R, Michel C, Chipperfield AJ , Clough GF. Dynamics of microvascular blood flow and oxygenation measured simultaneously in human skin. Microcirculation 2014;21:562-73.
26. Fuchs D, Dupon PP, Schaap LA , Draijer R. The association between diabetes and dermal microvascular dysfunction non-invasively assessed by laser Doppler with local thermal hyperemia: a systematic review with meta-analysis. Cardiovasc Diabetol 2017;16:11.
27. Hedin CA. Smokers' melanosis. Occurrence and localization in the attached gingiva. Arch Dermatol 1977;113:1533-8.
28. Helfrich YR, Maier LE, Cui Y, Fisher GJ, Chubb H, Fligiel S et al. Clinical, Histologic, and Molecular Analysis of Differences Between Erythematotelangiectatic Rosacea and Telangiectatic Photoaging. JAMA Dermatol 2015;151:825-36.

SUPPLEMENTARY MATERIAL (SALIA COHORT)

METHODS

Study population

The Study on the influence of Air pollution on Lung function, Inflammation and Ageing (SALIA) is a cohort study including middle-aged women from the urban Ruhr area (Dortmund, Duisburg, Essen, Gelsenkirchen and Herne) and two rural northern counties (Southern Münsterland) in West Germany. The baseline investigation started in 1985, when the women were about 55 years of age. Men were not recruited because of the high occupational exposure of many men in this area, where coal mining and steel industry constituted the predominant sources of income in the time period before the baseline examination[1]. The replication analysis is based on data from the clinical follow-up examination (2007–2010), in which 834 women participated. All participants gave written informed consent. The Medical Ethics Committee of the University of Bochum approved the follow-up examination[2].

Telangiectasia assessment

Severity of telangiectasia was manually graded using a photonumeric 0-5 scale, as part of the SCINEXA™ method[3].

Determinants

BMI was assessed by physical examination. Information on skin color type (based on the Fitzpatrick scale[4]), household education level (highest level of education of the participants and their partners combined) and lifestyle (use of sun protection cream and sunbeds, holidays in sunny regions, smoking and alcohol consumption) was collected via interview.

Statistical analysis

In SALIA, we investigated the influence of lifestyle and physiological factors on telangiectasia using a multivariable linear regression model including age, BMI, skin type, use of sun protection cream and tanning beds, holidays in sun rich regions, smoking history, education level and alcohol consumption as independent variables. Information on these variables and on telangiectasia were available for 784 women and we included only these complete cases. The analysis was performed in R. A two-sided P-value of <0.05 was considered statistically significant.

RESULTS

Between May 2007 and March 2010, 834 women were screened on telangiectasia. A number of 50 women were excluded due to missing data, leaving 784 women included in the final analysis. The women were slightly older than in the RS with a mean age of 73.5 years (Supplementary Table S3). The mean value of the telangiectasia was 2.1.

The age range in the SALIA cohort was smaller (66-79 years) than the age range in the RS (51-98 years) and showed a decrease in telangiectasia with increasing age, whereas the RS showed an overall increase in telangiectasia with increasing age. However, when zooming in on the age range of 60-75 years in the RS, a decrease in telangiectasia was seen, similar to the SALIA cohort in the comparable age range (Supplementary Figures S1 and S2). Light skin color type (skin type I/II vs. III/IV: ß=0.44 [95%CI 0.22 to 0.66]) and smoking (current vs. never smoking: ß=0.66 [95%CI: 0.002 to 1.33]) were replicated as potential determinants. Women using sun-cream protection showed less telangiectasia (ß=-0.21 [95%CI: -0.45 to 0.02]). Age was associated with less telangiectasia (ß=-0.09 [95%CI: -0.12 to -0.05], as opposed to the findings in the RS (Supplementary Table S4).

REFERENCES

1. Schikowski T, Sugiri D, Ranft U, Gehring U, Heinrich J, Wichmann HE et al. Long-term air pollution exposure and living close to busy roads are associated with COPD in women. Respir Res 2005;6:152.
2. Vossoughi M, Schikowski T, Vierkotter A, Sugiri D, Hoffmann B, Teichert T et al. Air pollution and subclinical airway inflammation in the SALIA cohort study. Immun Ageing 2014;11:5.
3. Vierkotter A, Ranft U, Kramer U, Sugiri D, Reimann V , Krutmann J. The SCINEXA: a novel, validated score to simultaneously assess and differentiate between intrinsic and extrinsic skin ageing. J Dermatol Sci 2009;53:207-11.
4. Fitzpatrick TB. The Validity and Practicality of Sun-Reactive Skin Types I Through VI. Archives of Dermatology 1988;124:869-71.

SUPPLEMENTARY TABLES

Supplementary Table S1. Sensitivity analysis complete cases RS (N=1146)

Determinant	%Δ[a]	95% CI	P-value
Sex			
Male	ref	ref	ref
Female	19.0	**[11.1, 27.4]**	**0.008**
Age (per year)	1.3	**[0.7, 2.0]**	**<0.001**
BMI (per point)	0.4	[-0.3, 1.1]	0.243
Skin color			
White-to-olive	ref	Ref	ref
White	8.8	[-0.6, 19.1]	0.068
Pale	27.7	**[12.6, 44.9]**	**<0.001**
Baldness			
No/mild baldness	ref	Ref	ref
Moderate	-2.2	[-9.6, 5.9]	0.588
Extensive	2.9	[-6.9, 13.8]	0.570
Tendency to develop sunburn	14.0	**[6.4, 22.2]**	**<0.001**
Outdoor work history	2.0	[-5.6, 10.3]	0.610
History of living in a sunny country	2.3	[-10.2, 16.5]	0.733
Tanning bed use >10 times	-3.0	[-11.4, 6.2]	0.508
Sun-protective behavior[b]	2.0	[-4.4, 8.9]	0.550
Spending winter in sunny country	-20.8	**[-31.4, -8.5]**	**0.002**
Smoking history[c]			
Never	ref	Ref	ref
Former	10.4	**[2.9, 18.3]**	**0.006**
Current	36.8	**[25.2, 49.5]**	**<0.001**
Education level[d]			
Low	ref	Ref	ref
Medium	-2.8	[-13.2, 8.9]	0.627
High	-5.3	[-16.1, 7.0]	0.384
Alcohol (per glass per day)	$4.0*10^{-4}$	[-2.0, 2.0]	0.999
Dry skin			
No	ref	Ref	ref
Yes	-1.2	[-7.2, 5.3]	0.714
Batch[e]	28.2	**[16.5, 41.0]**	**0.004**
Residual[f]	2.2	**[1.8, 2.5]**	**<0.001**

Abbreviations: 95% CI, 95% conference interval; BMI, body mass index; ref, reference variable. [a] %Δ: the percentage change in telangiectasia area (the % increase in the mean value of telangiectasia area per unit increase of the independent variable, calculated by the formula: $(\exp^{\beta}-1) * 100\%$. E.g. 1.7% increase in telangiectasia area per 1 year of age; [b] Wearing sunglasses and/or a brimmed hat in the sunshine; [c] Cigars, cigarettes

or pipe; [d] Low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [e] Technical variable which accounts for possible changes in resolution; [f] Technical variable which accounts for possible changes in flash light variability. **Boldface** indicates statistically significant determinants.

Supplementary Table S2. Sensitivity analysis women RS (N=1521)

Determinant	%Δ[a]	95% CI	P-value
Age (per year)	1.5	**[1.1, 1.8]**	**<0.001**
BMI (per point)	0.6	**[0.1, 1.2]**	**0.034**
Skin color			
White-to-olive	ref	Ref	ref
White	10.2	**[1.3, 20.0]**	**0.024**
Pale	31.4	**[16.2, 48.7]**	**<0.001**
Baldness			
No/mild baldness	ref	ref	ref
Moderate	2.4	[-3.8, 9.1]	0.453
Extensive	-3.3	[-12.5, 7.0]	0.521
Tendency to develop sunburn	7.8	**[1.7, 14.3]**	**0.012**
History of living in a sunny country	5.4	[-7.3, 19.8]	0.424
Sun-protective behavior[b]	1.9	[-3.8, 7.9]	0.524
Spending winter in sunny country	-7.7	[-18.7, 4.8]	0.218
Smoking history[c]			
Never	ref	ref	ref
Former	7.8	**[1.7, 14.3]**	**0.011**
Current	45.0	**[33.7, 57.3]**	**<0.001**
Education level[d]			
Low	ref	ref	ref
Medium	4.06	[-5.2, 14.2]	0.401
High	1.7	[-8.4, 13.0]	0.748
Alcohol (per glass per day)	0.9	[-1.5, 3.3]	0.477
Dry skin			
No	ref	ref	ref
Yes	-2.7	[-8.5, 3.5]	0.388
Free androgen index[e]	0.8	[-1.3, 2.8]	0.464
Estradiol (per pmol/l)	$3.0*10^{-3}$	[-0.01, 0.02]	0.682
Batch[f]	25.6	**[14.5, 37.9]**	**<0.001**
Residual[g]	2.0	**[1.7, 2.4]**	**<0.001**

Abbreviations: 95% CI, 95% conference interval; BMI, body mass index; ref, reference variable. [a] %Δ: the percentage change in telangiectasia area (the % increase in the mean value of telangiectasia area per unit increase of the independent variable, calculated by the formula: $(\exp^{\beta}-1) * 100\%$. E.g. 1.7% increase in telangiectasia area per 1 year of age; [b] Wearing sunglasses and/or a brimmed hat in the sunshine; [c] Cigars, cigarettes or pipe; [d] Low (primary education); medium (lower secondary education/lower vocational education/intermediate vocational education); high (general secondary education/higher vocational education/university); [e] Free androgen index (calculated as total testosterone in nmol/l divided by sex hormone binding globulin in nmol/l); [f] Technical variable which accounts for possible changes in resolution; [g] Technical variable which accounts for possible changes in flash light variability. **Boldface** indicates statistically significant determinants.

Supplementary Table S3. Characteristics of 784 female participants of the SALIA cohort with telangiectasia measurements

Characteristic	Women N=784
Telangiectasia score - mean (SD)	2.1 (1.5)
Age at photo in years - mean (SD)	73.6 (3.0)
BMI in kg/m^2 - mean (SD)	27.3 (4.5)
Skin color	
I/II (%)	437 (55.7)
III/IV (%)	347 (44.3)
Regular use of sun protection cream	
No (%)	309 (39.4)
Yes (%)	475 (60.6)
Tanning bed use	
Never:(%)	644 (82.1)
Ever (%)	140 (17.9)
Holidays in sunrich regions in weeks per year – mean (SD)	1.4 (2.6)
Smoking history	
Current (%)	21 (2.7)
Former (%)	138 (17.6)
Never (%)	625 (79.7)
Education level[3]	
Low: <10yrs education (%)	139 (17.7)
Medium: 10yrs education (%)	385 (49.1)
High: >10yrs education (%)	260 (33.2)
Alcohol	
Never (%)	130 (16.6)
Ever (%)	654 (83.4)

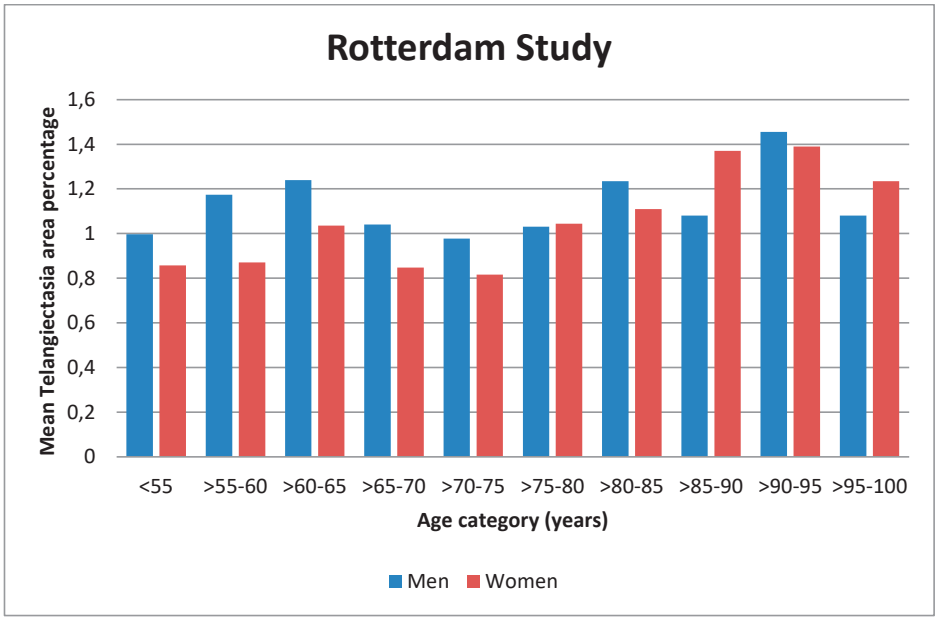
Abbreviations: BMI, body mass index; SD, standard deviation.

Supplementary Table S4. Multivariable linear regression of facial telangiectasia: determinants of facial telangiectasia among 784 women of the SALIA cohort

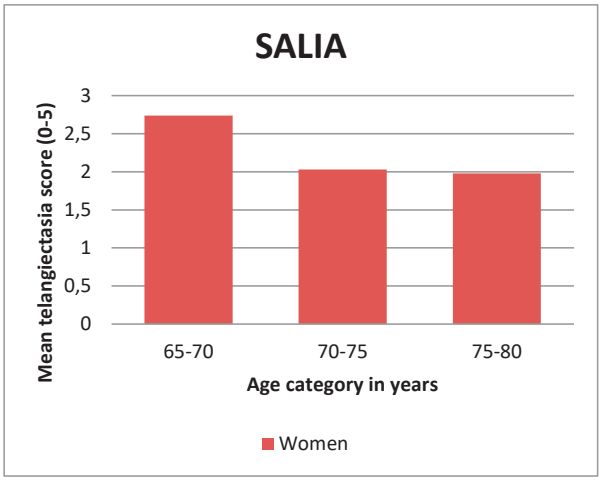
Determinant	ß	95% CI	P-value
Age (per year)	-0.09	**[-0.12, -0.05]**	**<0.001**
BMI (per point)	0.005	[-0.02, 0.03]	0.714
Skin type (Fitzpatrick)			
III/IV	ref	ref	ref
I/II	0.44	**[0.22, 0.66]**	**<0.001**
Regular use of sun protection cream			
No	ref	ref	ref
Yes	-0.21	[-0.45, 0.02]	0.074
Tanning bed use			
Never	ref	ref	ref
Ever	0.03	[-0.26, 0.33]	0.827
Holidays in sunrich regions in weeks (per year)	-0.01	[-0.05, 0.04]	0.797
Smoking history			
Never	ref	ref	ref
Former	0.20	[-0.09, 0.48]	0.174
Current	0.66	**[0.002, 1.33]**	**0.049**
Education level			
Low	ref	ref	ref
Medium	-0.01	[-0.30, 0.29]	0.958
High	-0.06	[-0.38, 0.26]	0.701
Alcohol consumption			
Never	ref	ref	ref
Ever	0.003	[-0.29, 0.29]	0.983

Abbreviations: BMI, body mass index; SD, ref, reference variable. **Boldface** indicates statistically significant determinants.

SUPPLEMENTARY FIGURES



Supplementary Figure 1. Distribution of telangiectasia per age category in the Rotterdam Study



Supplementary Figure 2. Distribution of telangiectasia per age category in the SALIA cohort

CHAPTER 2.2

Genetics of facial telangiectasia in the Rotterdam Study: a genome-wide association study and candidate gene approach

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ABSTRACT

Background: The severity of facial telangiectasia or red veins is associated with many lifestyle factors. However, the genetic predisposition remains unclear.

Objectives: We performed a genome-wide association study (GWAS) on facial telangiectasia in the Rotterdam Study (RS) and tested for replication in two independent cohorts. Additionally, a candidate gene approach with known pigmentation genes was performed.

Methods: Facial telangiectasia were extracted from standardized facial photographs (collected from 2010–2013) of 2842 northwestern European participants (median age 66.9, 56.8% female) from the RS. Our GWAS top hits (P-value $<10^{-6}$) were tested for replication in 460 elderly women of the SALIA cohort and in 576 additional men and women of the RS. Associations of top single nucleotide polymorphisms (SNPs) with expression quantitative trait loci (eQTL) in various tissues were reviewed (GTEx database) alongside phenotype associations in the UK biobank database. SNP-based associations between known pigmentation genes and facial telangiectasia were tested. Conditional analysis on skin color was additionally performed.

Results: Our most significant GWAS signal was rs4417318 (P-value $5.38*10^{-7}$), an intergenic SNP on chromosome 12 mapping to the *SLC16A7* gene. Other suggestive SNPs tagged genes *ZNF211*, *ZSCAN4*, *ICOS* and *KCNN3*; SNP eQTLs and phenotype associations tagged links to the vascular system. However, the top signals did not pass significance in the two replication cohorts. The pigmentation genes *KIAA0930*, *SLCA45A2* and *MC1R*, were significantly associated with telangiectasia in a candidate gene approach but not independently of skin color.

Conclusion: In this GWAS on telangiectasia in a northwestern European population, no genome-wide significant SNPs were found, although suggestive signals indicate genes involved in the vascular system might be involved in telangiectasia. Significantly associated pigmentation genes underline the link between skin color and telangiectasia.

INTRODUCTION

Telangiectasia is dilated small blood vessels visible in the skin, which vary in color from red to blue. These linear or branched-like vessels are typically located on the nose and cheeks. Risk factors for having more extensive facial telangiectasia include environmental factors such as smoking and UV-exposure and intrinsic factors such as aging, pale skin color and tendency to develop sunburn[1-4].

Facial telangiectasia is regarded as one of the skin aging features, together with wrinkles, pigmented spots, xerosis and skin sagging. Skin aging research shows that UV-exposure is an important risk factor for all signs of skin aging, but other determinants such as for example, skin color, have different effects in the different features of skin aging [5, 6]. Twin studies demonstrate that facial wrinkles are 55% heritable, highlighting a sizeable genetic background to this feature [7]. Genome-wide association studies (GWAS) performed on pigmented spots discovered that genetic variations in skin color genes *(IRF4*, *MC1R*, *ASIP* and *BNC2*) are important in the amount of facial pigmented spots [5]; moreover, melanocortin-1-receptor (*MC1R*) variants are associated with youthful looks [8].

Hence, different skin aging phenotypes are accounted by genes and environmental factors differently and therefore it makes sense to study these separately, in order to understand skin aging as a whole. Telangiectasia is a less well-studied phenotype and its aetiology and risk factors remain to be fully understood. A recent GWAS study in 1,534 Han Chinese women found single-nucleotide polymorphism (SNP) rs191497052 tagging the *KIDINS220* gene associated with having more facial telangiectasia [9]. In another recent study, the heritability of telangiectasia was estimated to be low [10]. However, this does not exclude that specific genetic variants may be associated with susceptibility for degree of telangiectasia.

In this study, we performed a GWAS on facial telangiectasia in 2,842 North-West European men and women of the Rotterdam Study (RS). Our results were tested for replication in 460 German women of the SALIA cohort and also in a separate group of 576 RS men and women. Since pigmentation genes are known to influence wrinkling and pigmented spots, we additionally reviewed the association between telangiectasia and known pigmentation genes.

METHODS

Study population

Subjects were included from the RS, a large population-based cohort, which started in 1990 in a suburb of Rotterdam. Today the RS comprises four cohorts (RSI-IV) and new subjects are still being added. Our GWAS includes participants from RSI-III. Extensive details and objectives of the RS have been described elsewhere. The Rotterdam Study has been approved by the

institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare and Sports [11].

Phenotyping

Collection of our phenotype, facial telangiectasia in the RS, has been validated and described in detail before [12]. In short, telangiectasia was digitally extracted from standardized high-resolution facial photographs using a semi-automated script in MATLAB. This resulted in a percentage area of the total facial area which is covered with telangiectasia. Between the start of the dermatological screening in 2010 and July 2013, we included 2,842 men and women, after quality control (QC).

Genotyping and imputation

DNA extraction was performed using whole blood samples following standardized and previously described protocols [13]. Genotyping in the RS was performed using both the Infinium II HumanHap550(-Duo) (RSI & RSII) and 610-Quad Genotyping BeadChip (RSI & RSIII; Illumina, San Diego, CA, USA). Imputation of markers was performed using the Haplotype Reference Consortium 1.1 as reference panel [14]. RSI, II and III were imputed separately on the Michigan imputation server. In total 39 117 105 genotypes or imputed variants were available. Additionally, markers with poor imputation quality scores (R2 < 0.3) or frequencies lower than 1% were removed.

Statistical analysis

We performed a GWAS separately for cohorts RSI, RSII and RSIII using a linear regression with the score test and RVTESTS software package [15]. Since the residuals of the linear regression on telangiectasia did not fit a normal distribution, we ln-transformed our outcome measure resulting in approximately normal distribution of the residuals of the regression. Our analyses were adjusted for age, sex and two technical variables which accounted for the variability in analyzed batches and flashlight. A conditional analysis was performed by additionally adjusting the analysis for skin color. Details of all variables have been published [6]. To account for possible population stratification and hidden relatedness between participants, we also adjusted for the first four genetic principal components. Subsequently, QC was performed using EasyQC software package with parameter defaults [16]. To bundle the results of our three cohorts, we performed a meta-analysis using software METAL and the inverse variance approach [17]. Meta-analysis was completed for 8 086 478 markers. P-values $< 0.05*10^{-8}$ were considered genome-wide statistically significant and P-values $0.05*10^{-8} < 0.05*10^{-5}$ genome-wide statistically suggestive.

Replication and power calculation

Replication of our top associated SNPs (P-value $< 5.0*10^{-6}$) was performed in two separate cohorts. The first cohort consisted of 460 German elderly women of the SALIA cohort, where

telangiectasia have been scored manually based on photonumeric grading as part of the SCINEXA™ method[18]. Details on this cohort have been described elsewhere [19, 20]. The GWAS was performed using linear regression, adjusted for age and the first 10 genetic principal components. The second replication cohort consisted of 576 RS participants where photographs were collected between September 2013 and May 2016, available after QC. Here, phenotyping, genotyping and statistical analysis were performed as described in detail above. Additionally, we conducted a power analysis to calculate the power of our analysis and the probability of replicating our top SNP in two independent cohort, using GWAPower tool [21].

Candidate gene approach

To assess whether telangiectasia is associated with known pigmentation genes, we reviewed the association between the SNPs on these genes known from their association with pigmented spots [5], tanning response [22] or hair color [23] in three recent state-of-the-art GWAS papers, and telangiectasia in the discovery cohort. This was performed by selecting the dosage of the alleles of the known variants and performing a linear regression. For the skin color gene *MC1R*, several functional SNPs have been discovered with known cumulative effects. Therefore, we combined four known functional *MC1R* variants (rs1805005, rs1805007, rs1805008, rs1805009) into one genetic risk score by adding up the number of risk alleles [8]. Additional analyses conditioned on skin color were performed. The SNP (rs191497052) which was associated with telangiectasia in female Han Chinese[9] is not present in our European cohort and therefore was not analyzed. P-values < 0.05 were regarded as statistically significant.

Bioinformatics

Single nucleotide polymorphisms were annotated to genes using UCSC genome browser (GRCh37/hg19). To assess how the found associations could influence mRNA expression levels, the association of our top SNPs with expression quantitative trait loci (eQTLs) in different tissues was investigated using the GTEx portal (https://gtexportal.org/) during Q1 2020, and SNP phenotype associations in the UK biobank via Open Targets (https://www.opentargets.org/) [24].

RESULTS

Population characteristics

Our population consisted of 1521 women (53.5%) and 1321 men (46.5%). The median age was 66.6 years, and the median percentage of facial telangiectasia area was slightly higher in women than in men [men: 0.77%, (interquartile range (IQR) 0.49–1.21); women: 0.96%, (IQR 0.62–1.41)].

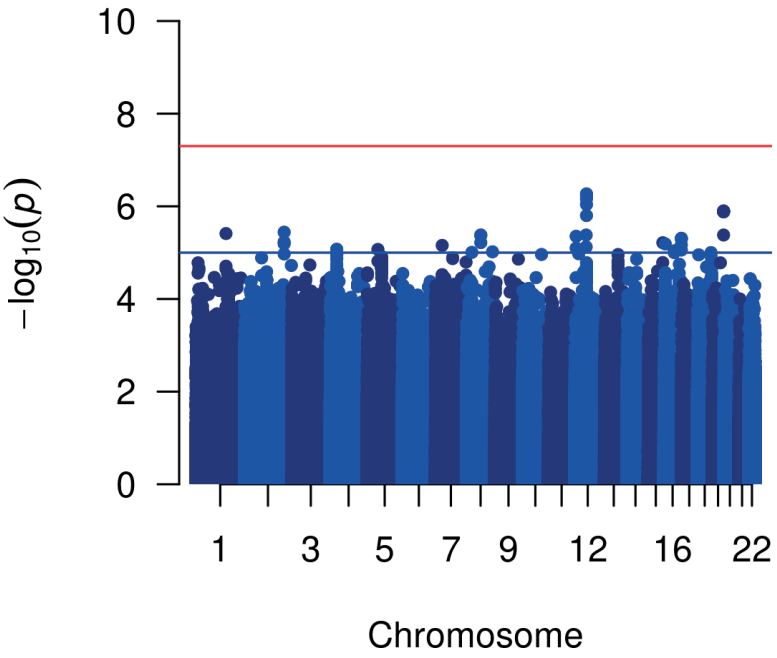

Figure 1. Manhattan plot representing the association between the single nucleotide polymorphisms (SNPs) and the ln-transformed percentage of the face, which is covered with telangiectasia for 2842 men and women. On the x-axis the chromosomes are plotted with each dot representing a SNP on corresponding chromosomal locations vs. the −log10(P-value) of the association. The red horizontal line represents the threshold for genome-wide-significant, indicating a P-value of $5*10^{-8}$. The blue horizontal line represents the threshold for genome-wide-suggestive.

GWAS results and replication

In our main GWAS, we did not find any genome-wide significant hits (Figure 1). The most significantly associated SNP was rs4417318 (P-value $5.38*10^{-7}$), an intergenic SNP located on chromosome 12. This SNP is significantly associated with variation in the expression (i.e. an eQTL) of the pseudogene *RP11-813P10.2* exclusively in coronary artery tissue as were the other suggestive hits in this locus (Table 1) supporting a vasculature role for this gene locus.

Other associated SNPs with a P-value < $5.0*10^{-6}$ were located on chromosome 12 as well but also on chromosomes 1, 2, 8, 16 and 19 (Table 1). The second strongest locus that was associated, on chromosome 19, had a significant association with the expression of the *ZNF211* in skin, which is the most significant of its eQTL associations. In addition, this SNP is associated with platelet and red cell distribution width in the UK biobank. On chromosome 2, the most significant SNP is nearby to the *ICOS* gene (inducible T-cell costimulatory) which is linked to skin wound healing including angiogenesis[25]. The strongest associating SNP on chromosome 1 is within the gene *KCNN3*, which is strongly linked with atrial fibrillation[26]. SNP rs7463003 on chromosome 8 is between the genes *RDH10* and *STAU2*, both genes are significantly associated

Table 1. Top hits GWAS telangiectasia Rotterdam Study, n=2842

SNP	CHR	BASE	EA	OA	fEA	P- value	p-value in SALIA replication	p-value in RS replication	Direction	Mapped gene	Most significant eQTL (tissue type)
rs4417318	12	60620713	c	g	0.6543	5.38E-07	0.091	0.525	---	*SLC16A7*	*RP11-813P10.2* (coronary artery)
rs12230938	12	60708785	a	c	0.3449	5.96E-07	0.125	0.535	+++	*SLC16A7*	*RP11-813P10.2* (coronary artery)
rs17602381	12	60567404	a	t	0.3448	6.87E-07	0.295	0.462	+++	*SLC16A7*	*RP11-813P10.2* (coronary artery)
rs12227514	12	60558709	t	c	0.3454	9.13E-07	0.273	0.434	+++	*SLC16A7*	*RP11-813P10.2* (coronary artery)
rs73573497	19	58165890	a	g	0.0529	1.26E-06	0.479	0.347	---	*ZNF211*	*ZNF211* (skin not sun-exposed)
rs73573500	19	58166262	t	c	0.0530	1.27E-06	0.479	0.349	---	*ZNF211*	*ZNF211* (skin not sun-exposed)
rs73573501	19	58166536	t	c	0.0529	1.27E-06	0.479	0.346	---	*ZNF211*	*ZNF211* (skin not sun-exposed)
rs12610258	19	58167451	c	g	0.9471	1.30E-06	0.479	0.343	+++	*ZSCAN4*	*ZNF211* (skin not sun-exposed)
rs12610292	19	58167753	t	c	0.0529	1.32E-06	0.479	0.342	---	*ZSCAN4*	*ZNF211* (Esophagus - Mucosa)
rs9710520	19	58168922	a	g	0.9472	1.32E-06	0.458	0.342	+++	*ZSCAN4*	*ZNF211* (skin not sun-exposed
rs11173337	12	60531507	a	g	0.3488	1.58E-06	0.092	0.379	+++	*SLC16A7*	*RP11-813P10.2* (coronary artery)
rs77938763	2	204962815	a	g	0.0210	3.61E-06	0.521	0.407	---	*ICOS*	None
rs77766535	1	154746441	a	c	0.0988	3.87E-06	0.995	0.494	+++	*KCNN3**	None
rs7463003	8	74315266	a	c	0.9794	4.16E-06	0.815	0.088	+++	*STAU2-AS1*	None
rs2106823	19	58168641	a	g	0.9379	4.16E-06	0.867	0.334	+++	*ZSCAN4*	Not present in database
rs73351721	12	58830976	a	g	0.0602	4.17E-06	0.843	0.086	+++	*AK093124*	None
rs118021692	8	74313389	a	g	0.0207	4.24E-06	0.815	0.086	---	*STAU2-AS1*	None
rs7198289	16	90134174	a	c	0.1768	4.89E-06	0.972	0.766	+++	*PRDM7**	*FAM157C* (whole blood)[$]
rs7198471	16	90134260	a	c	0.1768	5.00E-06	0.972	0.767	+++	*PRDM7**	*FAM157C* (whole blood) [$]

Main results of GWAS telangiectasia in n=2842 individuals (p-value <=5* 10^{-6}). SNP listed on rs number sorted by p-value (smallest through largest). CHR, chromosome; BASE, refers to position of SNP on the chromosome; EA, effect allele; OA, other allele; fEA, frequency of the effect allele; Direction, direction in which the effect of the SNP is per cohort of the Rotterdam Study (RSI, RSII, RSIII); Mapped gene according to UCSC genome browser where * indicates the SNP is in the gene, all others are intergenic SNPs mapped to closest gene/ transcript. [$] - most significant eQTL in skin was with *CDK10*.

with systolic blood pressure in the UK biobank although this SNP itself is not. Finally, the most significant SNP on chromosome 16 was significantly associated with ease of skin tanning in the UK biobank (P-value = $3.0*10^{-176}$) and is in the gene *PRDM7* but near the *MC1R* gene, and the most significant eQTL in skin is with the gene *CDK10*.

None of the top SNPs could be replicated in the two independent cohorts (Table 1), although this might be explained by lack of power. The power calculation performed indicated at least 950 subjects per cohort would be required to have an 80% power of replicating the associations that were found in the discovery cohort, since the top SNP only explained 2% of the total variance (data not shown). An additional GWAS conditioned on skin color, revealed similar effect sizes and P-values; however, the two SNPs in the *PRDM7* gene, near the *MC1R* gene dropped in significance. This suggests these hits were not (entirely) independent of skin color.

Candidate gene approach

Telangiectasia were significantly associated with known pigmentation SNPs with rs16891982 (p-value 0.03) mapping to the *SLC45A2* gene and rs11703668 (p-value 0.01) mapping to the *KIAA0930* gene. In addition, the combined *MC1R* genetic risk score was also significantly associated with having more telangiectasia (p-value 0.03) (Supplementary Table S1). Conditional analysis revealed that the *KIAA0930* gene signal might be partly skin color independent (p-value 0.03) whereas the *SCLC45A2* gene signal (p-value 0.08) and the *MC1R* genetic risk score (p-value 0.26) were not.

DISCUSSION

This GWAS study on facial telangiectasia did not reveal genome-wide significant associations between SNPs and facial telangiectasia in a northwestern European population. However, there are tentative links between the genes near some of the suggestive SNPs with the vasculature system, perhaps, indicating some of them are not false positives. In addition, in a candidate gene approach, several significant links with known pigmentation genes and telangiectasia were found, confirming the link between skin color and telangiectasia found in epidemiological studies.

Smoking habits and UV-exposure remain the most importantly associated life style factors associated with the presence of facial telangiectasia [1-4]. In addition, pigmentation and skin color seem to play a role because pale colored individuals are repeatedly most at risk. In support of this, the current study found two SNPs in known skin color genes (*KIAA0930* and *SLCA45A2*) and the *MC1R* genetic risk score to be associated with telangiectasia in addition to the genome-wide suggestive SNPs in the *PRDM7* gene which also covers the *MC1R* locus. The link between pale skin and telangiectasia might be explained by the increased risk of getting sunburn or UV-related damage which is more pronounced in individuals with pale skin. Photodamaged biopsies

in a recent study into photoaging show more elastic damage, sebaceous gland prominence, inflammation and dilated vessels compared to participant matched sun-protected buttock skin [27] which indicates that UV-damaged skin has more telangiectasia than sun-protected skin. The *KIAA0930* gene locus was recently discovered to be associated with tanning response to sun exposure [22], hair color and sunburn [28], revealing association with multiple pigmentation traits. The SNP tagging the *KIAA0930* gene remained significantly associated with greater telangiectasia when additionally correcting for skin color, although with marginal significance level (P-value 0.03) given the fairly large sample size. The results tagging the *MC1R* gene and the *SLC45A2* gene, in contrast, were more likely driven by their association with skin color. Overall these results indicate pigmentation genes do not associate fundamentally with telangiectasia independently of skin color. This is in contrast to other skin aging phenotypes, e.g. pigmented spots, where several pigmentation genes were very significantly associated with the amount of acquired facial pigmented spots, even when additionally adjusted for skin color [5]. This highlights different genetic pathways for different skin aging phenotypes.

Our GWAS results indicate that there are no single gene variants with strong association with telangiectasia. In conjunction, as the heritability of telangiectasia is low [10], it suggests that very large studies (e.g. >10 000 subjects) might be needed to identify any gene variants that do associate on a genome-wide level. The only SNP previously reported to be significantly associated with telangiectasia, rs191497052, could not be replicated in our discovery cohort nor in two other smaller Caucasian cohorts, since it was not present [9]. This highlights the need for further replication of rs191497052 in Asian populations and better understanding of the genetic background of skin aging features across different populations.

Although the genes tagged by the top SNPs are linked to the vasculature system, the links were quite disparate. For example, the top SNP linked to *RP11-813P10.2* expression in coronary artery tissue, *ICOS* is linked to angiogenesis, SNPs in *ZNC211* are linked with platelet and red cell parameters in the UK biobank, *KCNN3* is linked with atrial fibrillation, and *RDH10* and *STAU2* are linked with systolic blood pressure. Hence, replication of these SNP associations is required before vasculature associated variants can be determined to be driving the appearance of telangiectasia. Future, larger studies might also investigate the relation between pigmentation and the vasculature system as one can imagine vasculature differences in different skin colors (maybe resulting in more or less telangiectasia).

Strengths to this study are that the telangiectasia method has been validated and was successfully used in lifestyle studies [4, 12]. Also, we added two independent cohorts for external validation of our results. However, the sample size of our cohorts was too small to discover and replicate SNPs with small effect sizes in traits with low heritability. Alternatively, the suggestive SNPs in the discovery cohort could be false positives.

In conclusion, we conducted a GWAS on facial telangiectasia in a fairly large northwestern European population of men and women in an attempt to explore its' genetic background. We did not find significantly associated SNPs in this study, however, suggestive signals showed

tentative links with the vascular system. Significantly associated pigmentation genes *KIAA0930*, *SLCA45A2* and *MC1R* underline the link between skin color and telangiectasia in a candidate gene approach. Much larger studies are now required to replicate suggestive signals and to identify the influences of DNA sequence variants on telangiectasia.

REFERENCES

1. Green AC, Hughes MC, McBride P , Fourtanier A. Factors associated with premature skin aging (photoaging) before the age of 55: a population-based study. Dermatology 2011;222:74-80.
2. Isik B, Gurel MS, Erdemir AT , Kesmezacar O. Development of skin aging scale by using dermoscopy. Skin Res Technol 2013;19:69-74.
3. Kennedy C, Bastiaens MT, Bajdik CD, Willemze R, Westendorp RG, Bouwes Bavinck JN et al. Effect of smoking and sun on the aging skin. J Invest Dermatol 2003;120:548-54.
4. Mekic S, Hamer MA, Wigmann C, Gunn DA, Kayser M, Jacobs LC et al. Epidemiology and determinants of facial telangiectasia: a cross-sectional study. J Eur Acad Dermatol Venereol 2019.
5. Jacobs LC, Hamer MA, Gunn DA, Deelen J, Lall JS, van Heemst D et al. A Genome-Wide Association Study Identifies the Skin Color Genes IRF4, MC1R, ASIP, and BNC2 Influencing Facial Pigmented Spots. J Invest Dermatol 2015;135:1735-42.
6. Hamer MA, Pardo LM, Jacobs LC, Ikram MA, Laven JS, Kayser M et al. Lifestyle and physiological factors associated with facial wrinkling in men and women. J Invest Dermatol 2017.
7. Gunn DA, Rexbye H, Griffiths CE, Murray PG, Fereday A, Catt SD et al. Why some women look young for their age. PLoS One 2009;4:e8021.
8. Liu F, Hamer MA, Deelen J, Lall JS, Jacobs L, van Heemst D et al. The MC1R Gene and Youthful Looks. Curr Biol 2016;26:1213-20.
9. Liu Y, Gao W, Koellmann C, Le Clerc S, Hüls A, Li B et al. Genome-wide scan identified genetic variants associated with skin aging in a Chinese female population. Journal of Dermatological Science 2019;96:42-9.
10. Pardo LM, Hamer MA, Liu F, Velthuis P, Kayser M, Gunn DA et al. Principal component analysis of seven skin ageing features identifies three main types of skin ageing. Br J Dermatol 2019.
11. 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;32:807-50.
12. Hamer MA, Jacobs LC, Lall JS, Wollstein A, Hollestein LM, Rae AR et al. Validation of image analysis techniques to measure skin aging features from facial photographs. Skin Res Technol 2015;21:392-402.
13. Kayser M, Liu F, Janssens AC, Rivadeneira F, Lao O, van Duijn K et al. Three genome-wide association studies and a linkage analysis identify HERC2 as a human iris color gene. Am J Hum Genet 2008;82:411-23.
14. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet 2016;48:1279-83.
15. Zhan X, Hu Y, Li B, Abecasis GR , Liu DJ. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. Bioinformatics 2016;32:1423-6.
16. Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Magi R et al. Quality control and conduct of genome-wide association meta-analyses. Nat Protoc 2014;9:1192-212.
17. Willer CJ, Li Y , Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 2010;26:2190-1.
18. Vierkotter A, Ranft U, Kramer U, Sugiri D, Reimann V , Krutmann J. The SCINEXA: a novel, validated score to simultaneously assess and differentiate between intrinsic and extrinsic skin ageing. J Dermatol Sci 2009;53:207-11.
19. Vossoughi M, Schikowski T, Vierkotter A, Sugiri D, Hoffmann B, Teichert T et al. Air pollution and subclinical airway inflammation in the SALIA cohort study. Immun Ageing 2014;11:5.

20. Huls A, Abramson MJ, Sugiri D, Fuks K, Kramer U, Krutmann J et al. Nonatopic eczema in elderly women: Effect of air pollution and genes. J Allergy Clin Immunol 2019;143:378-85 e9.
21. Feng S, Wang S, Chen C-C , Lan L. GWAPower: a statistical power calculation software for genome-wide association studies with quantitative traits. BMC Genet 2011;12:12-.
22. Visconti A, Duffy DL, Liu F, Zhu G, Wu W, Chen Y et al. Genome-wide association study in 176,678 Europeans reveals genetic loci for tanning response to sun exposure. Nat Commun 2018;9:1684.
23. Hysi PG, Valdes AM, Liu F, Furlotte NA, Evans DM, Bataille V et al. Genome-wide association meta-analysis of individuals of European ancestry identifies new loci explaining a substantial fraction of hair color variation and heritability. Nature Genetics 2018;50:652-6.
24. Carvalho-Silva D, Pierleoni A, Pignatelli M, Ong C, Fumis L, Karamanis N et al. Open Targets Platform: new developments and updates two years on. Nucleic Acids Research 2018;47:D1056-D65.
25. Maeda S, Fujimoto M, Matsushita T, Hamaguchi Y, Takehara K , Hasegawa M. Inducible costimulator (ICOS) and ICOS ligand signaling has pivotal roles in skin wound healing via cytokine production. Am J Pathol 2011;179:2360-9.
26. Wang X, Nie Y, Ning S, Shi Y, Zhao Y, Niu S et al. [Rs17042171 at chromosome 4q25 is associated with atrial fibrillation in the Chinese Han population from the central plains]. Zhong Nan Da Xue Xue Bao Yi Xue Ban 2018;43:594-603.
27. Sachs DL, Varani J, Chubb H, Fligiel SEG, Cui Y, Calderone K et al. Atrophic and hypertrophic photoaging: Clinical, histologic, and molecular features of 2 distinct phenotypes of photoaged skin. Journal of the American Academy of Dermatology 2019;81:480-8.
28. Kichaev G, Bhatia G, Loh P-R, Gazal S, Burch K, Freund MK et al. Leveraging Polygenic Functional Enrichment to Improve GWAS Power. American journal of human genetics 2019;104:65-75.

SUPPLEMENTARY TABLES

Supplementary Table S1. Association with known skin pigmentation genes

Skin pigmentation gene	SNP	CHR	BASE	EA	fEA	p-value
KIAA0930	rs11703668	22	45630335	g	0.4600	**0.01**
MC1R	GRS*	16	-	-	-	**0.03**
SLC45A2	rs16891982	5	33951693	c	0.0215	**0.03**
FOSL2	rs71443018	2	28613302	g	0.0390	0.18
BNC2	rs62543565	9	16901067	a	0.6254	0.20
EMX2	rs35563099	10	119572403	t	0.1600	0.20
RALY/ASIP	rs6059655	20	32665748	a	0.0826	0.25
IRF4	rs12203592	6	396321	t	0.0973	0.29
PA2G4P4	rs9818780	3	156492758	c	0.4900	0.32
OCA2	rs1800407	15	28230318	t	0.0488	0.33
SLC24A4	rs12896399	14	92773663	t	0.5010	0.40
EDNRB	rs1279403	13	78391757	t	0.4060	0.40
TYRP1	rs1408799	9	12672097	t	0.2955	0.51
RIPK5	rs12078075	1	205163798	g	0.0900	0.56
DSTYK	rs2369633	1	205181062	t	0.0890	0.58
AHR/AGR3	rs117132860	7	17134708	a	0.0300	0.60
ATP11A	rs1046793	13	113539894	c	0.4600	0.64
BCAS1	rs73132911	20	52661068	t	0.0460	0.64
TRPS1	rs2737212	8	116621214	c	0.4500	0.69
SLC45A1	rs80293268	1	8207579	g	0.0470	0.72
KITLG	rs12821256	12	89328335	t	0.8700	0.76
TPCN2	rs35264875	11	68846399	a	0.8298	0.76
PPARGC1B	rs251464	5	149196234	c	0.2500	0.78
SHC4	rs1426654	15	48426484	g	0.0210	0.79
HERC2	rs12913832	15	28365618	a	0.1710	0.80
KRT31	rs117612447	17	39551099	t	0.0290	0.84
LHX2	rs58979150	9	126808006	t	0.1080	0.96
TYR	rs1393350	11	89011046	a	0.2325	0.99
PDE4B	rs1308048	1	66888542	c	0.4200	0.99
DCT	rs9561570	13	95156198	t	0.3100	0.99

Results of candidate gene approach regarding known skin pigmentation genes (first column) and ln-transformed telangiectasia percentage, matched on the most significant SNPs on these genes known from their association with pigmented spots, tanning response or hair color and telangiectasia, sorted by p-value (smallest through largest). CHR, chromosome; BASE, refers to position of SNP on the chromosome; EA, effect allele; fEA, frequency of the effect allele; p-value of SNP in linear regression in the discovery cohort of the RS (n=2842), the significant associations ($p<0.05$) are presented in **bold.** * For *MC1R* a genetic risk score using 4 functional *MC1R* SNPs (rs1805005, rs1805007, rs1805008, rs1805009) was applied.

Part III

DRY SKIN

CHAPTER 3.1

Prevalence and determinants for xerosis cutis in the middle-aged and elderly population: a cross-sectional study

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ABSTRACT:

Background: Determinants and the extent of dry skin in healthy middle-aged and elderly populations have not been well established.

Objective: We aimed to identify the prevalence and determinants for generalized dry skin (GDS) and localized dry skin (LDS) within a large prospective population-based cohort of middle-aged and elderly individuals of the Rotterdam Study.

Methods: Dry skin was physician-graded as none, localized, or generalized. For GDS and LDS, separate multivariable logistic regression analyses were performed to search for association with participant characteristics, lifestyle factors, environmental factors, several comorbidities, and drug exposure.

Results: Among the 5,547 eligible participants, 60% had dry skin, of whom a fifth had GDS. Age, female sex, skin color, body mass index, outside temperature, eczema, and chemotherapy in the past were significant determinants for both GDS and LDS. Smoking, the use of statins and diuretics, poorer self-perceived health, and several dermatologic conditions increased the likelihood of having GDS only. Daily cream use was associated with less LDS.

Limitations: Interobserver variability and residual confounding could have influenced our results. Because of our cross-sectional design, we could not infer causality.

Conclusion: We identified factors significantly associated with dry skin in a general middle-aged and elderly population, with health parameters more strongly associated with GDS.

INTRODUCTION

Dry skin (xerosis cutis) is one of the most common skin conditions in middle-aged and elderly populations and can be considered part of the physiologic aging of skin. As the worldwide overall prevalence of dry skin is estimated at 29% to 85% [1-5] it affects roughly every other person. Dry skin can be a very heterogeneous phenotype and can present with scaling, roughness, and even fissures. Patients usually experience itch, but the skin can also feel tight, painful, or burning. In addition, dry damaged skin can be a *porte d'entrée* for skin infections.

Xerosis may be a feature on its own, or it can co-occur with or be part of different skin diseases. Also, some chronic diseases, including diabetes, HIV, hypothyroidism, and renal insufficiency, and some therapies, including the use of statins, diuretics, or chemotherapeutic agents, can be accompanied by dry skin [2, 3, 6-9]. Therefore, dry skin not only is a very common condition but may also be an indicator of a person's health status.

Many different lifestyle and environmental factors are known to influence dry skin, including bathing behavior and weather conditions [10]. Others, such as smoking and alcohol consumption, are less well investigated. Genes also play a role, with the *filaggrin* gene (*FLG*) being the best-known associated gene [11].

Most observational research on xerosis in elderly individuals has been performed in selected and relatively small populations, such as nursing home residents and those with many comorbidities [1, 2]. Therefore, little evidence is provided for determinants of dry skin in general middle-aged and older populations, and few studies have investigated a broad range of possible determinants and how they relate to the extent of dry skin. In this study, we aimed to investigate the prevalence and determinants of localized dry skin (LDS) (ie, mild dry skin) or generalized dry skin (GDS) (ie, severe dry skin), as well as co-occurring diseases, in a large middle-aged and elderly population-based cohort.

METHODS

Study design

Participants were selected from the Rotterdam Study (RS) cohort, which is a large prospective population-based cohort situated in the Rotterdam suburb Ommoord. The study started in 1990 and is still ongoing. Details and objectives of the RS have been described elsewhere. The RS has been approved by the institutional review board (medical ethics committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare, and Sports[12].

Identification of dry skin

Between 2010 and 2016, during routine visits at the research center, a full-body skin examination (FBSE) was performed in 5,555 participants. The presence and extent of dry skin were graded in 5,547 individuals by a dermatology-trained physician by observing scaly or rough skin with or without erythema that did not fit any other known skin disease. We also collected data on self-reported dry skin, but because of poor agreement with our clinical judgment of dry skin, we chose physician-based dry skin as the outcome. Dry skin was scored as absent, localized (on the extensor side of the arms and legs), or generalized (LDS and GDS, respectively). All 5,547 RS participants were included in our analysis.

Characteristics

Sex and age at entry of the study were collected from the database. Self-perceived health, smoking, alcohol intake, and education level were collected from general interviews. Data on facial cream use were collected from dermatologic interviews. Skin color was graded by physicians as belonging to 1 of 3 darkness categories. Height and weight were measured at the research center and used to calculate body mass index (BMI). Mean outside temperature and air humidity over the last week before the center visit were calculated by using the weather data from Rotterdam the Hague airport.

Associated diseases

Dermatologic conditions were assessed during the FBSE. Eczema was defined as erythematous, scaly, lichenified, excoriated, and fissured patches. Seborrheic dermatitis was defined as erythema with greasy scaling on the typical locations of the scalp, face, or chest. Psoriasis was assessed as sharply demarcated erythematous, scaly thickened patches. Varicose veins were graded by using the clinical, etiology, anatomy, and pathophysiology classification (CEAP) and when present, assigned a clinical (C) score of C2 to C6. Self-reported history of itchy skin conditions, asthma, hay fever, and dust mite allergy were collected from dermatologic interviews. Diabetes mellitus was scored as present if at least 1 of the following criteria was present: fasting plasma glucose level of 7.0 mmol/L or higher, nonfasting glucose level of 11.1 mmol/L or higher, and use of antidiabetic medicine or dietary treatment for type 2 diabetes mellitus. Renal impairment was defined as having a glomerular filtration rate lower than 60. Hypothyroidism was graded as present depending on the combination of thyroid-stimulating hormone (TSH) and free thyroxine (fT4) levels as follows: high TSH level and normal or low fT4 level or normal TSH level and low fT4 level.

Associated medications

A trained nurse, who examined all the medication in use, assessed the current use of statins and diuretics. Ever receiving chemotherapy was self-reported in 1 of the interviews.

Statistical analysis

To investigate the various determinants in relation to dry skin, we performed a multivariable binary logistic regression, during which we adjusted for possible confounders. GDS and LDS were assessed separately. Use of an ordinal logistic regression model was considered but was not possible because the assumption of parallel lines was violated [13]. The rate of missing data was less than 15% per variable and was imputed by using multiple imputation with 20 imputations. First, we analyzed possible determinants for GDS and LDS, including age, sex, skin color, mean temperature, relative humidity, cream use, smoking, alcohol consumption, BMI, self-perceived health, and education level. Interaction between relative humidity and mean temperature was present in the GDS analysis, and therefore, the interaction term was added to the model.

Second, we investigated the role of common chronic diseases and concomitant medication use and presence of GDS and LDS in a logistic regression model that was adjusted for all significant factors from the multivariable analysis. Because we selected the investigated variables on the basis of prior hypotheses as well as plausible and previously reported associations in the literature, we did not correct for multiple testing and regarded P values of .05 or lower as significant. All analyses were conducted by using SPSS Statistics for Windows (version 24.0, IBM Inc, Armonk, NY).

RESULTS

Demographics

This cohort included 5547 middle-aged and elderly participants (age range, 51-101 years; mean age, 70 years; 57% of our participants were female, and 60% (95% confidence interval [CI], 58%-61%) had dry skin. Of the individuals with dry skin, 1 in 5 were severely affected and had GDS, whereas the rest had dry skin only on the extensor side of the extremities (ie, LDS) (Supplementary Table S1).

Lifestyle and demographic determinants

Age was significantly associated with dry skin, more so with GDS (odds ratio [OR], 1.04; 95% CI, 1.03-1.05) than with LDS (OR, 1.009; 95% CI, 1.003-1.016). Women were more commonly affected than men: they had a 50% higher likelihood of having GDS and a 30% higher likelihood of having LDS. Individuals with a brown-black skin color experienced GDS 3 times more often than did individuals with skin in the Mediterranean skin color group (OR, 3.62; 95% CI, 1.96-6.73). However, individuals with a white skin color had LDS more often than did individuals with skin in the Mediterranean skin color group (OR, 1.18; 95% CI, 1.01-1.38). A higher BMI was associated with less GDS and LDS (Table I).

A higher mean outside temperature was strongly associated with less GDS (OR, 0.70; 95% CI, 0.56-0.88) and moderately associated with less LDS (OR, 0.95; 95% CI, 0.94-0.96). Relative out-

Table 1. Multivariable logistic model.

Variable	Generalized VS no dry skin[1] OR (95%CI)	p	Localized VS no dry skin[2] OR (95%CI)	p
Age (years)[3]	**1.04 (1.03-1.05)**	**<0.01**	**1.009 (1.003-1.016)**	**<0.01**
Sex				
Female	**1.49 (1.16-1.93)**	**<0.01**	**1.29 (1.10-1.52)**	**<0.01**
Skin color				
Olive to light brown	Reference		Reference	
Very white to white	1.24 (0.94-1.64)	0.12	**1.18 (1.01-1.38)**	**0.049**
Brown to black	**3.62 (1.96-6.73)**	**<0.01**	1.20 (0.76-1.90)	0.43
BMI[4]	**0.96 (0.94-0.98)**	**<0.01**	**0.98 (0.97-0.99)**	**<0.01**
Humidity[5]	0.99 (0.96-1.02)	0.39	**0.988 (0.978-0.998)**	**0.01**
Temperature[6]	**0.70 (0.56-0.88)**	**<0.01**	**0.95 (0.94-0.96)**	**<0.01**
Humidity X temperature[7]	**1.003 (1.001-1.006)**	**0.01**		
Cream use				
No	Reference		Reference	
Yes, sometimes	1.29 (0.91-1.83)	0.16	1.09 (0.87-1.36)	0.46
Yes, daily	0.80 (0.60-1.02)	0.11	**0.77 (0.65-0.92)**	**<0.01**
Smoking[8]	**1.27 (1.02-1.57)**	**0.03**	0.99 (0.87-1.14)	0.94
Alcohol consumption[9]	1.00 (0.99-1.02)	0.64	0.995 (0.987-1.003)	0.20
Self-perceived health[10]	**0.993 (0.987-0.999)**	**0.02**	0.998 (0.994-1.002)	0.40
Education level[11]				
Low	Reference		Reference	
Medium	0.99 (0.74-1.34)	0.96	1.05 (0.86-1.28)	0.66
High	0.77 (0.55-1.08)	0.13	0.84 (0.67-1.04)	0.11

[1] The odds ratio expresses the odds for having a generalized dry skin versus no dry skin per tested variable. This analysis is adjusted for the following factors: sex, age, temperature, relative humidity, temperature X relative humidity, cream use, smoking, alcohol consumption, BMI, Quality Of Life and education level; [2] The odds ratio expresses the odds for having a localized dry skin versus no dry skin per tested variable. This analysis is adjusted for the following factors: sex, age, temperature, relative humidity, cream use, smoking, alcohol consumption, BMI, Quality Of Life and education level; [3] Per one year increase; [4] Body Mass Index in kg/m^2 per 1 point; [5] Rolling Relative humidity over the last week in %; [6] Rolling average temperature over the last week in Celsius; [7] Interaction term rolling relative humidity x rolling average temperature. Not significant in localized model, hence excluded; [8] Ever smoking versus never; [9] Alcohol consumption per gram/day; [10] Self perceived health score based on overall health, scores between 0-100 (0 is low quality – 100 is high quality) per 1 point; [11] Low= primary education. Medium= lower vocational/ lower secondary/ intermediate vocational education. High= general secondary/ higher vocational education or university. P-values <0.05 are presented in **bold**

side air humidity was related to temperature and had a significant interaction with temperature in the GDS model. A higher relative humidity did not significantly interact with temperature in LDS and had a small protective association (Table I).

Interestingly, although reported for facial cream use, participants who used cream on a daily basis, had less LDS (OR, 0.77; 95% CI, 0.65-0.92), but not GDS (OR, 0.80; 95% CI, 0.60-1.02).

Smokers had more GDS (OR, 1.27 1.02-1.57), whereas individuals with better self-perceived health, had less GDS (OR, 0.993; 95% CI, 0.987-0.999). Education level and alcohol use were not associated with presence of dry skin (Table I).

Comorbidities and associated medications

Of the assessed skin diseases, eczema was highly associated with having dry skin. Here, the probability of having LDS was 2.5 times higher (OR, 2.44; 95% CI, 1.85-3.25) and the likelihood of having GDS was 7 times higher in patients with eczema (OR, 7.04; 95% CI, 5.92-8.37). Other dermatologic diseases associated with GDS were seborrheic dermatitis (OR, 1.38; 95% CI, 1.06-1.79) and an itchy skin condition in the past (OR, 1.26; 95% CI, 1.14-1.39). Having psoriasis or higher C scores on the clinical, etiology, anatomy, and pathophysiology classification for venous insufficiency was not associated with dry skin (Table II).

Other tested medical conditions included diabetes, which was a determinant for LDS only (OR, 1.22; 95% CI, 1.04-1.45) (Table II). Renal impairment, hypothyroidism, and atopic constitution (asthma, hay fever, or dust mite allergy) were not associated with dry skin.

Table 2. Associated diseases and medication regression model

Disease or medication	OR (95%CI) generalized VS no dry skin[1]	p	OR (95%CI) localized VS no dry skin[2]	p
Dermatological diseases				
Eczema	**7.04 (5.92-8.37)**	**<0.01**	**2.44 (1.85-3.25)**	**<0.01**
Seborrheic derm[3]	**1.38 (1.06-1.79)**	**0.02**	1.05 (0.88-1.26)	0.57
Psoriasis	1.01 (0.60-1.68)	0.99	0.89 (0.64-1.24)	0.50
Itchy skin condition[4]	**1.26 (1.14-1.39)**	**0.02**	1.08 (0.95-1.22)	0.25
Varicose veins	0.90 (0.73-1.11)	0.34	0.97 (0.85-1.10)	0.62
Other diseases				
Diabetes	1.04 (0.80-1.36)	0.76	**1.22 (1.04-1.45)**	**0.02**
Renal impairment	1.20 (0.92-1.57)	0.18	1.08 (0.90-1.30)	0.42
Hypothyroidism	1.15 (0.85-1.56)	0.36	0.96 (0.78-1.18)	0.71
Atopy	0.95 (0.74-1.21)	0.68	1.01 (0.87-1.17)	0.91
Medication				
Statins	**1.28 (1.05-1.57)**	**0.02**	1.08 (0.95-1.24)	0.25
Diuretics	**1.37 (1.06-1.75)**	**0.01**	1.11 (0.94-1.32)	0.22
Chemotherapy	**1.69 (0.97-2.95)**	**0.07**	**1.56 (1.05-2.32)**	**0.03**

[1] The odds ratio represents the odds for having a generalized dry skin versus no dry skin when having a certain disease or when using a certain medicament. This analysis is adjusted for the following factors: age, sex, skin color, temperature, relative humidity, humidity X temperature, BMI, smoking and quality of life; [2] The odds ratio represents the odds for having a localized dry skin versus no dry skin when having a certain disease or when using a certain medicament. This analysis is adjusted for the following factors: age, sex, skin color, temperature, relative humidity, BMI and cream use; [3] Seborrheic dermatitis; [4] Ever having had an itchy skin condition, question from dermatological questionnaire

The use of certain medications were also linked to dry skin. Using statins (OR, 1.28; 95% CI, 1.05-1.57) and using diuretics (OR, 1.37; 95% CI, 1.06-1.75) were both significantly associated with GDS but not with LDS. Ever having received chemotherapy was associated with LDS (OR, 1.56; 95% CI, 1.05-2.32) and with GDS (OR, 1.69; 95% CI, 0.97-2.95), with similar odds ratios but with the odds of LDS being statistically significant.

DISCUSSION

In this study, the prevalence of dry skin in people with an average age of 70 years was 60%, which corresponds well with the range of 29% to 85% previously reported in the literature [1-4]. The known risk factors of increasing age, female sex, eczema, and lower outside temperature were replicated in this study. Less well known determinants included skin color (white and brown to black) and lower BMI. GDS was less common, but it was a more severe condition than LDS. Additional determinants for GDS included smoking, some dermatologic conditions, and use of certain medication. Moreover, self-perceived health was significantly poorer in individuals with GDS. Interestingly, individuals who used facial moisturizing cream on a daily basis over the past year had significantly less LDS, even though they were instructed not to wear cream 24 hours before the FBSE. If use of a facial moisturizer is assumed to be a proxy of use of a body moisturizer, this implies that emollients may have a beneficial effect on dry skin for longer than 24 hours.

Fluctuations in intercellular lipid levels, water metabolism, and changes in the keratinization process play a role in the development of dry skin [11, 14]. With aging, the skin's barrier function weakens as the lipid film on the skin surface decreases and keratinocyte proliferation declines, leading to transepidermal water loss (TEWL) and dry skin [15, 16]. Sebum production in male skin is higher and more stable throughout life, which could explain why men experience dry skin less than women do[17]. Cream use may to a certain extent mimic this lipid film and therefore prevent TEWL. Interestingly, light- and dark-skinned individuals have significantly more dry skin than do those in the group in between. Research into ethnic skin differences has shown that black skin has a higher TEWL and a 2.5 times greater desquamation rate compared with white skin[18]. Consistent evidence in Mediterranean skin color is lacking, although our results suggest that these individuals experience dry skin less than individuals with white and dark skin color do.

An increased BMI resulted in a lower risk of dry skin, which has been previously reported[2]. It is clear that malnourished individuals have drier skin on account of lack of sufficient nutrients to maintain a healthy skin barrier, with the mechanisms explaining the other side of the spectrum remaining to be fully understood. An increase in the availability of lipids for the stratum corneum with increased body mass could play a role.

Low outside temperature was highly associated with dry skin. Surprisingly, outside air humidity had a weaker association, which might be due to the difference between humidity outside

and that indoors. Therefore, we hypothesize that air humidity is important, but actual exposure to humidity indoors and out is required to further understand its importance for dry skin.

Smoking was associated with GDS. It is well known that smoking stimulates skin aging, but less well known is that it leads to dry skin. Recently, it was found that in animal models administration of nicotine disrupted the dermoepidermal junction, reduced the formation of rete ridges, and disorganized collagen bundles, all of which may affect the barrier function and increase the risk of dry skin[19].

Eczema is well known to occur with an impaired skin barrier, but seborrheic dermatitis is now also being recognized as an impaired barrier skin disease[20, 21]. This could clarify the increased likelihood of dry skin in both skin conditions. We also found that ever having had an itchy skin condition is associated with GDS. Itch is an important symptom of dry skin; this association is well known[2, 3, 22]and itch is most likely a symptom of dry skin.

Patients with diabetes showed more LDS. The association with dry skin is well known but not well understood, although it may be related to damage of dermal proteins and formation of advanced glycation end products in individuals with diabetes[7, 23]. That the association was seen only on the extremities might be due to alterations to the microvascularization in the arms and legs of individuals with diabetes. Previous studies showed that hypothyroidism and renal insufficiency are associated with xerosis[6, 9] but the prevalence of these conditions in our group of healthy subjects was very low, which could clarify why we did not find such an association. We did confirm known associations with culprit drugs, including statins, diuretics[24, 25] and chemotherapy agents (which have known toxic effects on the human body, including the skin)[26].

It was notable that GDS was more strongly associated with several comorbidities than LDS was. The systemic effects of most diseases could explain this finding, and conversely, it would imply that GDS is a symptom of diseases or even a biomarker of deteriorating health. This suggests that dry skin on the extremities is mainly a cosmetic condition in healthy individuals but becomes more widespread over the body with decreasing health. Hence, longitudinal studies are required to determine the degree to which dry skin might be pre-empting the prevalence of skin and systemic disease.

The main strength of our study is that we investigated a large population-based sample of middle-aged and elderly individuals living in the community. Also, the diagnoses were done by physicians and stratified on the basis of severity of dry skin to assess differences in a wide range of determinants for severe dry skin and LDS. We assume that physician scoring of skin as dry is more reliable than self-reporting of dry skin, as it is an independent evaluation that should be more comparable across individuals than self-report diagnoses are. Case definition might be a limitation of the study because we did not use validated questionnaire diagnostic criteria for dry skin and it was not feasible to measure TEWL. Nevertheless, consensus on the best scoring method for dry skin is lacking and the outcome assessment might suffer from inter-rater and intrarater variability, which also makes it difficult to compare the observations with those of other studies. Residual confounding, such as the lack of data on bathing behavior,

could also have influenced our results. Finally, because of the cross-sectional design of our study, no causal relationship can be proved.

In conclusion, dry skin is a highly prevalent skin condition, affecting 60% of our middle-aged and elderly population. We have identified new and replicated known determinants for dry skin, which are similar between healthy community-based populations and nursing home populations, and that dry skin is more strongly associated with health parameters such as drug use and skin disease when more widely spread across the body.

REFERENCES

1. Hahnel E, Lichterfeld A, Blume-Peytavi U , Kottner J. The epidemiology of skin conditions in the aged: A systematic review. J Tissue Viability 2017;26:20-8.
2. Lichterfeld A, Lahmann N, Blume-Peytavi U , Kottner J. Dry skin in nursing care receivers: A multi-centre cross-sectional prevalence study in hospitals and nursing homes. International Journal of Nursing Studies 2016;56:37-44.
3. Paul C, Maumus-Robert S, Mazereeuw-Hautier J, Guyen CN, Saudez X , Schmitt AM. Prevalence and risk factors for xerosis in the elderly: a cross-sectional epidemiological study in primary care. Dermatology 2011;223:260-5.
4. Smith DR, Atkinson R, Tang S , Yamagata Z. A survey of skin disease among patients in an Australian nursing home. J Epidemiol 2002;12:336-40.
5. Augustin M, Kirsten N, Korber A, Wilsmann-Theis D, Itschert G, Staubach-Renz P et al. Prevalence, predictors and comorbidity of dry skin in the general population. J Eur Acad Dermatol Venereol 2019;33:147-50.
6. Chaker L, Bianco AC, Jonklaas J , Peeters RP. Hypothyroidism. The Lancet 2017;390:1550-62.
7. de Macedo GMC, Nunes S , Barreto T. Skin disorders in diabetes mellitus: an epidemiology and physiopathology review. Diabetology & Metabolic Syndrome 2016;8:63.
8. Lee D, Benson CA, Lewis CE, Grunfeld C , Scherzer R. Prevalence and factors associated with dry skin in HIV infection: the FRAM study. AIDS 2007;21:2051-7.
9. Solak B, Acikgoz SB, Sipahi S , Erdem T. Epidemiology and determinants of pruritus in pre-dialysis chronic kidney disease patients. International Urology and Nephrology 2016;48:585-91.
10. Pons-Guiraud A. Dry skin in dermatology: a complex physiopathology. Journal of the European Academy of Dermatology and Venereology 2007;21:1-4.
11. Sandilands A, Sutherland C, Irvine AD , McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci 2009;122:1285-94.
12. 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;32:807-50.
13. Harrell FE. Ordinal Logistic Regression. Regression Modeling Strategies: With Applications to Linear Models, Logistic and Ordinal Regression, and Survival Analysis. Cham: Springer International Publishing; 2015. p. 311-25.
14. Verdier-Sévrain S , Bonté F. Skin hydration: a review on its molecular mechanisms. Journal of Cosmetic Dermatology 2007;6:75-82.
15. Chalyk NE, Bandaletova TY, Kyle NH , Petyaev IM. Age-related differences in morphological characteristics of residual skin surface components collected from the surface of facial skin of healthy male volunteers. Skin Research and Technology 2017;23:212-20.
16. Kottner J, Lichterfeld A , Blume-Peytavi U. Maintaining skin integrity in the aged: a systematic review. British Journal of Dermatology 2013;169:528-42.
17. Luebberding S, Krueger N , Kerscher M. Skin physiology in men and women: in vivo evaluation of 300 people including TEWL, SC hydration, sebum content and skin surface pH. International Journal of Cosmetic Science 2013;35:477-83.
18. Wesley NO , Maibach HI. Racial (Ethnic) Differences in Skin Properties. American Journal of Clinical Dermatology 2003;4:843-60.
19. Eltony SA , Ali SS. Histological study on the effect of nicotine on adult male guinea pig thin skin. Anat Cell Biol 2017;50:187-99.

20. Cork MJ, Danby SG, Vasilopoulos Y, Hadgraft J, Lane ME, Moustafa M et al. Epidermal Barrier Dysfunction in Atopic Dermatitis. Journal of Investigative Dermatology 2009;129:1892-908.
21. DeAngelis YM, Gemmer CM, Kaczvinsky JR, Kenneally DC, Schwartz JR , Dawson TL. Three Etiologic Facets of Dandruff and Seborrheic Dermatitis: Malassezia Fungi, Sebaceous Lipids, and Individual Sensitivity. Journal of Investigative Dermatology Symposium Proceedings 2005;10:295-7.
22. Reich A, Ständer S , Szepietowski JC. Pruritus in the elderly. Clinics in Dermatology 2011;29:15-23.
23. Gkogkolou P , Böhm M. Advanced glycation end products. Dermato-Endocrinology 2012;4:259-70.
24. Golomb BA , Evans MA. Statin Adverse Effects. American Journal of Cardiovascular Drugs 2008;8:373-418.
25. Elias PM , Ghadially R. The Aged Epidermal Permeability Barrier: Basis for Functional Abnormalities. Clinics in Geriatric Medicine 2002;18:103-20.
26. Heidary N, Naik H , Burgin S. Chemotherapeutic agents and the skin: An update. Journal of the American Academy of Dermatology 2008;58:545-70.

SUPPLEMENTARY TABLES

Supplementary Table S1. Population characteristics

Variable	Generalized dry skin[1] (N=642)	Localized dry skin (N=2667)	No dry skin (N=2238)
Age - median [IQR]	72.4 [64.0-81.7]	69.3 [62.3-77.7]	67.6 [60.7-76.8]
Sex - *N*(%)			
Male	259 (40.3)	1128 (42.3)	1020 (45.6)
Female	383 (59.7)	1539 (57.7)	1218 (54.4)
Skin color – N (%)			
Very white-white	544 (84.7)	2253 (84.5)	1829 (81.7)
White to olive-light brown	77 (12.0)	369 (13.8)	368 (16.4)
Brown-black	21 (3.3)	45 (1.7)	41 (1.8)
Temperature- median [IQR]	8.2 [4.2-12.2]	8.9 [4.8-13.0]	11.0 [7.2-14.7]
Humidity- median [IQR]	83.9 [81.0-86.8]	82.9 [79.3-86.5]	82.6 [78.7-86.5]
Cream use – N (%)			
No	164 (25.5)	777 (29.1)	721 (32.2)
Yes, few times a week	67 (10.4)	244 (9.1)	192 (8.6)
Yes, daily	292 (45.5)	1284 (48.1)	1238 (55.3)
Missing	119 (18.5)	362 (13.6)	87 (3.9)
Smoking[2] – N (%)			
No	445 (69.3)	1939 (72.7)	1595 (71.3)
Yes	195 (30.4)	721 (27.0)	640 (28.6)
Missing	2 (0.3)	7 (0.3)	3 (0.1)
Alcohol consumption[3]			
Median [IQR]	8.6 [1.6-8.6]	8.6 [1.6-8.6]	8.6 [1.6-8.6]
Missing – N (%)	103 (16.0)	391 (14.7)	343(15.3)
BMI – N (%)[4]			
<20	19 (3.0)	40 (1.5)	39 (1.7)
20-25	177 (27.6)	713 (26.7)	607 (27.1)
>25	446 (69.4)	1906 (71.5)	1589 (71.0)
Missing	0 (0.0)	8 (0.3)	3 (0.1)
QoL[5]			
Mean (SD)	77.11 (13.9)	78.13 (14.1)	78.52 (14.7)
Missing – N (%)	2 (0.3)	9 (0.3)	9 (0.4)
Education level[6]- N (%)			
Low	75 (11.7)	267 (10.0)	212 (9.5)
Medium	410 (63.9)	1667 (62.5)	1300 (58.1)
High	149 (23.2)	696 (26.1)	698 (31.2)
Missing	8 (1.2)	37 (1.4)	28 (1.2)

Supplementary Table S1. Population characteristics (continued)

Variable	Generalized dry skin[1] (N=642)	Localized dry skin (N=2667)	No dry skin (N=2238)
Self-reported dry skin[7] – N (%)			
No	278 (43.3)	1517 (56.9)	1464 (65.4)
Yes	326 (50.8)	1073 (40.2)	746 (33.3)
Missing	38 (5.9)	77 (2.9)	28 (1.3)
Dermatological diseases			
Eczema – N (%)			
No	535 (83.3)	2475 (92.8)	2167 (96.8)
Yes	107 (16.7)	191 (7.2)	71 (3.2)
Missing	0 (0.0)	1 (0.0)	0 (0.0)
Seborrheic dermatitis- N (%)			
No	536 (83.5)	2326 (87.2)	1972 (88.1)
Yes	103 (16.0)	338 (12.7)	265 (11.8)
Missing	3 (0.5)	3 (0.1)	1 (0.1)
Psoriasis – N (%)			
No	621 (96.7)	2586 (97.0)	2166 (96.8)
Yes	21 (3.3)	79 (3.0)	72 (3.2)
Missing	0 (0.0)	2 (0.0)	0 (0.0)
Varicose veins[8]			
Yes	464 (72.3)	1949 (73.1)	1629 (72.8)
No	177 (27.6)	716 (26.8)	605 (27.0)
Missing	1 (0.2)	2 (0.1)	4 (0.2)
Itchy skin condition[9] – N (%)			
No	390 (60.7)	1740 (65.2)	1516 (67.7)
Yes	216 (33.6)	845 (31.7)	692 (30.9)
Missing	36 (5.6)	82 (3.1)	30 (1.3)
Other diseases			
Diabetes[10]- N (%)			
Yes	94 (14.6)	429 (16.1)	304 (13.6)
No	538 (83.8)	2201 (82.5)	1890 (84.5)
Missing	10 (1.6)	37 (1.4)	44 (1.9)
Renal impairment[11]- N(%)			
Yes	108 (16.8)	346 (13.0)	254 (11.3)
No	485 (75.5)	2163 (81.1)	1852 (82.8)
Missing	49 (7.6)	158 (5.9)	132 (5.9)
Hypothyroidism[12]- N(%)			
Yes	68 (10.6)	233 (8.7)	196 (8.7)
No	517 (80.5)	2227 (83.5)	1879 (84.0)
Missing	57 (8.9)	207 (7.8)	163 (7.3)

Supplementary Table S1. Population characteristics (continued)

Variable	Generalized dry skin[1] (N=642)	Localized dry skin (N=2667)	No dry skin (N=2238)
Atopy [13] – N (%)			
Yes	110 (17.1)	502 (18.8)	433 (18.8)
No	515 (80.2)	2107 (79.0)	1757 (78.5)
Missing	17 (2.6)	58 (2.2)	48 (2.7)
Medication			
Statins- N(%)			
Yes	206 (32.1)	725 (27.2)	580 (25.9)
No	432 (67.3)	1920 (72.0)	1642 (73.4)
Missing	4 (0.6)	22 (0.8)	16 (0.7)
Diuretics- N(%)			
Yes	118 (18.4)	399 (15.0)	301 (13.5)
No	520 (81.0)	2246 (84.2)	1921 (85.8)
Missing	4 (0.6)	22 (0.8)	16 (0.7)
Chemotherapy[14]-N(%)			
Yes	21 (3.3)	76 (2.9)	39 (1.7)
No	614 (95.6)	2556 (95.8)	2173 (97.1)
Missing	7 (1.1)	35 (1.3)	26 (1.2)

[1] Dry skin graded by physician, localized, generalized or no dry skin; [2] No= never, Yes = ever smoking; [3] Alcohol consumption in grams/day; [4] Body Mass Index in kg/m^2 per 1 point; [5] Self perceived health score based on overall health, scores between 0-100 (0 is low quality – 100 is high quality); [6] Low= primary education. Medium= lower vocational/ lower secondary/ intermediate vocational education. High= general secondary/ higher vocational education or university; [7] Question from questionnaire: Have you experienced dry skin over the last year?; [8] Varicose veins = C(EAP) 2-6. CEAP classification of chronic venous disorders. 0= No signs of venous disease. 1=Spider or reticular veins. 2= Varicose veins. 3= Edema without skin lesions. 4= Skin changes without ulceration. 5= Skin changes with healed ulceration. 6= Skin changes with active ulceration; [9] Question from questionnaire: Have you ever had an itchy skin condition?; [10] Diabetes mellitus present if at least one of the following criteria was present: fasting plasma glucose ≥7.0 mmol/L, non-fasting glucose ≥11.1 mmol/L, use of antidiabetic medicine or dietary treatment for type 2 DM; [11] Renal impairment. Yes: Glomerular Filtration Rate (GFR) = <60 No: GFR≥60; [12] Hypothyroidism if ↑TSH & ↓/=fT4 or =TSH &↓fT4; [13] Atopic constitution: self-reported asthma, hay fever and dust mite allergy; [14] Ever having received chemotherapy

CHAPTER 3.2

Genetic susceptibility to dry skin in a general middle-aged to elderly population: a GWAS

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TO THE EDITOR,

Dry skin (xerosis cutis) is a common skin condition associated with aging, affecting 30-85% of the world population [1-6]. Still, little is known about the genetic predisposition for having dry skin, and its exacerbation by the skin aging process. The *FLG* gene, located in the Epidermal Differentiation Complex (EDC) on chromosome 1, is the best known gene involved in skin disorders characterized by severe dry skin including ichthyosis vulgaris (IV) and atopic dermatitis (AD) [7, 8]. Nevertheless, whether polymorphisms within the *FLG* gene or other genes are associated with having clinically detectable dry skin in the general population, remains unknown. Therefore, we performed a GWAS to search for SNPs associated with dry skin in participants from the Rotterdam Study, a prospective population-based cohort of middle-aged to elderly individuals. The Rotterdam Study has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center (Rotterdam, The Netherlands) and by the review board of The Netherlands Ministry of Health, Welfare and Sports [9].

During one visit to the research center, dry skin was physician graded as absent, localized (extensor side of arms and legs), or generalized (more extensive across the body than the extensor side of extremities). Between 2010 and 2016, a total of 5,547 participants were screened for having dry skin by observing scaly or rough skin with or without erythema, of which 4,586 were eligible for our study. Detailed materials and methods are presented in Supplementary Materials and Methods. First, we performed a logistic regression GWAS on the totally dry skin group (localized and generalized; n = 2,736) versus 1,850 controls who were free of dry skin. Secondly, we performed a GWAS on the more severe phenotype, generalized dry skin only (n = 530) versus the 1,850 controls. This we did to help exclude the variation in dry skin influenced by nongenetic factors (air humidity and skin-cream use that are both known to especially influence localized dry skin) [6]. Quality control, linkage disequilibrium analysis, and (functional) annotation were additionally performed. Population demographics are presented in Supplementary Table S1. The first GWAS comparing all dry skin cases (localized and generalized) with the controls did not yield any genome-wide significant signals (Supplementary Figure S1). The second GWAS only using the generalized dry skin cases versus controls identified several genome-wide significant associations on chromosome 1 as shown on a Manhattan plot (Figure 1). SNPs with $P \leq 5 \times 10^{-7}$ associating with generalized dry skin are shown in Table 1.

Our top SNP association rs12123821 ($P = 3.05 \times 10^{-10}$) is an intergenic variant mapping closest to the *HRNR* gene in the EDC locus. Other significant SNPs ($P < 5.0 \times 10^{-8}$) on chromosome 1 mapped to different EDC genes, including *TCHH* and *FLG*. In addition, five SNPs with highly suggestive associations ($5.0 \times 10^{-8} < P < 5.0 \times 10^{-7}$) were also found on chromosome 1, all tagging EDC genes (Supplementary Figure S2). Other highly suggestive associations were found for SNPs on chromosomes 16, 18, and 2.

Linkage disequilibrium analysis and corresponding expression quantitative trait loci analysis indicated that the significant SNPs on chromosome 1 probably comprise two independent

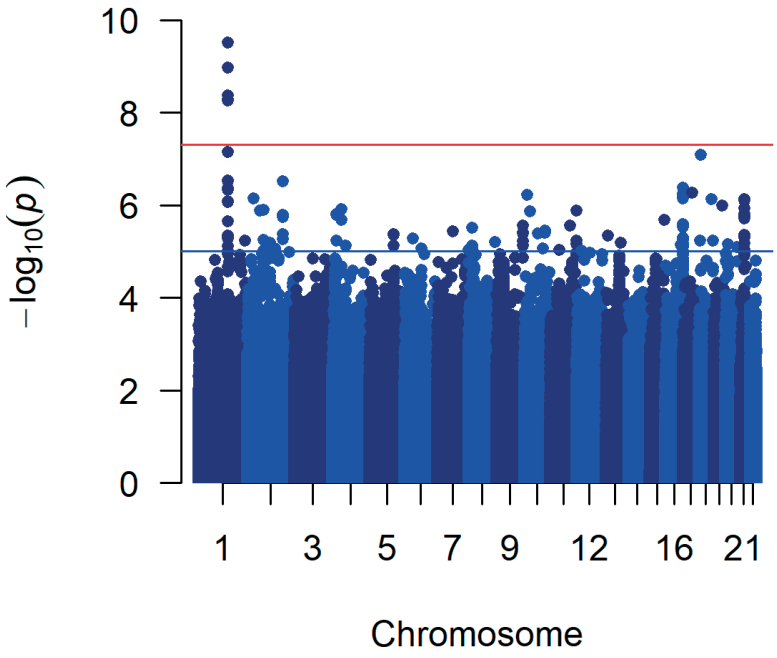


Figure 1. Figure 1. Manhattan plot of GWAS of generalized dry skin. Manhattan plot representing the association between the SNPs and having a generalized dry skin for 530 cases and 1,850 controls. On the x-axis, the chromosomes are plotted with each dot representing an SNP on corresponding chromosomal locations versus the –log10 (P-value) of the association with having a generalized dry skin. The red horizontal line represents the threshold for genome-wide-significance, indicating $P = 5.0 \times 10^{-8}$. The blue horizontal line represents the threshold for genome-wide suggestive associations, indicating $P = 5.0 \times 10^{-5}$.

signals: one located near the *HRNR* gene with *LINGO4* expression quantitative trait loci and the other comprising the *FLG* locus with *FLG/FLG-AS-1* expression quantitative trait loci (Supplementary Results). Conditional analysis on the top SNP did not reveal any new signals. Adjusting for the only available *FLG* loss-of-function mutation in our GWAS did not decrease the top signals, and adjusting for eczema cases did not decrease the top signal (rs12123821; $P = 4.22 \times 10^{-9}$), suggesting that it is not primarily driven by known EDC eczema variants (Supplementary Tables S2 and S3).

Conditional analyses showed that SNPs on chromosome 16 were driven by known *MC1R* pigmentation and aging variants (results not shown). If the link between dry skin and *MC1R* genotypes can be validated, this finding would suggest that more biologically aged skin has a greater susceptibility to dry skin. The signals on chromosomes 2 and 18 represent, to our knowledge, previously unreported links to skin biology. On chromosome 18, rs144079954 was mapped to pseudogene *NPIPB1P*. The function of pseudogenes is not yet fully elucidated; however, there is accumulating evidence for a regulatory function on other genes [10]. Rs62195431 on chromosome 2 maps to *NUP35*, which codes for a nucleoporin protein. Several nucleoporins

Table 1. Top genetic hits from GWAS of generalized dry skin

SNP	CHR	BASE	EA	OA	fEA	Pvalue	Direction	Functional effect	Mapped gene or closest gene symbol	eQTL
rs12123821	chr1	152179152	t	c	0.047	3.05E-10	+++	Intergenic variant	*HRNR*	*LINGO4*
rs115045402	chr1	152029548	a	g	0.027	1.06E-09	+++	Intergenic variant	*AC2* (pseudogene precursor RNA sequence)	*LINGO4*
rs115288876	chr1	152000117	a	g	0.041	4.24E-09	+++	Upstream transcript variant	*AC2* (pseudogene precursor RNA sequence)	*LINGO4*
rs12122629	chr1	152074116	a	c	0.957	5.23E-09	---	Intergenic variant	*TCHH*	*LINGO4*
rs61816761	chr1	152285861	a	g	0.018	5.40E-09	+++	Missense variant	*FLG*	*FLG**
rs12731336	chr1	152448098	a	g	0.046	7.06E-08	+++	Intergenic variant	*LCE5A*	*LINGO4*
rs144079954	chr18	11619623	t	g	0.026	7.99E-08	+++	Intergenic variant	*DQ594439/ piRNA -59696*	*RP11-64C12.8*
rs61815559	chr1	152271219	a	t	0.972	2.93E-07	---	Intergenic variant	*FLG*	*FLG-AS1*
rs62195431	chr2	184254708	a	c	0.060	3.04E-07	+++	Intergenic variant	*NUP35*	None in any tissue
rs61814884	chr1	151976836	a	g	0.970	3.05E-07	---	Intron variant	*AC2* (pseudogene precursor RNA sequence)	*C1orf68*
rs75687828	chr16	89618876	a	g	0.089	4.11E-07	+++	Intron variant	*SPG7*	*CDK10*
rs80324518	chr16	89614534	t	c	0.089	4.13E-07	+++	Intron variant	*SPG7*	*CDK10*
rs61814899	chr1	152069131	a	g	0.029	4.34E-07	+++	Intergenic variant	*TCHHL1*	*FLG**
rs77426698	chr1	151908055	a	g	0.039	4.61E-07	+++	Intergenic variant	*THEM4*	*LINGO4*

* no significant hit in skin, most significant eQTL in nerve - tibial tissue. GWAS results showing highly suggestive SNPs (p-value<5.0* 10^{-7}) for generalized dry skin (n=530) versus no dry skin (n=1850) sorted by P-value of the association (smallest to largest). CHR, chromosome; BASE, refers to position of SNP on the chromosome; EA, effect allele; OA, other allele; fEA, frequency of the effect allele; Pvalue, p-value of association in GWAS; Direction, direction in which the effect of the SNP is per cohort of the Rotterdam Study (RSI, RSII, RSIII); Mapped gene or closest gene symbol, annotation of SNP using UCSC genome browser (Hg 19); Functional Effect, effect of the SNP; eQTL: expression quantitative trait loci, these are genomic loci that explain a part of the variation in expression levels of mRNAs in various tissues

have been associated with nonhematological malignancies, including skin cancer [11], but their role in skin barrier formation remains unknown.

Our study population, however well-defined and including both sexes, was of limited statistical power for a GWAS. Nevertheless, discovering multiple significant SNPs with this sample size indicates relatively large effect sizes. Other limitations include the visual grading of dry skin, which ideally would have been supported by a technical measurement, e.g. skin electrical impedance. Furthermore, correcting for common atopic dermatitis–associated *FLG* loss-of-function SNPs in our conditional analysis was of limited accuracy because of known difficulties in imputing these SNPs. Genotyping all of these mutations for the conditional analysis would have been more powerful. Despite measuring dry skin only once, we showed that generalized dry skin determinants were more systemic or robust, whereas in the localized dry skin group, these were more environmental or variable [6]. In addition, in our study, the group with eczema was heterogeneous because it was not limited to atopic dermatitis cases only. Finally, it is hard to predict generalizability to other populations because the cohort is predominantly of North-European descent.

We find evidence that the presence of generalized dry skin has a genetic predisposition and particularly with genes in the EDC. Ichthyosis vulgaris could not have driven the results on its own because the prevalence in the general population is low. We showed that our findings are not driven by known eczema gene variants, although we cannot exclude that there is a genetic overlap between dry skin and eczema, as seen in the clinical presentation. Replication of the SNPs detected in this study would strengthen these assumptions and provide more direction for future research into the biological drivers of dry skin and its treatment.

REFERENCES

1. Hahnel E, Lichterfeld A, Blume-Peytavi U , Kottner J. The epidemiology of skin conditions in the aged: A systematic review. J Tissue Viability 2017;26:20-8.
2. Augustin M, Kirsten N, Korber A, Wilsmann-Theis D, Itschert G, Staubach-Renz P et al. Prevalence, predictors and comorbidity of dry skin in the general population. J Eur Acad Dermatol Venereol 2019;33:147-50.
3. Lichterfeld A, Lahmann N, Blume-Peytavi U , Kottner J. Dry skin in nursing care receivers: A multi-centre cross-sectional prevalence study in hospitals and nursing homes. International Journal of Nursing Studies 2016;56:37-44.
4. Paul C, Maumus-Robert S, Mazereeuw-Hautier J, Guyen CN, Saudez X , Schmitt AM. Prevalence and risk factors for xerosis in the elderly: a cross-sectional epidemiological study in primary care. Dermatology 2011;223:260-5.
5. Smith DR, Atkinson R, Tang S , Yamagata Z. A survey of skin disease among patients in an Australian nursing home. J Epidemiol 2002;12:336-40.
6. Mekic S, Jacobs LC, Gunn DA, Mayes AE, Ikram MA, Pardo LM et al. 'Prevalence and determinants for xerosis cutis in the middle-aged and elderly population: a cross-sectional study'. J Am Acad Dermatol 2018.
7. Sandilands A, Sutherland C, Irvine AD , McLean WH. Filaggrin in the frontline: role in skin barrier function and disease. J Cell Sci 2009;122:1285-94.
8. McGrath JA. Profilaggrin, Dry Skin, and Atopic Dermatitis Risk: Size Matters. Journal of Investigative Dermatology 2012;132:10-1.
9. Ikram MA, Brusselle G, Ghanbari M, Goedegebure A, Ikram MK, Kavousi M et al. Objectives, design and main findings until 2020 from the Rotterdam Study. Eur J Epidemiol 2020;35:483-517.
10. Pink RC, Wicks K, Caley DP, Punch EK, Jacobs L , Carter DRF. Pseudogenes: pseudo-functional or key regulators in health and disease? RNA 2011;17:792-8.
11. Roy A , Narayan G. Oncogenic potential of nucleoporins in non-hematological cancers: recent update beyond chromosome translocation and gene fusion. J Cancer Res Clin Oncol 2019;145:2901-10.

SUPPLEMENTARY MATERIALS AND METHODS

Study Population

Participants were included from the Rotterdam Study (RS), a large prospective population-based cohort of middle-aged to elderly individuals that comprises a suburb of Rotterdam, as described previously [1]. The first cohort started in 1990. The second (RSII), third (RSIII), and fourth (RSIV) cohorts were added with the ongoing study. The dermatological screening started in 2010 and consists of participants across all the four cohorts. This study includes participants from RSI–III. The RS has been approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center (Rotterdam, The Netherlands) and by the review board of The Netherlands Ministry of Health, Welfare and Sports.

Phenotyping

Identification of dry skin cases in the RS has been described in detail before [2]. In short, dry skin was physician graded as absent, localized (extensor side of arms and legs), or generalized if it was more extensive than the extensor side of extremities only. Between 2010 and 2016, a total of 5,547 participants were screened for having dry skin by observing scaly or rough skin with or without erythema. Of this group, 4,595 had provided eligible genetic material, of which 4,586 had no missing covariate data and were included in our analysis.

First, we performed a GWAS defining individuals with both localized and generalized dry skin as cases (n = 2,736) and defining 1,850 controls without dry skin. Localized dry skin might be a more variable skin phenotype and more easily influenced by external factors such as weather, humidity, and moisturizer cream use than generalized dry skin [2]. Therefore, we performed another GWAS by only using the more severe phenotype, generalized dry skin (n = 530) and 1,850 controls, and excluded participants with localized dry skin only.

Covariates

Sex and age were collected from the database. Other covariates were selected on the basis of known significant associations with dry skin, and they included body mass index, outside temperature, and skin color [2]. Skin color was graded by physicians and clustered into two categories. Height and weight were measured at the research center, and body mass index was calculated. Mean outside temperature over the last week before the center visit was calculated using weather data from WeatherOnline collected at the Rotterdam The Hague Airport (https://www.weatheronline.co.uk/). Eczema was defined as erythematous, scaly, lichenified, excoriated, and fissured patches on the trunk, extremities, or hands or in skin folds during full-body skin examination.

Genotyping and Imputation of GWAS data

DNA was extracted from whole-blood samples according to standard protocols, which has been described previously [1]. In the RS, genotyping was done using both the Infinium II HumanHap550(-Duo) (RSI and RSII) and 610-Quad Genotyping BeadChip (RSI and RSIII) (Illumina, San Diego, CA). Quality control for genotyping has been described previously [3]. Imputation was carried out using Haplotype Reference Consortium 1.1, which is a reference panel of 64,976 haplotypes for genotype imputation [4]. The three cohorts were imputed separately on the Michigan Imputation Server where a faster algorithm for imputed large reference datasets was implemented in mac3 [5]. In total 39,117,105 genotyped and/ or imputed variants were available. In total, 39,117,105 genotyped and/or imputed variants were available. Additional quality control included the removal of markers with frequencies <1% and low imputation quality scores (r2 < 0.3).

Statistical analysis

We performed the GWAS using a logistic regression with RVTESTS [6] software package using the score test, while adjusting for age, sex, body mass index, skin color, temperature, and four genetic principal components, the latter was to account for possible population stratification or hidden relatedness among participants [1]. Next, we performed quality control on the three GWAS per cohort using EasyQC software package [7] with parameter defaults. In total, 8,021,997 markers that were present at least in one of the cohorts were available for further analysis. Because analyses were done per cohort separately, we performed a meta-analysis using the inverse variance approach with the software METAL [8]. SNPs were only presented in the results if the direction of the effect was the same over all the three cohorts.

Linkage Disequilibrium analysis

We calculated the patterns of Linkage Disequilibrium (LD) of the top hits (5.0×10^{-6}) for chromosome 1. First, we reformatted the imputed data into best guess genotypes using GCTA software with parameter defaults [9]. Next, we extracted the genotypes of the SNPs and calculated pairwise LD (r^2>=08) between them around a distance of 1 megabase. We also calculated the LD blocks (genomic regions of two or more SNPs in moderate to high LD) using the same thresholds. Briefly, the function finds SNPs that tag other correlated SNPs left and right according to a distance and a pairwise LD.

Conditional association and sensitivity analysis

We performed a conditional GWAS, conditioned on the top SNP rs12123821 to investigate whether any new signals would be revealed. To assess whether the top hits on chromosome 1 were independent of the *FLG* gene, we performed a conditional analysis by adjusting the associations on chromosome 1 for *FLG* mutations reported in Europeans as reported at the Online Mendelian Inheritance in Man (OMIM) website (https://www.omim.org/allelicVariants/135940) that were present in the RS. Of the five variants reported on the site, only two,

namely, R501X (rs61816761) and R2447X (rs146466242), were present in the RS at a frequency of at least 1%. In addition, rs146466242 had a bad imputation quality; thus, we performed the conditional analysis by adding only rs61816761 (Imputation quality: $r^2 = 0.76$) as an additional covariate. The same approach was applied for the top hits on chromosome 16 because the top hits were located in the region around the skin color gene *MC1R*, which is also known to be associated with skin aging [10]. Therefore, we performed an additional analysis on chromosome 16 by adjusting for rs1805007, rs35096708, and rs139810560, which are known *MC1R* functional SNPs. Because having severely dry skin is strongly associated with having eczema and genetic signals could thus be driven by eczema cases, we also included a sensitivity analysis where we additionally adjusted for having active eczema lesions.

Bioinformatics

To annotate SNPs to human genes, we downloaded the University of California Santa Cruz gene table (Genome browser; hg19; downloaded on December 2018) and mapped the genomic coordinates of the main results. Intergenic SNPs were mapped to the closest gene using the same tool.

To evaluate how genetic variants could be influencing mRNA expression levels, we mapped each SNP to expression quantitative trait loci (eQTLs); eQTLs are genomic loci that explain a part of the variation in expression levels of mRNAs in various tissues [11]. The Genotype-Tissue Expression data used for the analyses were obtained from the Genotype-Tissue Expression Portal (http://www.gtex.org) on 23 January 2020 and were restricted to eQTLs with a significance $P < 0.05$ in the tissues skin (sun exposed [lower leg]) or the skin (not sun exposed [suprapubic]).

SUPPLEMENTARY RESULTS

Population characteristics

During a full skin examination, physicians stratified the participants into three groups regarding their dry skin status: generalized dry skin, localized dry skin, and no dry skin (also named as the control group). The percentage of women was slightly higher than that of men in all the groups, ranging from 53.6% in the group without dry skin to 60.2% in the group with generalized dry skin (Supplementary Table S1). The median age ranged from 67.6 (interquartile range = 61.0–76.9) years in the control group to 72.5 (interquartile range = 64.1–81.8) years in the group with generalized dry skin.

Main results GWAS

We first compared all dry skin cases (localized and generalized; n = 2,736) with the controls (n = 1,850). No genome-wide significant hits were found; the most significant SNP was rs35070517 ($P = 1.03 \times 10^{-6}$) located on chromosome 20 in an intergenic region (Supplementary Figure S1).

Second, a GWAS on the more severe phenotype, focusing only on the generalized dry skin cases (n = 530) versus the controls (n = 1,850), was performed. The most significant SNPs associating with generalized dry skin with $P \leq 5 \times 10^{-7}$ are presented in Table 1. As shown on a Manhattan plot (Figure 1), we identified several genome-wide significant associations on chromosome 1. These SNPs mapped to the epidermal differentiation complex region, a gene-rich cluster involved in epidermal differentiation. Our top SNP association rs12123821 ($P = 3.05 \times 10^{-10}$) was an intergenic variant and mapped closest to the *HRNR* gene. Other SNPs with significant associations ($P < 5.0 \times 10^{-8}$), all on chromosome 1, were rs115045402 ($P = 1.06 \times 10^{-9}$), rs115288876 ($P = 4.24 \times 10^{-9}$), rs12122629 ($P = 5.23 \times 10^{-9}$), and rs61816761 ($P = 5.40 \times 10^{-9}$). These mapped to different epidermal differentiation complex genes, including *TCHH* and *FLG*. In addition, five SNPs with highly suggestive associations ($5.0 \times 10^{-8} < P < 5.0 \times 10^{-7}$) were also found on chromosome 1. These tagged epidermal differentiation complex genes *TCHHL1, THEM4, LCE5A*, and *FLG* (Supplementary Figure S2)

Other highly suggestive associations were found for chromosome 16 (two SNPs), chromosome 18 (one SNP), and chromosome 2 (one SNP) (Table 1). On chromosome 16, we found SNPs rs75687828 ($P = 4.11 \times 10^{-7}$) and rs80324518 ($P = 4.13 \times 10^{-7}$) where the closest gene was *SPG7*, mutations of which can cause autosomal recessive hereditary spastic paraplegia [12]. Although defects in *SPG7* itself do not cause xerosis, the clinical spectrum of hereditary spastic paraplegia can include ichthyosis [13]. Furthermore, *SPG7* is located in a region of extended LD that includes the *MC1R* locus. *MC1R* is a known skin color gene but is also known to influence many other skin aging–related phenotypes, such as perceived aging as well as skin cancer [10, 14, 15]. SNP rs144079954 ($P = 7.99 \times 10^{-8}$) on chromosome 18 mapped to Piwi-interacting RNA (Piwi-interacting RNA-596996). The relevance of this RNA gene to skin biology is unknown. On chromosome 2, rs62195431 ($P = 3.04 \times 10^{-7}$) mapped to *NUP35*, a gene that codes for nucleoporins, which modulate cellular and physiological pathways involved in tumorigenesis, including skin cancer [16].

LD analysis on chromosome 1

To assess whether our top signals from chromosome 1 were independent of one another, we performed LD analysis of the genomic regions around the most significant SNP associations on chromosome 1 down to $P = 5 \times 10^{-6}$. We found one large region of strong LD ($r2 > 0.8$) in which six of our top SNPs were located: rs115288876, rs12122629, rs61815559, rs61814884, rs61814899, and rs77426698. These SNPs were in strong LD with each other and were significantly associated with generalized dry skin (Supplementary Table S2). Genes in this region were *TCHH*, *RPTN*, *HRNR*, *FLG*, and *FLG-AS*. Although the top SNP (rs12123821; $P = 3.05 \times 10^{-10}$) mapped within this region, it was not part of these blocks in strong LD, suggesting that it might be a separate signal. Other signals with mapped genes and not in strong LD with other SNPs were rs115045402 (*AC2*), rs61816761 (*FLG*), and rs12731336 (*LCE5A*) (Supplementary Table S3).

Conditional association and sensitivity analyses

Adjusting the GWAS for the top associated SNP, rs12123821, did not reveal any new signals. It weakened the association for SNPs tagging *AC2*, *TCHH*, *THEM4*, and *LCE5A*, suggesting that these were not entirely independent from the top hit. However, *FLG*-associated signals became more significant, indicating their independence from the main signal (Supplementary Table S3). Adjusting for a common *FLG*-associated mutation in Europeans that was present in the RS and of sufficient imputation quality did not significantly affect the top five signals. This confirms that these were not driven by this *FLG* mutation (Supplementary Table S3). Adjusting for having active eczema lesions showed that the top hit remained highly significant with $P = 4.22 \times 10^{-9}$ (results not shown).

A conditional analysis using three known *MC1R* SNPs weakened the chromosome 16 associations, suggesting that *MC1R* SNPs at least partly drove the associations (results not shown).

Bioinformatics

To help determine whether any identified SNPs were influencing the expression of nearby genes in the skin, the most significant eQTLs for each SNP were investigated in Genotype-Tissue Expression skin datasets. Of the eQTLs identified, *LINGO4* was expressed in the skin for multiple top SNPs (Table 1). *LINGO4* is a homologous gene of the *LINGO1* and *LINGO2* genes, which are known to play a role in the susceptibility of essential tremors [17 18]; however, their role in skin biology is not yet known. *FLG*-associated signals were verified with both rs61816761 and rs61815559 corresponding with the expression of *FLG* and *FLG-AS1* genes. The eQTL between SNPs rs75687828 and rs80324518 on chromosome 16 and *CDK10* expression implicates differences in cell cycle and other important cellular processes such as transcription and metabolism with dry skin [19] (Table 1).

REFERENCES

1. 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;32:807-50.
2. Mekic S, Jacobs LC, Gunn DA, Mayes AE, Ikram MA, Pardo LM et al. 'Prevalence and determinants for xerosis cutis in the middle-aged and elderly population: a cross-sectional study'. J Am Acad Dermatol 2018.
3. Lango Allen H, Estrada K, Lettre G, Berndt SI, Weedon MN, Rivadeneira F et al. Hundreds of variants clustered in genomic loci and biological pathways affect human height. Nature 2010;467:832-8.
4. McCarthy S, Das S, Kretzschmar W, Delaneau O, Wood AR, Teumer A et al. A reference panel of 64,976 haplotypes for genotype imputation. Nat Genet 2016;48:1279-83.
5. Das S, Forer L, Schönherr S, Sidore C, Locke AE, Kwong A et al. Next-generation genotype imputation service and methods. Nature Genetics 2016;48:1284.
6. Zhan X, Hu Y, Li B, Abecasis GR , Liu DJ. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. Bioinformatics 2016;32:1423-6.

7. Winkler TW, Day FR, Croteau-Chonka DC, Wood AR, Locke AE, Magi R et al. Quality control and conduct of genome-wide association meta-analyses. Nat Protoc 2014;9:1192-212.
8. Willer CJ, Li Y , Abecasis GR. METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 2010;26:2190-1.
9. Yang J, Lee SH, Goddard ME , Visscher PM. GCTA: A Tool for Genome-wide Complex Trait Analysis. The American Journal of Human Genetics 2011;88:76-82.
10. Liu F, Hamer MA, Deelen J, Lall JS, Jacobs L, van Heemst D et al. The MC1R Gene and Youthful Looks. Curr Biol 2016;26:1213-20.
11. Cookson W, Liang L, Abecasis G, Moffatt M , Lathrop M. Mapping complex disease traits with global gene expression. Nature reviews Genetics 2009;10:184-94.
12. Elleuch N, Depienne C, Benomar A, Hernandez AM, Ferrer X, Fontaine B et al. Mutation analysis of the paraplegin gene (SPG7) in patients with hereditary spastic paraplegia. Neurology 2006;66:654-9.
13. Garcia-Cazorla À, Mochel F, Lamari F , Saudubray J-M. The clinical spectrum of inherited diseases involved in the synthesis and remodeling of complex lipids. A tentative overview. Journal of Inherited Metabolic Disease 2015;38:19-40.
14. Han J, Kraft P, Nan H, Guo Q, Chen C, Qureshi A et al. A Genome-Wide Association Study Identifies Novel Alleles Associated with Hair Color and Skin Pigmentation. PLOS Genetics 2008;4:e1000074.
15. Bastiaens MT, Huurne JACt, Kielich C, Gruis NA, Westendorp RGJ, Vermeer BJ et al. Melanocortin-1 Receptor Gene Variants Determine the Risk of Nonmelanoma Skin Cancer Independently of Fair Skin and Red Hair. The American Journal of Human Genetics 2001;68:884-94.
16. Roy A , Narayan G. Oncogenic potential of nucleoporins in non-hematological cancers: recent update beyond chromosome translocation and gene fusion. J Cancer Res Clin Oncol 2019;145:2901-10.
17. Stefansson H, Steinberg S, Petursson H, Gustafsson O, Gudjonsdottir IH, Jonsdottir GA et al. Variant in the sequence of the LINGO1 gene confers risk of essential tremor. Nature Genetics 2009;41:277-9.
18. Wu Y-W, Prakash KM, Rong T-Y, Li H-H, Xiao Q, Tan LC et al. Lingo2 variants associated with essential tremor and Parkinson's disease. Human Genetics 2011;129:611-5.
19. Lim S , Kaldis P. Cdks, cyclins and CKIs: roles beyond cell cycle regulation. Development 2013;140:3079-93.

SUPPLEMENTARY TABLES

Supplementary Table S1. Characteristics of the 4595 participants

Characteristic	No dry skin	Localized dry skin	Generalized dry skin
Sex			
Male – n (%)	859 (46.4)	931 (42.1)	211 (39.8)
Female – n (%)	993 (53.6)	1282 (57.9)	319 (60.2)
Age[1] – median [IQR]	67.6 [61.0- 76.9]	68.9 [62.3-77.9]	72.5 [64.1-81.8]
BMI[2]– mean (SD)	27.8 (4.5)	27.5 (4.2)	27.1 (4.1)
Temperature[3]- mean (SD)	10.1 (5.7)	8.6 (5.8)	8.0 (5.6)
Skin color[4]			
Very white / white – n (%)	1605 (86.7)	1945 (87.9)	469 (88.5)
White to olive / brown – n (%)	247 (13.3)	268 (12.1)	61 (11.5)
Total	1852	2213	530

[1] Age in years, not normally distributed, hence median and interquartile range presented; [2] Body Mass Index in kg/m^2 (data missing in 9 participants, these individuals were excluded from further analysis); [3] Mean outside temperature over the past week in degrees Celsius; [4] Skin color merged in two categories, scored at the research center.

Supplementary Table S2. LD blocks on chromosome 1

SNP	SNP ID	Pvalue	NTAG	LEFT	RIGHT	KBSPAN	TAGS	REGION
1:151908055	rs77426698	4.61E-07	1	151908055	152000117	92.063	rs115288876	REGION 1
1:152000117	rs115288876	4.24E-09	2	151908055	152074116	166.062	rs77426698|rs12122629	REGION 1
1:151976836	rs61814884	3.05E-07	2	151976836	152098428	121.593	rs61814899|rs140371183	REGION 1
1:152069131	rs61814899	4.34E-07	2	151976836	152098428	121.593	rs61814884|rs140371183	REGION 1
1:152074116	rs12122629	5.23E-09	1	152000117	152074116	74	rs115288876	REGION 1
1:152029548	rs115045402	1.06E-09	0	152029548	152029548	0.001	NONE	x
1:152271219	rs61815559	2.93E-07	2	152098428	152319572	221.145	rs140371183|rs61816766	REGION 1
1:152179152	rs12123821	3.05E-10	0	152179152	152179152	0.001	NONE	x
1:152319572	rs61816766	8.40E-07	1	152271219	152319572	48.354	rs61815559	REGION 1
1:152285861	rs61816761	5.40E-09	0	152285861	152285861	0.001	NONE	x
1:152448098	rs12731336	7.06E-08	0	152448098	152448098	0.001	NONE	x

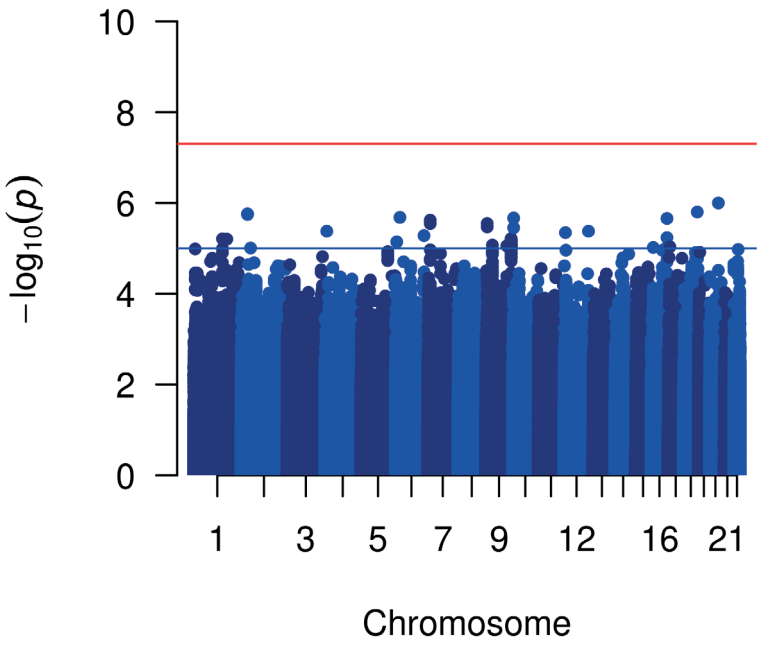
For the highly suggestive SNPs on chromosome 1 (p-value< 5.0*10^{-7}), SNP ID according to RS number, Pvalue = p-value of the association of the GWAS generalized dry skin, NTAG represents the number of SNPs in Linkage Disequilibrium (LD), LEFT and RIGHT present the left and right border of the area of LD in kilo base pair, KBSPAN represents the width of the LD in kilo base pair, REGION represents the region where the LD blocks are situated or where they overlap, x meaning LD R^2< 0.8

Supplementary Table S3. Conditional analyses

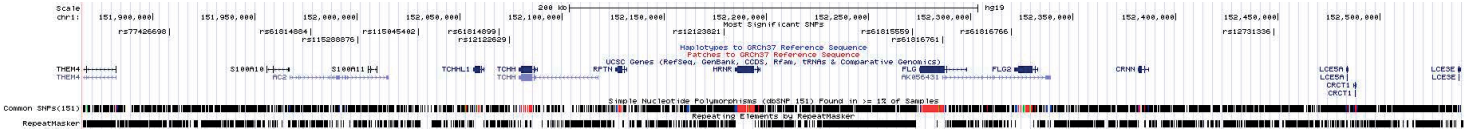
SNP	Gene	LD	P-value GWAS generalized dry skin	P-value GWAS generalized dry skin adjusting for top hit	P-value GWAS generalized dry skin adjusting for *FLG* SNPs
rs12123821	*HRNR*	NOT IN REGION	3.05E-10	x	3.26E-10
rs115045402	*AC2*	NOT IN REGION	1.06E-09	n.s.	1.24E-09
rs115288876	*AC2*	REGION 1	4.24E-09	n.s.	5.41E-09
rs12122629	*TCHH*	REGION 1	5.23E-09	n.s.	6.96E-09
rs61816761	*FLG*	NOT IN REGION	5.40E-09	1.24E-09	x
rs12731336	*LCE5A*	NOT IN REGION	7.06E-08	n.s.	7.75E-08
rs61815559	*FLG*	REGION 1	2.93E-07	4.72E-08	2.27E-05
rs61814884	*AC2*	REGION 1	3.05E-07	7.68E-08	2.62E-05
rs61814899	*TCHHL1*	REGION 1	4.34E-07	9.87E-08	n.s.
rs77426698	*THEM4*	REGION 1	4.61E-07	n.s.	n.s.

For the highly suggestive SNPs on chromosome 1 (p-value< $5.0*10^{-7}$) the mapped gene or closest gene (if intergenic) is presented. LD presents whether the SNPS are in Linkage Disequilibrium in region 1 (shaded grey) or not tagging any other SNPs. The first column of p-values presents the main results. In the second column, p-values are presented for the GWAS generalized dry skin adjusted for top SNP rs12123821. The final column presents the p-values for the GWAS generalized dry skin when adjusted for two *FLG* SNPs (rs146466242; R2447X and rs6181671*;* R501X) common in Europeans and present in the RS. n.s. = non-significant indicating a p-value below $5.0*10^{-5}$.

SUPPLEMENTARY FIGURES



Supplementary Figure S1. Manhattan plot GWAS localized and generalized dry skin
Manhattan plot representing the association between the SNPs and having a localized or generalized dry skin for 2736 cases and 1850 controls. On the X-axis the chromosomes are plotted with each dot representing a SNP on corresponding chromosomal locations versus the –log10 p-value of the association with having a localized or generalized dry skin. The red horizontal line represents the threshold for genome-wide-significant, indicating a p-value of $5x10^{-8}$. The blue horizontal line represents the threshold for genome-wide-suggestive, indicating a p-value of $5.0 x10^{-5}$.



Supplementary Figure S2. Genomic region top SNPs chromosome 1

Figure displaying genomic region on chromosome 1, which shows the location of the highly suggestive associated SNPs (p-value<5.0 x 10^{-7}) with below the names and position of the mapping genes.

Part IV

HEALTH

CHAPTER 4.1

A healthy diet in women is associated with less facial wrinkles in a large Dutch population-based cohort

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ABSTRACT

Background: Little is known about the effects of different dietary patterns on facial wrinkling.

Objective: We aimed to investigate the association between diet and facial wrinkles in a population-based cohort of 2,753 elderly participants of the Rotterdam Study.

Methods: Wrinkles were measured in facial photographs by digitally quantifying the area wrinkles occupied as a percentage of total skin area. Diet was assessed by the Food Frequency Questionnaire. Adherence to the Dutch Healthy Diet Index (DHDI) was calculated. In addition, we used principal component analysis (PCA) to extract relevant food patterns in men and women separately. All food patterns and the DHDI were analyzed for an association with wrinkle severity using multivariable linear regression

Results: Better adherence to the Dutch guidelines was significantly associated with less wrinkles among women but not in men. In women, a red meat and snack-dominant PCA pattern was associated with more facial wrinkles, whereas a fruit-dominant PCA pattern was associated with fewer wrinkles.

Limitations: Due to the cross-sectional design of our study, causation could not be proven. Other health-conscious behaviors of study participants could have influenced the results

Conclusion: Dietary habits are associated with facial wrinkling in women. Global disease prevention strategies might benefit from emphasizing that a healthy diet is also linked to less facial wrinkling.

INTRODUCTION

Maintaining a healthy body and youthful appearance is increasingly becoming popular because the longevity and wealth of the global population is still rising. The rise of functional foods claiming various skin benefits suggests that certain nutrients could help to prevent skin aging and enhance cosmesis[1].

While several small studies have investigated the effects of dietary supplements on skin aging [2-4], large nutritional studies on this topic are lacking. To our knowledge, only three previous studies have investigated features of skin aging in association with diet [5-7]. In these studies, intake of vegetables, foods high in carotenoids and vitamin C, olive oil, linoleic acid, and fish were associated with less photoaging and intake of saturated fats and sugar with more wrinkling.

On the basis of these observations, a healthy diet appears associated with less skin aging. However, in these previous studies, researchers investigated separate nutrients or food groups that were prone to false-positive associations because of co-linearity with the causative nutrient and the interaction between single nutrients. Also, the effect sizes of single nutrients are often small, making it difficult to discover associations. Studying complete dietary patterns in epidemiologic nutritional research, therefore, can be preferred over studying single nutrients [8]. Dietary pattern analysis can be conducted a priori, in which the healthiest pattern is predefined using existent guidelines, eg, the Dutch Healthy Diet Index (DHDI)[9]. However, in case of little prior knowledge, an a posteriori approach, in which formed patterns were data driven, could be more appropriate, eg, using a principal component analysis (PCA)[8].

In our study, we investigated the association between digitally quantified facial wrinkling, dietary patterns, and healthy lifestyle parameters in a large population-based cohort of 2,753 elderly participants of the Rotterdam study using both an a priori and an a posteriori approach.

METHODS

Study population

Participants were selected from the Rotterdam study, a prospective population-based cohort study in Rotterdam, the Netherlands. The Rotterdam study was approved by the institutional review board (Medical Ethics Committee) of the Erasmus Medical Center and by the review board of The Netherlands Ministry of Health, Welfare, and Sports. Objectives and details of the study design have been described elsewhere[10].

During 2010-2014, standardized high-resolution digital facial photographs were taken of 4,649 participants by trained physicians. From these pictures, we obtained wrinkle data for 3831 participants, and nutrition data was available for 2,813 of these participants. We excluded 60 of the 2,813 participants because of unrealistic caloric intakes (<500 and >5,000 kcal/day). The remaining 2,753 participants were included in our analysis.

Wrinkles

Using full-face photographs, we digitally quantified the area detected as wrinkles as a percentage of the total facial skin area using a semi-automated script in MATLAB (MathWorks, Natick, MA). Our wrinkle data has been validated [11] and utilized in other analyses [12, 13].

Dietary intake and food pattern analysis

Dietary intake was assessed using a validated semi-quantitative Food Frequency Questionnaire (FFQ) [9]. We defined the a priori healthiness of the diet in our population, using the DHDI [14, 15]. For the a posteriori approach, we used a PCA (Supplementary Methods).

Statistical analysis

For all analyses, we used a basic (age adjusted) and a multivariable linear regression model, stratified by sex because men and women have different risk factors for wrinkling [12]. First, we tested the effect of known and possible new risk factors (physical activity, daily energy intake) on facial wrinkling. Second, all PCA patterns and DHDI were used to test associations between wrinkles and diet (Supplementary Methods).

Additional analysis

Ultraviolet (UV) exposure data was missing for 45% of the study participants. In a complete case analysis in women (N = 849), we adjusted our main analysis for UV exposure variables (Supplementary Methods). Association tests with physical activity were additionally adjusted for UV to reduce residual confounding. Also, we tested single food groups separately in women to understand which food groups drive the association of a food pattern (Supplementary Table S1).

RESULTS

The study population consisted of 2753 middle-aged and elderly Dutch men (41%) and women (59%), with a median age of 67.3 (interquartile range [IQR] 62.6-72.3) years. Known risk factors, such as age, sex, body mass index, and smoking status, showed a significant association with wrinkle area in both the basic and the multivariable model. Of the newly investigated risk factors, daily energy intake did not have an effect on wrinkles, even when not adjusting for body mass index. Strikingly, more physical activity resulted in more wrinkles in the multivariable model in men and in women (Table 1).

Adherence to the predefined healthy diet, shown by higher DHDI scores, resulted in significantly less facial wrinkling in women (−4.19%, 95% confidence interval [CI] −7.30 to −1.08; Table 2) but not men. In women and in men, we extracted 4 and 3 food patterns, respectively, using PCA of the 34 food groups. The first 3 PCA patterns in women and men were comparable. The

Table 1. Population characteristics women and men

WOMEN N=1613	N/Mean/ Median	Wrinkle%Δ Univariable	* 95%CI	Wrinkle%Δ Multivariable	** 95%CI
Wrinkle %Δ-median [IQR]	3.7 [2.3-5.8]				
Age - median [IQR]	67.1 [62.5-72.0]	4.524	**[4.102, 4.948]**	4.726	**[4.294, 5.159]**
Daily energy intake (Kcal)-mean (SD)[1]	2027 (636)	0.0004	[-0.004, 0.005]	-0.001	[-0.005, 0.004]
Physical activity (METhours/week)-median [IQR][2]	46.6 [18.8-87.4]	0.093	**[0.029, 0.157]**	0.082	**[0.019, 0.144]**
BMI in kg/m²-mean (SD)	27.4 (4.8)	-2.138	**[-2.726, -1.546]**	-2.057	**[-2.652, -1.458]**
Smoking					
Never (%)	634 (39)	Ref	-	Ref	-
Former (%)	739 (46)	-1.392	[-6.992, 4.545]	10.413	**[3.798, 17.450]**
Current (%)	237 (15)	32.692	**[22.300, 43.967]**	37.473	**[25.978, 50.016]**
Education[3]					
Low (%)	140 (9)	Ref	-	Ref	-
Medium (%)	1087 (67)	2.235	[-4.018, 8.894]	-3.142	[-12.156, 6.798]
High (%)	370 (23)	-5.111	[-11.610, 1.865]	-9.263	[-18.754, 1.337]
MEN N=1150					
Wrinkle %Δ-median [IQR]	4.6 [3.1-6.5]				
Age - median [IQR]	67.7 [62.7-72.7]	2.395	**[1.980, 2.811]**	2.555	**[2.113, 2.999]**
Daily energy intake (Kcal)-mean (SD)[1]	2312 (706)	0.005	[0.0003, 0.009]	0.004	[-0.001, 0.008]
Physical activity (METhours/week)-median [IQR][2]	40.7 [17.9-73.3]	0.111	**[0.035, 0.186]**	0.108	**[0.033, 0.183]**
BMI in kg/m²-mean (SD)	27.4(3.5)	-1.898	**[-2.757, -1.031]**	-1.816	**[-2.680, -0.946]**
Smoking					
Never (%)	242 (21.2)	Ref	-	Ref	-
Former (%)	679 (59.6)	-6.149	[-11.978, 0.066]	0.524	[-6.935, 8.620]
Current (%)	218 (19.1)	13.610	**[5.102, 22.807]**	15.279	**[4.898, 26.686]**
Education[3]					
Low (%)	67 (5.9)	Ref	-	Ref	-
Medium (%)	630 (55.3)	0.858	[-5.131, 7.225]	9.157	[-3.092, 23.017]
High (%)	428 (37.5)	1.194	[-5.008, 7.800]	8.463	[-4,140, 22.721]

Wrinkle area percentage delta per characteristic, 'univariable' and multivariable linear regression. Significant results (p=<0.05) are presented in **bold.** [1] Daily energy intake in kilocalories (Kcal). 1Kcal= 4184 joules; [2] METhours/week= Metabolic Equivalent of Task, a physiological measure expressing the energy cost of physical activity; [3] Categories of education; low=primary education, medium=lower vocational education/lower secondary education/intermediate vocational education, high=general secondary education/higher vocational education/university. * 'Univariable' analysis is adjusted for technical variation and age; ** Multivariable analysis is adjusted for technical variation, age, daily energy intake, physical activity, BMI, smoking and education.

first pattern consisted of high consumption of mainly healthy food groups (including vegetables, fish and poultry, nuts and seeds, and mineral water) and wine. The second pattern was an unhealthy pattern consisting of consumption of mainly meat, grains, snacks, soft drinks, coffee, and other alcoholic drinks. The third pattern was an intermediate mix of healthy and unhealthy foods that resembled a typical Dutch diet, which included a high intake of cheese, potatoes, grains, and fats (Table 3). The fourth PCA pattern, which was seen in women, was a diet high in fruit, supplemented with yogurt, milk, and some vegetables (Table 3).

In men, no a posteriorly defined food pattern was associated with increased or decreased wrinkling, but in women, the unhealthy pattern was significantly associated with more wrinkling (3.32%, 95% CI 0.06 to 6.68) and the fruit pattern was significantly associated with less facial wrinkling (−3.20%; 95% CI −6.25 to −0.06) (Table 2). We also calculated the same fruit PCA pattern in men, but there was no significant protective effect on wrinkles for this food pattern (−0.41%, 95% CI −3.67 to 2.96).

UV exposure and physical activity did not significantly alter effect size of food patterns in our sensitivity analysis (data not shown). The single food group analysis detected single food groups associated with facial wrinkling (Supplementary Table S1).

Table 2. Association of the Dutch Healthy Diet Index (DHDI) and dietary patterns with facial wrinkles.

	Women (N=1613)			**Men (N=1150)**		
Dietary pattern	Wrinkle Δ%	95%CI	p.	Wrinkle Δ%	95%CI	p.
A priori						
DHDI	-4.48	**[-7.58, -1.36]**	**0.005**	0.61	[-2.79, 4.03]	0.724
A posteriori						
'Healthy'	-0.56	[-3.55, 2.54]	0.723	0.76	[-2.62, 4.26]	0.664
'Unhealthy'	3.32	**[0.06, 6.68]**	**0.046**	2.72	[-0.58, 6.12]	0.107
'Intermediate'	-1.84	[-5.39, 1.83]	0.322	-0.67	[-4.79, 3.63]	0.755
'Fruit'	-3.20	**[-6.25, -0.06]**	**0.046**	-	-	-

*Percentage increase/decrease in wrinkle area percentage(Δ%) per 10 points increase on the DHDI, when committing to a dietary pattern in the female and male group. **P-values< 0.05 are considered to be significant and are presented in **bold.** *** Adjusted for technical variation, age, physical activity, BMI, daily energy intake, smoking and education level.

DISCUSSION

We found a healthy diet to be associated with less facial wrinkling in women, shown by both the predefined DHDI and the healthy fruit pattern in women. In addition, the unhealthy food pattern was associated with more facial wrinkling in the same group, providing more evidence of the link between a healthy diet and wrinkling. These observations are in-line with previous studies showing that high intake of animal source products, fats, and carbohydrates increased skin aging [5, 6] and vitamin C and carotenoids decreased wrinkles [7].

Table 3. Dietary patterns (eigenvalue≥1.5) with factor loadings of the contributing food groups in women and men.

	Women (N=1613)				**Men (N=1150)**		
	'Healthy'	'Unhealthy'	'Intermediate'	'Fruit'	'Healthy'	'Unhealthy'	'Intermediate'
Citrus fruits	-	-	-	**0.836**	**0.448**	-0.418	-
Other fruits	-	-	-	**0.826**	**0.515**	-0.455	-
Yellow vegetables	**0.754**	-	-	0.204	**0.718**	-	-
Greenleafy vegetables	**0.739**	-	-	-	**0.691**	-	-
Other vegetables	**0.688**	-	-	-	**0.653**	-	-
Pulses	0.227	-	-	-	-	-	-
Milk	-	-	-	0.251	-	-	0.323
Yoghurt	-	-	-	0.208	0.267	-0.230	-
Cheese	-	0.370	-	-	-	-	0.343
Soy	0.297	-0.277	-	-	-	-	-
Nuts and seeds	-	-	-	-	0.317	-	-
Eggs	-	0.350	-	-	-	0.228	-
Poultry	0.262	0.252	-	-	0.271	0.251	-
Unprocessed meat	-	**0.546**	-	-	-	**0.471**	-
Processed meat	-	**0.575**	-	-	-	**0.505**	0.306
Lean fish	0.394	0.227	-	-	0.339	0.289	-
Fatty Fish	**0.469**	-	-	-	0.291	0.248	-
Shellfish	0.272	0.275	-	-	-	0.378	-
Whole grains	-	-	0.365	-	-	-	0.374
Refined grains	-	0.338	0.246	-	-	**0.425**	0.210
Potatoes	-	0.204	**0.417**	-	-	0.097	0.394
Soups and sauces	-	-	0.343	-	-	0.181	0.397
Savoury snacks	-	**0.446**	-	-	-	**0.454**	-
Sweets	-	0.229	**0.441**	0.285	-	-	**0.521**
Soft drinks	-	0.286	-	-	-	0.288	0.237
Wine	0.246	0.233	-	-	0.246	-	-0.299
Other alcoholic drinks	-	-	-	-	-	**0.404**	-
Mineral water	0.303	-	-	-	0.289	0.100	-
Herb tea	0.299	-0.314	-	-	0.214	-	-
Black tea	-	-	0.209	-	-	-0.259	0.235
Coffee	-	0.338	-	-	-	0.328	-
Olive oil	-	-	**0.494**	-	-	-	0.266
Healthy fats	-	-	**0.619**	-	-	-	**0.532**
Unhealthy fats	-	-	**0.589**	-	-	-	**0.507**
Eigenvalues	3.051	2.302	1.753	1.588	3.140	2.326	1.776
Explained variance (%)	8.973	6.770	5.155	4.671	9.236	6.842	5.223

[1] Food groups with a factor loading ≥0,2 or ≤-0,2 are considered to have an important association with a dietary pattern and are presented. Weak associations are displayed as (-); [2] Factor loadings >0.4 are presented in **bold**. They represent the highest and most explanatory factor loadings for the specific pattern; [3] Food patterns are respectively named 'Healthy', 'Unhealthy', 'Intermediate' and 'Fruit'.

Both a healthy diet preventing wrinkles and an unhealthy diet aggravating wrinkles were found in women but not in men. Men and women are known to show distinct wrinkling patterns and different dietary habits, which could help explain the sex differences in the wrinkle associations[12, 16]. Although, no wrinkle-protecting effect was found when applying the fruit-based food pattern to the male subgroup, this difference might be explained by men consuming less fruits than women, making an association harder to detect.

The posteriorly defined healthy food PCA pattern was not associated with less facial wrinkling in women. The single food group analysis showed that of nutrients in the healthy PCA pattern, yellow vegetables and soy were significantly associated with less wrinkling and wine was significantly associated with more wrinkling. Thus, this common food pattern includes food groups that associate with both less and greater wrinkling, and is therefore not associated with wrinkling overall.

Examining patterns of nutrient intake can be valuable in the interpretation of nutrition associations. For example, although processed meat does not associate with wrinkling in the single nutrient analysis, it does associate via the unhealthy PCA pattern in women, which suggests that in concert with other (unhealthy) nutrients, processed meat could be promoting skin wrinkling.

The biological mechanism responsible for the unhealthy PCA pattern association could be increased oxidative stress load [17], an upregulated inflammatory state [18] or the effect of Advanced Glycation Endproducts which can disrupt cell metabolism and weaken antioxidant defense [19]. In contrast, vitamins and flavonoids in a healthy diet provide protection from photoaging and stimulate collagen production and DNA repair mechanisms[20, 21].

We found more physical activity to be associated with more facial wrinkling in both sexes. As many sports are practiced outside, UV exposure could play a role through residual confounding. However, the effect was independent of UV exposure in our sensitivity analysis.

The main strength of our study is that we used 2 validated methods to capture dietary patterns associated with facial wrinkles in a large population-based cohort. Also, wrinkles were digitally quantified in a standardized and validated way, reducing interobserver bias and measurement error.

However, nutrition intake is difficult to capture accurately and our Food Frequency Questionnaire data correlates less with intake of vegetables and better with snacks in a validation study [9]. We tried to reduce confounding by adjusting for possible and known confounders in our analyses. Nonetheless, there are other possible residual confounders, which were not available in our data set, such as stress and hours of sleep per night. Another possible confounder is health-conscious behavior, as it is possible that people who eat healthy also tend to use sunscreen more often. Although our sensitivity analysis excluded confounding by UV protection behaviors, 45% of the data was missing, giving some uncertainty to the accuracy of this analysis. Finally, due to the cross-sectional design of our study, we cannot exclude reverse causality.

In conclusion, our findings imply that type of diet influences the severity of facial wrinkles in women, where an unhealthy diet significantly increases wrinkling and a healthy diet decreases facial wrinkling. This creates opportunities to stimulate adherence to a healthy dietary pattern in women who want to maintain a youthful appearance, which simultaneously could improve overall health and decrease mortality risk [22].

REFERENCES

1. Cho S. The Role of Functional Foods in Cutaneous Anti-aging. Journal of Lifestyle Medicine 2014;4:8-16.
2. Yoon H-S, Kim JR, Park GY, Kim J-E, Lee DH, Lee KW et al. Cocoa Flavanol Supplementation Influences Skin Conditions of Photo-Aged Women: A 24-Week Double-Blind, Randomized, Controlled Trial. The Journal of Nutrition 2016;146:46-50.
3. Schwartz S, Frank E, Gierhart D, Simpson P , Frumento R. Zeaxanthin-based dietary supplement and topical serum improve hydration and reduce wrinkle count in female subjects. Journal of Cosmetic Dermatology 2016;15:e13-e20.
4. Žmitek K, Pogačnik T, Mervic L, Žmitek J , Pravst I. The effect of dietary intake of coenzyme Q10 on skin parameters and condition: Results of a randomised, placebo-controlled, double-blind study. BioFactors 2017;43:132-40.
5. Purba MB, Kouris-Blazos A, Wattanapenpaiboon N, Lukito W, Rothenberg EM, Steen BC et al. Skin wrinkling: can food make a difference? J Am Coll Nutr 2001;20:71-80.
6. Cosgrove MC, Franco OH, Granger SP, Murray PG , Mayes AE. Dietary nutrient intakes and skin-aging appearance among middle-aged American women. The American Journal of Clinical Nutrition 2007;86:1225-31.
7. Nagata C, Nakamura K, Wada K, Oba S, Hayashi M, Takeda N et al. Association of dietary fat, vegetables and antioxidant micronutrients with skin ageing in Japanese women. British Journal of Nutrition 2010;103:1493-8.
8. Hu FB. Dietary pattern analysis: a new direction in nutritional epidemiology. Curr Opin Lipidol 2002;13:3-9.
9. Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, Sturmans F 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.
10. 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;32:807-50.
11. Hamer MA, Jacobs LC, Lall JS, Wollstein A, Hollestein LM, Rae AR et al. Validation of image analysis techniques to measure skin aging features from facial photographs. Skin Research and Technology 2015;21:392-402.
12. Hamer MA, Pardo LM, Jacobs LC, Ikram MA, Laven JS, Kayser M et al. Lifestyle and physiological factors associated with facial wrinkling in men and women. J Invest Dermatol 2017.
13. Liu F, Hamer Merel A, Deelen J, Lall Japal S, Jacobs L, van Heemst D et al. The MC1R Gene and Youthful Looks. Current Biology 2016;26:1213-20.
14. van Lee L, Feskens EJ, Meijboom S, Hooft van Huysduynen EJ, van't Veer P, de Vries JH et al. Evaluation of a screener to assess diet quality in the Netherlands. Br J Nutr 2016;115:517-26.
15. van Lee L, Geelen A, van Huysduynen EJ, de Vries JH, van't Veer P , Feskens EJ. The Dutch Healthy Diet index (DHD-index): an instrument to measure adherence to the Dutch Guidelines for a Healthy Diet. Nutr J 2012;11:49.
16. Wardle J, Haase AM, Steptoe A, Nillapun M, Jonwutiwes K , Bellisle F. Gender differences in food choice: the contribution of health beliefs and dieting. Ann Behav Med 2004;27:107-16.
17. Rinnerthaler M, Bischof J, Streubel MK, Trost A , Richter K. Oxidative Stress in Aging Human Skin. Biomolecules 2015;5:545-89.
18. Baylis D, Bartlett DB, Patel HP , Roberts HC. Understanding how we age: insights into inflammaging. Longevity & Healthspan 2013;2:8-.

19. Clarke RE, Dordevic AL, Tan SM, Ryan L , Coughlan MT. Dietary Advanced Glycation End Products and Risk Factors for Chronic Disease: A Systematic Review of Randomised Controlled Trials. Nutrients 2016;8:125.
20. Schagen SK, Zampeli VA, Makrantonaki E , Zouboulis CC. Discovering the link between nutrition and skin aging. Dermato-endocrinology 2012;4:298-307.
21. Rahman K. Studies on free radicals, antioxidants, and co-factors. Clinical Interventions in Aging 2007;2:219-36.
22. Gunn DA, Larsen LA, Lall JS, Rexbye H , Christensen K. Mortality is Written on the Face. J Gerontol A Biol Sci Med Sci 2016;71:72-7.

SUPPLEMENTARY MATERIALS & METHODS

Covariate selection

Sex and age were collected from the database. Education level, smoking habits, ultraviolet (UV) exposure and physical activity were retrieved from the interviews. Body mass index (BMI) was calculated from weight and height measured at the research center. Total energy intake per day in kilocalories was calculated using the Dutch Food Composition Table (NEVO) of 2006.[1].

Food Frequency Questionnaire (FFQ)

The FFQ gives information on the consumption frequency and the average consumed amounts of 389 food items. The FFQ score was validated against dietary records over a 3-day period (4-5 months apart) in another Dutch population aged 55-69 years[2].

A priori food pattern

The DHDI is a composed measure of healthy nutritional behavior taking the Dutch governmental guidelines of a healthy diet into account, where a higher score correlates with the highest diet qualit[3, 4]. Physical activity and fish, fruit, vegetable, and fiber consumption are adequacy components, and saturated fatty acids, trans-fatty acids, number of consumption occasions of acidic drinks and foods, sodium, and alcohol are moderation components [4]. In our study, we calculated the DHDI from the nutritional data out of the FFQ, leaving the physical activity component out, resulting in a healthiness grade 0-90.

A posteriori food pattern

The 389 food items were first subdivided into 34 food groups by a nutritionist (Supplementary Table S1), on the basis of their nutritional characteristics and hypothesized association with skin aging [1] . The principal component analysis (PCA) with varimax rotation extracted food patterns from the 34 food groups, explaining the maximum variation of food intake in women and men separately, since men and women tend to eat differently [5]. Food patterns were considered relevant when showing an eigenvalue>1.5.

Statistical analysis

Associations between dietary intake and wrinkle area percentage were assessed using linear regression. Wrinkle area percentage was natural logarithm–transformed to normalize the distribution. For a more intuitive interpretation of the betas, we used the formula (expβ-1) × 100%, which results in a wrinkle percentage change. This is the percentage increase or decrease in wrinkle area per unit increase of the tested variable. All analyses were adjusted for technical variation, explained by 2 variables, which accounted for variations in resolution and flash light, as described in detail previously [6]. We tested the association of the wrinkle percentage in both a basic model (adjusted for technical variation and age) and a multivariable model including all

covariates (age, sex, BMI, daily energy intake, physical activity, smoking habit, education level). We tested for effect modification by BMI, which did not alter our results. All covariates had <8% missing data, which we replaced with multiple imputation. In our main analysis, we tested the association of DHDI (per 10 points increase in DHDI) and the relevant nutritional patterns from the PCA with wrinkle area adjusted for all covariates. The extra relevant food pattern in women was also tested in men. All analyses were conducted using IBM SPSS Statistics for Windows version 21.0.

UV variables

UV exposure variables included tanning bed use, hibernating in a sunny country, sunburn tendency, outdoor work, and UV protection behavior.

REFERENCES

1. Voedingscentrum R. Nederlands Voedingsstoffenbestand 2006/ Stichting Nederlands Voedingsstoffenbestand. Den Haag2006.
2. Goldbohm RA, van den Brandt PA, Brants HA, van't Veer P, Al M, Sturmans F 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.
3. van Lee L, Feskens EJ, Meijboom S, Hooft van Huysduynen EJ, van't Veer P, de Vries JH et al. Evaluation of a screener to assess diet quality in the Netherlands. Br J Nutr 2016;115:517-26.
4. van Lee L, Geelen A, van Huysduynen EJ, de Vries JH, van't Veer P , Feskens EJ. The Dutch Healthy Diet index (DHD-index): an instrument to measure adherence to the Dutch Guidelines for a Healthy Diet. Nutr J 2012;11:49.
5. Wardle J, Haase AM, Steptoe A, Nillapun M, Jonwutiwes K , Bellisle F. Gender differences in food choice: the contribution of health beliefs and dieting. Ann Behav Med 2004;27:107-16.
6. Hamer MA, Pardo LM, Jacobs LC, Ikram MA, Laven JS, Kayser M et al. Lifestyle and physiological factors associated with facial wrinkling in men and women. J Invest Dermatol 2017.

SUPPLEMENTARY TABLES

Supplementary Table S1. Single food group analysis women

Food group	B	95% LB	95% UB	p.
Citrusfruits	-0.022	-0.051	0.007	0.14
All other fruits	-0.009	-0.021	0.003	0.14
Total Fruit*	-0.007	-0.017	0.002	0.11
Greenleafy vegetables	-0.028	-0.081	0.024	0.29
Yellow vegetables	-0.061	-0.112	-0.009	0.02
All other vegetables	-0.005	-0.036	0.026	0.76
Total vegetables*	-0.011	-0.028	0.006	0.20
Pulses	0.048	-0.046	0.142	0.32
Milk	0.008	-0.007	0.022	0.29
Yoghurt	0.015	-0.007	0.037	0.19
Cheese	0.165	0.038	0.293	0.01
Soy products	-0.064	-0.119	-0.009	0.02
Refined grains	-0.060	-0.129	0.010	0.09
Whole grains	0.002	-0.038	0.042	0.91
Soft drinks	0.002	-0.026	0.030	0.87
Eggs	0.187	0.013	0.361	0.04
Unprocessed meat	0.014	-0.091	0.118	0.80
Processed meat	-0.114	-0.295	0.067	0.22
Poultry	-0.196	-0.382	-0.009	0.04
Fatty fish	0.053	-0.110	0.216	0.53
Lean fish	0.043	-0.143	0.230	0.65
Shellfish	0.548	-0.137	1.238	0.12
Total fish*	0.045	-0.056	0.147	0.38
Savoury snacks	0.115	-0.023	0.254	0.10
Sweets	-0.048	-0.121	0.024	0.19
Nuts & seeds	0.053	-0.156	0.262	0.62
Coffee	0.016	0.003	0.029	0.02
Black tea	-0.002	-0.016	0.012	0.79
Herbal tea	-0.005	-0.026	0.017	0.68
Mineral water	0.009	-0.002	0.019	0.11
Alcoholic drinks other than wine	0.015	-0.018	0.048	0.37
Wine	0.041	0.011	0.070	0.01
Soups & sauces	-0.048	-0.095	-0.002	0.04
Potatoes	0.002	-0.048	0.053	0.93
Olive oil	0.060	-0.467	0.589	0.82
Healthy fats	0.015	-0.184	0.214	0.88

Supplementary Table S1. Single food group analysis women (continued)

Food group	B	95% LB	95% UB	p.
Unhealthy fats	0.237	0.043	0.432	0.02
Total fats*	0.103	-0.019	0.226	0.10

[1] Wrinkle %Δ per 100 grams intake of a food group (N=1613); [2] Multivariable linear regression adjusted for technical variation, age, BMI, energy intake, physical activity, smoking and education. Significant ($p<0.05$) associations are highlighted; green if associated with less wrinkle % and red if associated with more wrinkle %. * Because of their different nutritional characteristics, some of the food groups are presented both as a subgroup defined by a nutritionist (for example Citrus fruits that are high in vitamin C) and as a total together with all other fruits.Total fruits = Citrus fruits + All other fruits. Total vegetables = Greenleafy vegetables + Yellow vegetables + All other vegetables. Total fish = Fatty fish + Lean fish + Shellfish. Total fats = Olive oil + Healthy fats+ Unhealthy fats.